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Estimación del riesgo cardiovascular en pacientes con diabetes tipo 1: de la estrategia colectiva a la individualizada

Antonio Serés Noriega



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TESIS DOCTORAL

**Estimación del riesgo cardiovascular en pacientes
con diabetes tipo 1: de la estrategia colectiva a la
individualizada**

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I. Abreviaturas y acrónimos

A:

AAS: ácido acetil salicílico

ACC: *American College of Cardiology*

ACC/AHA-2013: *pooled cohort equation of the American College of Cardiology/American Heart Association 2013 Guideline on the Assessment of Cardiovascular Risk*

ACTC: angiografía coronaria por tomografía computarizada

ADA: *American Diabetes Association*

AHA: *American Heart Association*

AIT: accidente isquémico transitorio

AOVE: aceite de oliva virgen extra

ARA-II: antagonistas del receptor de angiotensina II

C:

CAC score: puntuación de calcio aorto-coronario

CACTI: *Coronary Artery Calcification in Type 1 Diabetes study*

cHDL: colesterol contenido en las lipoproteínas de alta densidad (HDL)

cLDL: colesterol contenido en las lipoproteínas de baja densidad (LDL)

c-no-HDL: colesterol no-HDL

CORDIOPREV: *CORonary Diet Intervention With Olive Oil and Cardiovascular PREvention trial*

D:

DCCT: *Diabetes Control and Complications Trial*

DT1: diabetes tipo 1

DT2: diabetes tipo 2

E:

EAP: enfermedad arterial periférica

EASD: *European Association for the Study of Diabetes*

ECG: electrocardiografía

ECV: enfermedad cardiovascular

ECVA: enfermedad cardiovascular aterosclerótica

EDC: *Pittsburgh Epidemiology of Diabetes Complications study*

EDIC: *Epidemiology of Diabetes Interventions and Complications*

eGDR: *estimated Glucose Disposal Rate*

ERC: enfermedad renal crónica

ERD: enfermedad renal diabética

ESC: *European Society of Cardiology*

ESC/EASD-2019: guías de prevención cardiovascular de la *European Society of Cardiology* en colaboración con la *European Association for the Study of Diabetes* de 2019

EURODIAB: *European Diabetes Prospective Complications Study*

F:

FGe: filtrado glomerular estimado

FinnDiane: *Finnish Diabetic Nephropathy Study*

FLI: *Fatty Liver Index*

FRCV: factores de riesgo cardiovascular

G:

GGT: gamma glutamil transferasa

GIM: grosor íntima-media

GLP-1: *glucagón-like peptide-1*

GlycA: señal emitida por los grupos acetilo -COCH₃ de la N-acetilglucosamina y la N-acetilgalactosamina en el estudio mediante ¹H-RMN

GlycB: señal emitida por los grupos acetilo -COCH₃ del ácido N-acetilneuramínico en el estudio mediante ¹H-RMN

H:

¹H-RMN: espectroscopia de resonancia magnética nuclear

HbA1c: hemoglobina glicada

HTA: hipertensión arterial

I:

IAM: infarto agudo de miocardio

IC 95%: intervalo de confianza al 95%

IECA: inhibidor de la enzima convertidora de angiotensina

IL-6: interleucina 6

ISCI: infusión subcutánea continua de insulina

ITB: índice tobillo-brazo

M:

MASLD: enfermedad hepática esteatósica asociada a disfunción metabólica

MCG: monitorización continua de glucosa

MESA: *Multi-Ethnic Study of Atherosclerosis*

N:

NAC: neuropatía autonómica cardíaca

NT-proBNP: fracción N-terminal del propéptido natriurético de tipo B

P:

PCSK9: proproteína convertasa subtilisina/kexina tipo 9

PREDIMED: ensayo clínico “Prevención con Dieta Mediterránea”

PREVENT: ecuación *Predicting Risk of CVD EVENTS*

PCRus: proteína C reactiva ultrasensible

R:

RCV: riesgo cardiovascular

RMN: resonancia magnética nuclear

RNL: razón entre neutrófilos y linfocitos circulantes (neutrófilos/linfocitos séricos)

S:

SEA: Sociedad Española de Arteriosclerosis

SCORE2: *Systematic Coronary Risk Evaluation 2*

Steno-Risk: *Steno Type 1 Risk Engine*

Swedish NDR: *Swedish National Diabetes Register*

T:

T1D-ECR: *Type 1 diabetes exchange clinic registry*

TAC: tomografía axial computerizada

TNF- α : factor de necrosis tumoral alfa

TnTas: troponina T de alta sensibilidad

U:

UA: unidades de Agatston

UKPDS: *UK Prospective Diabetes Study*

V:

VLDL: lipoproteína de muy baja densidad

II. Enumeración de los artículos de la tesis

Tesis en formato de compendio de publicaciones. La tesis consta de cinco objetivos y ocho artículos.

1. **Serés-Noriega T**, Perea V, Amor AJ. Screening for Subclinical Atherosclerosis and the Prediction of Cardiovascular Events in People with Type 1 Diabetes. J Clin Med. 2024 Feb 15;13(4). Factor de impacto (2022) = 3,9 (Q1)

2. **Serés-Noriega T**, Giménez M, Perea V, Boswell L, Viñals C, Blanco J, Vinagre I, Pané A, Esmatjes E, Conget I, Amor AJ. Use of the Steno T1 Risk Engine Identifies Preclinical Atherosclerosis Better Than Use of ESC/EASD-2019 in Adult Subjects With Type 1 Diabetes at High Risk. Diabetes Care. 2022 Oct 1;45(10):2412–21. Factor de impacto (2022) = 16,2 (Q1)

3. **Serés-Noriega T**, Viñals C, Perea V, Mesa A, Boswell L, Mariaca K, Blanco J, Vinagre I, Pané A, Milad C, Solà C, Esmatjes E, Conget I, Giménez M, Amor AJ. Adherence to an energy-restricted Mediterranean diet is associated with the presence and burden of carotid atherosclerosis in people with type 1 diabetes. Diabetes Metab Res Rev. 2024 Mar 1;40(3):e3783. Factor de impacto (2022) = 8 (Q1)

4. Perea V, Vinagre I, **Serés-Noriega T**, Viñals C, Mesa A, Pané A, Milad C, Esmatjes E, Conget I, Giménez M, Amor AJ. Impact of preeclampsia and parity on sex-based discrepancies in subclinical carotid atherosclerosis in type 1 diabetes. J Clin Endocrinol Metab. 2023 Dec 27 dgad755. Factor de impacto (2022) = 5,8 (Q1)

5. **Serés-Noriega T**, Ortega E, Giménez M, Perea V, Boswell L, Mariaca K, Font C, Mesa A, Viñals C, Blanco J, Vinagre I, Pané A, Esmatjes E, Conget I, Amor AJ. Advanced lipoprotein profile identifies atherosclerosis better than conventional lipids in type 1 diabetes at high cardiovascular risk. Nutr Metab Cardiovasc Dis. 2023 Jun 1 ;33(6):1235–44. Factor de impacto (2022) = 3,9 (Q1)

6. **Serés-Noriega T**, Ortega E, Perea V, Giménez M, Boswell L, Mariaca K, Font C, Mesa A, Viñals C, Blanco J, Vinagre I, Pané A, Esmatjes E, Conget I, Amor AJ. Nuclear Magnetic Resonance-Based Lipidomics in the Assessment of

Cardiometabolic Risk in Type 1 Diabetes: An Exploratory Analysis. *Diabetes Ther.* 2023 Mar 1;14(3):553–67. Factor de impacto (2022) = 3,8 (Q2)

7. Mariaca K, **Serés-Noriega T**, Viñals C, Perea V, Conget I, Mesa A, Boswell L, Font C, Pané A, Vinagre I, Blanco J, Esmatjes E, Giménez M, Amor AJ. Neutrophil-to-lymphocyte ratio is independently associated with carotid atherosclerosis burden in individuals with type 1 diabetes. *Nutr Metab Cardiovasc Dis.* 2024 Feb 21;34(2):395–403. Factor de impacto (2022) = 3,9 (Q1)

8. **Serés-Noriega T**, Giménez M, Perea V, Blanco J, Vinagre I, Pané A, Ruiz S, Cofán M, Mesa A, Esmatjes E, Conget I, Ortega E, Amor AJ. Quantification of glycoproteins by nuclear magnetic resonance associated with preclinical carotid atherosclerosis in patients with type 1 diabetes. *Nutr Metab Cardiovasc Dis.* 2021 Jun 30;31(7):2099–108. Factor de impacto (2021) = 4,666 (Q1)

III. Resumen de la tesis

Título

Estimación del riesgo cardiovascular en pacientes con diabetes tipo 1: de la estrategia colectiva a la individualizada

Introducción

Las personas con diabetes tipo 1 (DT1) presentan un riesgo de enfermedad cardiovascular aterosclerótica (ECVA) de hasta ocho veces superior al de la población general. Además, incluso en aquellos con un control glucémico óptimo, este riesgo es tres veces superior, lo cual sugiere que deben de existir otros factores implicados en este elevado riesgo residual.

Actualmente, la estimación del riesgo cardiovascular (RCV) en estos pacientes se basa en clasificaciones arbitrarias o basadas en evidencias provenientes de cohortes antiguas, cuando existía un peor control de los diversos factores de riesgo cardiovascular (FRCV), o bien de extrapolaciones de estudios llevados a cabo en personas con diabetes tipo 2 (DT2). Estas estimaciones generalizadas impiden identificar correctamente a aquellos sujetos con DT1 que más se puedan beneficiar de un manejo precoz y agresivo de sus FRCV.

Hipótesis

La estrategia de prevención cardiovascular actual, basada en escalas de riesgo utilizadas en población general, no identifica correctamente a los sujetos con DT1 susceptibles de presentar futuros eventos cardiovasculares. El uso de distintos factores y biomarcadores podría permitir la reclasificación de los pacientes y, con ello, guiar de forma más precisa las medidas preventivas.

Objetivos

Ante este panorama, los objetivos de esta tesis son: revisar los principales métodos de detección de aterosclerosis subclínica y escoger una técnica útil para diagnosticar a nuestra cohorte. Una vez escogida, comparar las principales escalas de riesgo cardiovascular y su capacidad para identificar a aquellos con mayor carga

aterosclerótica (Objetivo 1). Finalmente, analizar el papel de otros factores de riesgo no clásicos (dietéticos, obstétricos) y biomarcadores (lipoproteicos, lipídicos e inflamatorios) en este RCV incrementado en adultos con DTI (Objetivos 3-5).

Métodos

Se incluye una cohorte de pacientes con DTI en seguimiento por la Unidad de Diabetes del Servicio de Endocrinología y Nutrición del Hospital Clínic de Barcelona. Los criterios de inclusión son: ≥ 40 años de edad, presencia de enfermedad renal diabética y/o evolución de la diabetes ≥ 10 años asociado a la presencia de otro FRCV. Se ha realizado una evaluación estandarizada, incluyendo el estudio de la aterosclerosis subclínica/preclínica mediante ecografía carotídea, recolección de datos mediante cuestionarios validados y de muestras sanguíneas para la evaluación de los diferentes biomarcadores. Se ha analizado de forma transversal la asociación de las diferentes variables (escalas de riesgo, factores obstétricos o dietéticos) o biomarcadores (lipídicos, inflamatorios) con la aterosclerosis (presencia y número de placas carotídeas). Se han realizado modelos de regresión logística para estudiar el papel independiente de los diferentes factores/biomarcadores en la aterosclerosis.

Resultados principales

Objetivo 1

a) Dentro de las técnicas para cribar la aterosclerosis subclínica en personas con DTI, la que parece ser de mayor utilidad en cuanto a la predicción de eventos cardiovasculares es la puntuación de calcio aorto-coronario (CAC score). Sin embargo, con esta técnica pasan desapercibidas las fases más iniciales en la formación de las placas de ateroma. El uso de la ecografía carotídea permite ver estos cambios precoces, que ocurren con prontitud en las personas con DTI. Además, se trata de una técnica con bajo coste y que consigue caracterizar rasgos de vulnerabilidad de las placas. Escogemos esta técnica como principal variable resultado en el resto de objetivos de esta tesis.

b) La escala de riesgo genérica de las guías de prevención cardiovascular de la “European Society of Cardiology” en colaboración con la “European Association for the Study of Diabetes” de 2019 (ESC/EASD-2019) muestra una pobre concordancia con la ecuación “Steno Type 1 Risk Engine” (Steno-Risk), específica de DTI. Además, el uso de Steno-Risk se asocia de manera independiente a la presencia y número de placas de ateroma independientemente de múltiples FRCV y del riesgo estimado por ESC/EASD-2019.

Objetivo 2

a) Una mayor adherencia a la dieta mediterránea hipocalórica, evaluada mediante el cuestionario de 17 ítems del ensayo PREDIMED-Plus, se asocia a la presencia y número de placas carotídeas en los modelos de regresión logística ajustados por múltiples confusores. El mayor consumo de frutas y de nueces también se asocia de forma inversa e independiente con la aterosclerosis.

b) Las mujeres con preeclampsia previa muestran una prevalencia de aterosclerosis carotídea superior a las que no la tuvieron y similar a la de los hombres. Además, la ecuación específica Steno-Risk muestra un peor rendimiento en este subgrupo. La inclusión de la paridad o de la historia de preeclampsia previa no mejora el rendimiento de la ecuación Steno-Risk.

Objetivo 3

Variables del perfil avanzado de lipoproteínas relacionadas con el HDL, LDL y cantidad total de partículas aterogénicas medidas mediante espectroscopia por resonancia magnética nuclear (^1H -RMN) se asocian de manera independiente con la aterosclerosis preclínica.

Objetivo 4

El estudio de la lipidómica mediante ^1H -RMN muestra la asociación inversa e independiente de algunos marcadores (e.g. esfingomielina, ácidos grasos omega-6) con la carga aterosclerótica.

Objetivo 5

a) Varios biomarcadores inflamatorios séricos aumentan conforme lo hace el número de placas carotídeas. Únicamente la razón entre los neutrófilos y los linfocitos circulantes (RNL) se asocia con la presencia y número de placas carotídeas.

b) Diversos parámetros de glicoproteínas inflamatorias medidas por ¹H-RMN se asocian de forma independiente con la presencia y número de placas de ateroma en aquellos sin tratamiento hipolipemiente.

Conclusiones

La estimación del RCV en pacientes con DTI que se realiza actualmente no permite identificar a aquellos que más se podrían beneficiar de un manejo precoz y agresivo. El uso de ecuaciones específicas de la enfermedad, técnicas de imagen y de varios factores/biomarcadores podrían ser de utilidad para su correcta fenotipación.

IV. Introducción

1. La importancia de la enfermedad cardiovascular en personas con diabetes tipo 1

Las personas con diabetes tipo 1 (DT1) presentan un riesgo cardiovascular (RCV) de entre cuatro y ocho veces superior al observado en la población sin diabetes, siendo la enfermedad cardiovascular aterosclerótica (ECVA), mayoritariamente de ubicación coronaria, la principal causa de mortalidad total y prematura [1-18]. Se trata del principal condicionante de la disminución en la esperanza de vida, estimada en una diferencia de más de diez años, en comparación con la población general [19,20]. Cabe destacar que, pese a que las mejoras en el manejo y prevención se han traducido en una menor incidencia de ECVA y mortalidad cardiovascular en países desarrollados [1,2,4,21], la de diagnósticos de DT1 ha aumentado globalmente, lo cual implica una mayor cantidad de población con riesgo de desarrollar ECVA [22-24]. Adicionalmente, la edad temprana a la que se suele diagnosticar la DT1, condiciona una exposición prolongada a los efectos deletéreos de la enfermedad en la formación y progresión de la aterosclerosis [25].

Clásicamente, se ha considerado la hiperglucemia crónica como el principal, e incluso único, factor de riesgo cardiovascular (FRCV). Ello se debe a que el ensayo clínico aleatorizado (ECA) y multicéntrico *Diabetes Control and Complications Trial* (DCCT) y su prospección observacional *Epidemiology of Diabetes Interventions and Complications* (EDIC), demostraron que el tratamiento intensivo de la diabetes durante 6,5 años, optimizando el control glucémico (objetivo de hemoglobina glicada [HbA1c] de 7% frente a 9%), se tradujo en una disminución del riesgo de ECVA, el cual perdura incluso tras treinta años de seguimiento [26,27]. Otros análisis de grandes cohortes prospectivas, como la del estudio *Pittsburgh Epidemiology of Diabetes Complications* (EDC) o *Finnish Diabetic Nephropathy Study* (FinnDiane), realizados en distintas poblaciones, apuntan en la misma dirección, mostrando la importancia del control glucémico en la prevención cardiovascular [28,29]. Estos resultados no son de extrañar teniendo en cuenta el papel etiopatogénico de la hiperglucemia crónica en la aparición y evolución de otro de los principales FRCV, la enfermedad renal diabética (ERD)

[30]. Sin embargo, datos más recientes, indican que incluso consiguiendo un control glucémico óptimo ($HbA_{1c} \leq 6.9\%$), el riesgo de muerte por causa cardiovascular supera entre dos y cuatro veces al de los controles sin diabetes, incluso tras ajustar por múltiples FRCV [5]. Además, un estudio de la cohorte FinnDiane, objetivó que entre aquellos pacientes con DTI y sin albuminuria, el riesgo de muerte por cardiopatía isquémica fue cuatro veces superior al de sus homólogos sin diabetes [7]. Adicionalmente, tras seguir durante treinta años a los sujetos del estudio EDC, la disminución, cercana al 60%, en la incidencia de insuficiencia renal, no se tradujo en una menor incidencia de ECVA [31]. Estos hallazgos ponen de manifiesto un elevado RCV residual, aquel que perdura tras mejorar los principales FRCV, e invita a abandonar el enfoque glucocéntrico imperante.

Por último, mencionar que el uso de tecnología aplicada a la diabetes (e.g. monitorización continua de la glucosa [MCG], sistemas de asa cerrada) ha irrumpido velozmente en la práctica clínica habitual. Pese a que su uso ha demostrado mejorar el control glucémico [32–34], todavía es pronto para evaluar objetivos cardiovasculares. Analizar cohortes actuales permitirá desgranar mejor este RCV residual.

2. Estrategia actual de predicción de eventos cardiovasculares en personas con diabetes tipo 1

Pese a la diferente fisiopatología, la mayoría de recomendaciones en cuanto a prevención cardiovascular se basan en extrapolaciones de estudios realizados en personas con diabetes tipo 2 (DT2) o bajo el antiguo término de “diabetes insulín dependiente”, donde las personas con DTI han estado pobremente representadas. Ello se debe a la escasez de ECA con objetivos cardiovasculares en personas con DTI y a que, como se ha mencionado anteriormente, los estudios clásicos han reclutado indistintamente a personas con DTI y DT2, siendo esta última mayoritaria por motivos meramente epidemiológicos. Ello supone un error fundamental, atendiendo a que ambas entidades no comparten mucho más allá de

la hiperglucemia, y eso queda plasmado en las diferencias de su perfil de RCV [35]. Además, en muchos de los ECA que han evaluado fármacos cardioprotectores (e.g. hipolipemiantes, antiagregantes, antihipertensivos) se ha excluido a las personas con DTI, por lo que desconocemos los objetivos terapéuticos óptimos en esta población.

Las principales guías de práctica clínica en prevención cardiovascular, ofrecen clasificaciones y recomendaciones cerradas del RCV (e.g. para un riesgo determinado se debe iniciar tratamiento hipolipemiante para un objetivo lipídico concreto), o menos estrictas y categóricas (e.g. si se cumplen ciertas características se puede valorar iniciar tratamiento hipolipemiante). En ellas se recomienda realizar cambios cardiosaludables en el estilo de vida (e.g. cese tabáquico, control ponderal, dieta cardiosaludable como la dieta mediterránea, actividad física) e inicio de fármacos cardioprotectores en determinados subgrupos (e.g. ácido acetil salicílico [AAS], inhibidores de la enzima convertidora de angiotensina [IECA], antagonistas del receptor de angiotensina II (ARA-II), estatinas). En la **Tabla 1** se resumen las categorizaciones del riesgo y recomendaciones de tratamiento hipolipemiante de las guías más actuales y utilizadas. En su mayoría, no se hace distinción entre el tipo de diabetes (DTI o DT2) y, en otras, se esbozan algunas diferencias entre ambas entidades, ofreciendo recomendaciones, en algunos casos poco claras, en cuanto al manejo terapéutico.

Tabla 1. Resumen de la categorización del riesgo cardiovascular y recomendaciones de tratamiento hipolipemiante en personas con diabetes tipo 1 según a las principales guías de práctica clínica

Guía	Categoría de riesgo	Recomendaciones de tratamiento hipolipemiante
<i>American Diabetes Association (ADA) 2024 [36]</i>	Edad 20-39 años y ≥ 1 FRCV	Razonable iniciar estatina
	Edad 40-75 años sin ECVA	Estatina de moderada intensidad
	Edad 40-75 años y ≥ 1 FRCV	Estatina de alta intensidad para objetivos de cLDL <

		70 mg/dL y $\geq$ 50% de reducción Valorar añadir ezetimibe o iPCSK9 si no se alcanza objetivo
	Asociar ECVA	Estatina de alta intensidad para objetivos de cLDL < 55 mg/dL y $\geq$ 50% de reducción Añadir ezetimibe o iPCSK9 si no se alcanza objetivo
<i>European Society of Cardiology (ESC) y European Association for the Study of Diabetes (EASD) 2019 [37]</i>	Moderado: Edad < 35 años con < 10 años de evolución y sin otros FRCV	Las estatinas pueden considerarse en > 30 años de edad asintomáticos, para objetivos de cLDL < 100 mg/dL
	Alto: $\geq$ 10 años de evolución sin lesión de órgano diana y $\geq$ 1 FRCV	Tratamiento para objetivos de cLDL < 70 mg/dL y $\geq$ 50% de reducción
	Muy alto: Asociar ECVA o Lesión de órgano diana o > 20 años de evolución y debut en la infancia	Tratamiento para objetivos de cLDL < 55 mg/dL y $\geq$ 50% de reducción
ESC 2021 [38]	Edad $\leq$ 40 años + lesión de órgano diana y/o cLDL > 100 mg/dL	Puede considerarse el uso de estatina
	Moderado: Edad > 40 años + < 10 años de evolución, sin lesión de órgano diana ni FRCV adicionales	-
	Alto: Edad > 40 años sin ECVA o lesión grave de órgano diana y que no cumplan criterios de riesgo moderado	Tratamiento para objetivos de cLDL < 100 mg/dL (inicio) y < 70 mg/dL y $\geq$ 50% de reducción (intensificación)
	Muy alto: > 40 años + ECVA o lesión grave de órgano diana: -FGe < 45 mL/min/1,73 m ² -FGe 45-59 mL/min/1,73 m ² + cociente	Tratamiento para objetivos de cLDL < 70 mg/dL (inicio) y < 55 mg/dL y $\geq$ 50% de reducción (intensificación)

	albúmina/creatinina en orina 30-300 mg/g -Cociente albúmina/creatinina en orina > 300 mg/g -Presencia de enfermedad microvascular en ≥ 3 territorios	
ESC 2023 [39]	Edad > 40 años sin ECVA o Edad < 40 años + (otros FRCV o complicaciones microvasculares o riesgo calculado de ECVA a 10 años $\geq 10\%$)	Debe considerarse el uso de estatina
Sociedad Española de Arteriosclerosis (SEA) 2024 [40]	Moderado: Edad < 35 años y < 10 años de evolución Alto: Si no moderado o muy alto Muy alto: Asociar ECVA o Lesión de órgano diana o Enfermedad vascular subclínica o Otros FRCV o ERC grado 4-5 o > 20 años de evolución	Valorar tratamiento hipolipemiente si cLDL > 116 mg/dL Iniciar tratamiento hipolipemiente si cLDL > 70 mg/dL Iniciar tratamiento hipolipemiente para objetivos de cLDL < 55 mg/dL y $\geq 50\%$ de reducción
American Association of Clinical Endocrinology (AACE) 2022 [41]	Bajo y moderado: No aplicable en DTI Alto: < 20 años de evolución con < 2 FRCV y sin lesión de órgano diana Muy alto: > 20 años de evolución y edad > 40 años (sin ECVA ni lesión de órgano diana) o ≥ 2 FRCV Extremo: Asociar ECVA o lesión grave de órgano diana (cociente albúmina/creatinina en orina ≥ 300 mg/g o	- Estatina de moderada intensidad de inicio para objetivos de cLDL < 100 mg/dL Estatina de alta intensidad +/- ezetimiba o ácido bempedoico para objetivos de cLDL < 70 mg/dL Estatina de alta intensidad +/- ezetimiba o ácido bempedoico para objetivos de cLDL < 55 mg/dL

	FGe < 45 mL/min/1,73 m ² o ITB < 0,9 o disfunción ventricular izquierda sistólica o diastólica	
Canadian Cardiovascular Society 2021 [42]	Edad ≥ 30 años y ≥ 15 años de evolución o Edad ≥ 40 años o Asociar complicaciones microvasculares	Iniciar estatinas si cLDL ≥ 77 mg/dL (≥ 2 mmol/mol)
	Asociar ECVA	Iniciar estatinas si cLDL ≥ 70 mg/dL (≥ 1,8 mmol/mol)
American Heart Association (AHA) and American College of Cardiology 2018 (ACC) [43] y 2019 [44]	Edad 20-39 años y: ≥ 20 años de evolución o cociente albúmina/creatinina en orina (≥ 30 mg/g) o FGe < 60 mL/min/1,73 m ² o Retinopatía o Neuropatía o ITB < 0,9	Razonable iniciar estatina
	Edad 40-75 años sin otros FRCV	Iniciar estatina de moderada intensidad
	Edad 40-75 años y múltiples FRCV o riesgo calculado de ECVA a 10 años ≥ 20%	Iniciar estatina de alta intensidad para reducir cLDL ≥ 50%
	Asociar ECVA	Iniciar estatina de alta intensidad para reducir cLDL ≥ 50% y adicionar otros hipolipemiantes si no se logran objetivos de < 70 mg/dL
The National Institute for Health and Care Excellence (NICE) 2023 [45]	Edad 18-40 años y <10 años de evolución	Considerar estatinas
	Edad > 40 años o > 10 años de evolución o Presencia de ERD o Presencia de otros FRCV	Ofrecer estatinas: iniciar con atorvastatina 20 mg
	Asociar ECVA	Iniciar tratamiento para objetivos de cLDL ≤ 77 mg/dL (≤ 2 mmol/mol)

cLDL: colesterol LDL; DTI: diabetes tipo 1; ECVA: enfermedad cardiovascular aterosclerótica; ERC: enfermedad renal crónica; ERD: enfermedad renal diabética; FGe:

filtrado glomerular estimado; FRCV: factores de riesgo cardiovascular; iPCSK9: inhibidores de proproteína convertasa subtilisina/kexina tipo 9; ITB: índice tobillo-brazo

Por otro lado, existen ecuaciones para la estimación del RCV, validadas en población general, como la *Systematic Coronary Risk Evaluation 2* (SCORE2), propuesta por la ESC [38], la cual no es aplicable en personas con diabetes. Posteriormente, se ha desarrollado la ecuación SCORE2-Diabetes, validada únicamente en personas con DT2 [46]. Otras, como las *pooled cohort equations* de la ACC y la AHA [47], incluyen aproximadamente un 10% de sujetos con diabetes, con una representación residual de la población con DT1. Además, incluyen la diabetes como variable predictora dicotómica (si/no), sin tener en cuenta otros posibles FRCV, como el control glucémico o la presencia de complicaciones microangiopáticas. En 2024, se ha publicado la ecuación *Predicting Risk of CVD EVENTS* (PREVENT) por parte de la AHA [48], la cual se ha estudiado y validado en un porcentaje similar de personas con diabetes, y aporta como novedad la inclusión opcional de la HbA1c y el cociente albúmina/creatinina en orina en el cálculo.

Que el horizonte temporal de estimación del RCV sea, en su mayoría, de diez años, y que no se permita el cálculo en menores de 30 - 40 años de edad, constituye un obstáculo para su uso en DT1, considerando que la edad temprana al diagnóstico se ha visto fuertemente asociada a la ECVA [25], y que la formación de las placas ateroscleróticas puede haberse iniciado en la infancia y adolescencia, fomentada por los efectos acumulativos de la DT1 [49]. Además, se ha observado que el uso de las ecuaciones no específicas de la población con DT1, pueden infraestimar el riesgo de cardiopatía isquémica [50].

Datos de los mayores estudios observacionales indican que, además del control glucémico y la presencia de ERD, los principales FRCV en DT1 son: los años de evolución de la diabetes, la presión arterial sistólica, los niveles séricos de colesterol LDL (cLDL), el uso de IECA y la historia familiar de enfermedad cardiovascular prematura [51–53]. También se conoce la pérdida de la protección

cardiovascular inherente al sexo femenino que se observa en la población general [6,54]. En este sentido, una revisión sistemática y metaanálisis de estudios observacionales, en una muestra de cerca de 200 000 participantes, estimó que el exceso de riesgo de ECVA, fue prácticamente el doble en las mujeres en comparación a los hombres con DTI [6]. Incluso, un reciente estudio de aleatorización mendeliana, objetivó una relación causal entre la presencia de la DTI y la aterosclerosis, lo cual sugiere la presencia de FRCV intrínsecos a la propia enfermedad [55].

A la luz de estas evidencias, eminentemente observacionales, algunas de las guías (**Tabla 1**), recomiendan un manejo terapéutico más agresivo en presencia de algunos de estos FRCV. Recientemente, la ESC elaboró una guía de prevención cardiovascular específicamente en personas con diabetes [39]. En ella se pone de manifiesto la escasez de ECA en esta población y es comedida en sus recomendaciones, sugiriendo el uso precoz de estatinas en casos de larga evolución de la DTI, presencia de dos o más FRCV o de cociente albúmina/creatinina en orina A2 (30-300 mg/g). También se deja la puerta abierta al uso de ecuaciones de estimación del RCV específicas de población con DTI, como se comentará en los próximos apartados.

En resumen, la estrategia de prevención del RCV en DTI actual presenta diversos inconvenientes:

- 1) Se dispone de múltiples guías clínicas las cuales son extensas, enrevesadas y no ofrecen clasificaciones ni recomendaciones homogéneas.

- 2) La evidencia en personas con DTI es escasa y de naturaleza observacional. Ello impide establecer recomendaciones basadas en el suficiente nivel de evidencia y lleva a que se extrapolen datos de estudios realizados en población con DT2.

- 3) Las principales ecuaciones poblacionales de estimación del RCV no han sido validadas en personas con DTI, no son aplicables en personas jóvenes y suelen estimar el riesgo a 5 o 10 años, lo cual puede llevar a infraestimar el riesgo en personas con DTI con un prolongado tiempo de evolución de la enfermedad.

Adicionalmente, comentar que, pese a no disponer de ECA de intervenciones farmacológicas cardiovasculares llevados a cabo específicamente en personas con DT1, los datos del metaanálisis de ECA realizado por la *Cholesterol Treatment Trialists' (CTT) Collaboration*, mostraron que el tratamiento con estatinas redujo el riesgo de ECVA de manera consistente en 18 686 personas con diabetes [56]. La reducción de eventos en el subgrupo con DT1 (n= 1466) fue similar a las personas con DT2, aunque el bajo número de eventos no permitió establecer conclusiones definitivas. Por otro lado, un reciente estudio de cohorte retrospectiva en > 11 000 personas con DT1 en prevención primaria, utilizando los datos del registro coreano, objetivó una reducción del riesgo de ECVA del 24% en aquellos en tratamiento con estatinas [57].

Finalmente, dado en parte por las limitaciones que se han expuesto, las dudas en cuanto al manejo terapéutico se manifiestan en la práctica clínica diaria. Por ejemplo, el uso de estatinas u otros fármacos cardioprotectores y la consecución de objetivos terapéuticos basados en algunas de las guías, se ubican muy por debajo de lo recomendado [58–65].

3. Hacia una predicción precisa e individualizada: ecuaciones de riesgo

Las guías de prevención cardiovascular de la ESC/EASD de 2013, ya recomendaban no utilizar las ecuaciones de riesgo generales en personas con diabetes [66]. Existen varias herramientas en personas con DT2, como la propuesta por la *UK Prospective Diabetes Study* (UKPDS) [67] o la, también británica, QRISK2 [68]. Esta última, actualizada anualmente, incluye a personas con DT1 desde 2017, y en la renovada QRISK3 [69]. En personas con DT1, el desarrollo de ecuaciones predictoras ha sido más lento y se ha iniciado más tarde (**Tabla 2**).

Tabla 2. Características de las principales ecuaciones de estimación del riesgo cardiovascular validadas en población con diabetes tipo 1 y ordenadas cronológicamente.

Ecuación	Estimación	Población	Variables incluidas	Resultados
<i>The Pittsburgh CHD in Type 1 Diabetes Risk Model</i> 2010 [70]	Cardiopatía isquémica (muerte, IAM fatal/no fatal, ondas Q patológicas en ECG. Seguimiento: 8 años	Derivación: n = 603 (EDC, no ECV, edad ~27 años, 50,4% hombres, duración de la diabetes ~19 años, HbA1c ~10,4%) Validación: n = 2328 (<i>EURODIAB</i> , sin ECV, edad media ~32,2 años, 48,4% hombres, duración de la diabetes ~14,3 años, HbA1c NR)	Hombres (4 predictores): leucocitos circulantes, cociente albúmina/creatinina en orina $\geq$ 30 mg/g, cHDL, duración de la diabetes Mujeres (5 predictores): razón cintura/cadera, c-no-HDL, presión arterial sistólica, tratamiento para la HTA, duración de la diabetes	Predicción a 10 años: Derivación: estadístico-C = 0,89 (hombres) y 0,84 (mujeres) Validación: estadístico-C = 0,77 (hombres) y 0,78 (mujeres)
<i>Cederholm J, et al., 5-year CVD risk model</i> 2011 [71]	ECV fatal o no fatal: IAM, ictus, angina inestable, revascularización coronaria Seguimiento medio: 4,9 años	Derivación: n = 3661 (Swedish NDR, 6,1% con ECV previa, edad media 44,6 años, 55,6% hombres, duración de la diabetes 28 años, HbA1c 7,9%) Validación: n = 4484 (Swedish NDR, 6,2% con ECV previa, edad media 44,6 años, 54,9% hombres, duración de la diabetes 28,1 años, HbA1c 8,1%)	8 predictores: duración de la diabetes, edad al diagnóstico de la DTI, razón colesterol total/cHDL, HbA1c, presión arterial sistólica, tabaquismo, cociente albúmina/creatinina en orina > 300 mg/g, ECV previa	Predicción a 5 años: Derivación: estadístico-C = 0,83 Validación: estadístico-C = 0,80
<i>Soedamah-Muthu S, et</i>	IAM fatal o no fatal, ondas Q patológicas	Derivación:	5 predictores: edad, HbA1c, razón cintura/cadera, cociente	Predicción a 7 años:

<i>al., Prognostic model 2014 [72]</i>	en ECG, ictus fatal o no fatal, ERC avanzada, amputaciones, ceguera y mortalidad por cualquier causa Seguimiento mediano: 7,3-8,1 años	n = 1973 (EURODIAB, no ECV, edad media 30,3 años, 52% hombres, duración de la diabetes 11,5 años, HbA1c 8,3%) Validación: n = 554 (EDC original, no ECV, edad media 28 años, 49% hombres, duración de la diabetes 18,6 años, HbA1c 9,1%) n = 324 (EDC reciente, no ECV, edad media 36,2 años, 48% hombres, duración de la diabetes 26,4 años, HbA1c 8,4%) n = 2999 (FinnDiane, no ECV, edad media 37,3 años, 51% hombres, duración de la diabetes 19 años, HbA1c 8,4%) n = 580 (CACTI, no ECV, edad media 36,3 años, 45% hombres, duración de la diabetes 22,7 años, HbA1c 8%)	albúmina/creatinina en orina, cHDL	Derivación: estadístico-C = 0,74 Validación: estadístico-C = 0,79 (EDC original); 0,74 (EDC reciente); 0,82 (FinnDiane) y 0,73 (CACTI)
<i>Steno Type 1 Risk Engine (Steno-Risk) 2016 [73]</i>	ECV fatal o no fatal: cardiopatía isquémica, ictus isquémico,	Derivación: n = 4306 (Dinamarca, no ECV, edad media 42,2 años, 54,2% hombres, duración de la diabetes 16,6 años, HbA1c 8,4%)	10 predictores: edad, sexo, duración de la diabetes, presión arterial sistólica, cLDL, HbA1c, cociente albúmina/creatinina en orina,	Predicción a 5 años: Derivación: estadístico-C = 0,826 (IC 95%, 0,807-0,845)

	insuficiencia cardíaca y EAP Seguimiento mediano: 6,8 años	Validación: n = 2119 (Dinamarca, no ECV, edad media 44,2 años, 57,7 % hombres, duración de la diabetes 13,6 años, HbA1c 8,1%)	FGe, tabaquismo, actividad física (>30 minutos diarios)	Validación: estadístico-C = 0,803 (IC 95%, 0,767-0,839) Predicción a 10 años*: Derivación: estadístico-C = 0,818 (IC 95%, 0,802-0,833)
QRISK3 2017 [69]	Evento de ECV fatal o no fatal: cardiopatía isquémica, ictus isquémico, AIT Seguimiento mediano: 4,4 años (~30% con ≥ 10 años)	Población general de Inglaterra de entre 25-84 años, sin ECV ni uso de estatinas Derivación (n = 7,89M): n = 21 677 con DTI (46% hombres) Validación (n = 2,67M) n = 7283 con DTI (46% hombres)	21 predictores: edad, sexo, etnia, deprivación, presión arterial sistólica, IMC, razón colesterol total/cHDL, tabaquismo, historia familiar de ECV prematura, DTI, DT2, tratamiento para la HTA, artritis reumatoide, fibrilación auricular, ERC, variabilidad presión sistólica, migraña, tratamiento con corticoesteroides, lupus eritematoso sistémico, tratamiento con antipsicóticos atípicos, patología psiquiátrica grave y disfunción eréctil	Predicción a 10 años: Validación: Mujeres: estadístico-C = 0,823 (IC 95%, 0,789-0,857) Hombres: estadístico-C = 0,804 (IC 95%, 0,776-0,832)
McGurnaghan S, et al., CVD risk prediction tool 2021 [74]	Ingreso hospitalario o muerte por: IAM, ictus, angina inestable, AIT, EAP, revascularización coronaria/carotídea/	Derivación: n = 27 527 (registro escocés, no ECV, edad media 35 años, 57% hombres, duración de la diabetes 13 años, HbA1c 8,7%)	15 predictores: edad, sexo, duración de la diabetes, deprivación, HbA1c, IMC, presión arterial sistólica, razón colesterol total/cHDL, FGe, cociente albúmina/creatinina	Predicción a 10 años: Derivación: estadístico-C = 0,82 (IC 95%, 0,81-0,83) Validación:

	periférica o amputación mayor	Validación: n = 33 183 (Swedish NDR, no ECV, edad media 31,9 años, 54,6% hombres, duración de la diabetes 16,9 años, HbA1c 8%)	en orina, presencia de retinopatía, tabaquismo, tratamiento para la HTA, tratamiento para la dislipemia y fibrilación auricular	estadístico-C = 0,85 (IC 95%, 0,85-0,86)
	Seguimiento: ~7-8 años ^f			
LIFE-T1D model 2024 [75]	IAM no fatal, ictus no fatal o muerte cardiovascular (incluyendo muerte por insuficiencia cardíaca)	Derivación: n = 39 756 (Swedish NDR, no ECV, edad media 28 años, 55% hombres, duración de la diabetes 14 años, HbA1c 8,1%) Validación: n = 2709 (Dinamarca, no ECV, edad media 43 años, 58% hombres, duración de la diabetes 19 años, HbA1c 8,1%) n = 1022 (UK Biobank, no ECV, edad media 53 años, 56% hombres, duración de la diabetes 33 años, HbA1c 7,9%)	9 predictores: edad del diagnóstico de la diabetes, tabaquismo, IMC, presión arterial sistólica, HbA1c, FGe, c-no-HDL, cociente albúmina/creatinina en orina, presencia de retinopatía	Predicción a 10 años: Derivación: estadístico-C = 0,846 (IC 95%, 0,839-0,853) en los hombres y 0,851 (IC 95%, 0,843-0,859) en las mujeres Validación: Dinamarca, estadístico-C = 0,767 (IC 95%, 0,723-0,812) en los hombres y 0,774 (IC 95%, 0,716-0,833) en las mujeres UK Biobank, estadístico-C = 0,711 (IC 95%, 0,663-0,760) en los hombres y 0,756 (IC 95%, 0,692-0,819) en las mujeres
	Seguimiento: 8-12 años			

AIT: accidente isquémico transitorio; CACTI: *Coronary Artery Calcification in Type 1 Diabetes*; cHDL: colesterol HDL; cLDL: colesterol LDL; DTI: diabetes tipo 1; DT2: diabetes tipo 2; EAP: enfermedad arterial periférica; ECG: electrocardiograma; ECV: enfermedad cardiovascular; EDC: *Pittsburgh Epidemiology of Diabetes Complications study*; ERC: enfermedad renal crónica; EURODIAB: *European Diabetes Prospective Complications Study*; FGe: filtrado glomerular estimado; FinnDiane: *Finnish Diabetic Nephropathy Study*; HTA: hipertensión arterial; IAM:

infarto agudo de miocardio; IC 95%: intervalo de confianza al 95%; IMC: índice de masa corporal; NDR: National Diabetes Register; NR: no reportado.

IC 95%: intervalo de confianza al 95%; NR: no reportado.

* La predicción a 10 años no se llevó a cabo en la cohorte de validación externa dado que $< 19\%$ de la muestra llegó a seguirse durante ≥ 10 años.

[‡] cálculo aproximado a partir del seguimiento reportado en personas-años.

Uno de los primeros intentos se llevó a cabo en 2010, en una muestra pequeña de la cohorte EDC, formada por personas con DTI que debutaron en la infancia, mostrando un correcto rendimiento en la predicción de coronariopatía isquémica a 10 años [70]. Un año más tarde, basada en el *Swedish National Diabetes Register* (Swedish NDR), se desarrolla y valida a 5 años una ecuación en personas en prevención primaria y secundaria que, pese a discriminar correctamente el riesgo de ECVA, excluyó a las personas cuyo diagnóstico de DTI se hizo a una edad de 30 años o superior, lo cual supone hasta un tercio de los individuos con DTI [76,77].

Posteriormente, *Soedamah-Muthu S, et al.* [72], publican una ecuación en base a la cohorte del *European Diabetes Prospective Complications Study* (EURODIAB), y validada en muestras del estudio FinnDiane, del *Coronary Artery Calcification in Type 1 Diabetes* (CACTI), y en dos muestras temporalmente distintas del EDC. Este ambicioso estudio, se llevó a cabo en una muestra joven y relativamente sana, por lo que se observaron pocos eventos, y el rendimiento de la ecuación propuesta se vio posiblemente mermado. Además, se incluyó la mortalidad por cualquier causa, las amputaciones y la ceguera (de cualquier etiología), como variables resultado, en base a la hipótesis de un origen fisiopatológico común, lo cual no tiene por qué ser necesariamente cierto.

Posteriormente, en Dinamarca se lleva a cabo la ecuación *Steno Type 1 Risk Engine* (Steno-Risk) [73]. Esta ecuación supone un gran avance de cara a su uso práctico e implementación clínica por diversos motivos: 1) se realiza en una gran muestra no seleccionada y representativa de la población con DTI; 2) predice eventos cardiovasculares mayores clásicos (muerte cardiovascular, infarto agudo de miocardio [IAM] o ictus no fatales) y añade episodios de insuficiencia cardíaca y eventos de enfermedad arterial periférica (EAP), siendo estos últimos una forma frecuente de debut de la ECVA en nuestro entorno [78], y 3) es la primera y única herramienta que incluye la actividad física como variable predictora, pese a no haber sido recogida de manera estandarizada y ser de naturaleza dicotómica. Sin embargo, cuenta con dos grandes inconvenientes. Por un lado, su desarrollo y validación fueron en población con más del 90% de ascendencia danesa, por lo que

se requieren estudios en otros entornos previo a implementar su uso generalizado. Por el otro lado, en la cohorte de validación se dispuso de menos del 19% de datos de seguimiento a más de diez años, por lo que tan solo se pudo validar a 5 años.

Aunque sin tratarse de una herramienta específica para población con DTI, la ecuación QRISK3 [69] se ha desarrollado en base a los datos de más de 21 000 personas con DTI provenientes de una muestra de más de 7 millones de británicos. La disponibilidad de datos y el tamaño de la muestra han permitido introducir hasta 21 predictores de RCV y logrando una ecuación con una buena calibración y capacidad discriminatoria. Más recientemente, en 2021 [74], se desarrolla una ecuación en una gran muestra de adultos jóvenes del registro escocés (n = 27 527) y validada en la cohorte del Swedish NDR (n = 33 183). Como variable resultado, incluye muerte o ingreso hospitalario por causa cardiovascular y la herramienta muestra un buen poder de discriminación a diez años en ambas cohortes.

Como se ha ido desgranando, la gran mayoría de ecuaciones predictivas de eventos cardiovasculares, se centran en el corto o medio plazo (5 - 10 años). Teniendo en cuenta que el principal FRCV es la edad, la mayoría de predicciones muestran un RCV bajo o, como mucho, moderado, en las personas jóvenes, las cuales pueden, frecuentemente, albergar placas ateroscleróticas [49,79,80]. En este sentido, la comunidad científica del campo de la prevención cardiovascular, va abrazando, cada vez con más fuerza, el concepto del RCV vitalicio o a muy largo plazo como una perspectiva más realista del impacto de la ECVA en todos aquellos con una esperanza de vida estimada superior al horizonte de diez años [38,44].

En la misma dirección, este mismo año 2024, se publica la ecuación LIFE-TID [75], la cual hace una primera aproximación de la estimación vitalicia del RCV (hasta los 90 años de edad) en base a diversos supuestos y, obviamente, sin ser factible su validación. Las condiciones predichas fueron eventos cardiovasculares mayores clásicos de tres puntos (IAM no fatal, ictus no fatal o muerte cardiovascular), en cerca de 40 000 sujetos con DTI de la Swedish NDR, y validada en población danesa y británica. Las variables predictoras fueron la edad del diagnóstico de la diabetes, consumo de tabaco, IMC, presión arterial sistólica,

HbA1c, FGe, niveles de c-no-HDL, cociente albúmina/creatinina en orina y la presencia de retinopatía. El rendimiento a diez años fue bueno, especialmente en la cohorte original y, una de las novedades aportadas en comparación a sus antecesoras, fue el ajustar las predicciones al riesgo competitivo de muerte por causas no cardiovasculares. No realizar este ajuste puede conducir a sobreestimar el riesgo real de ECVA [81]. Por ejemplo, la ecuación QRISK3, mantiene un correcto rendimiento en global, con o sin ajuste por riesgos competitivos, pero se torna más imprecisa cuando se aplica sin ajuste en las personas con mayor riesgo de muerte no cardiovascular, como son los mayores y los que presentan más comorbilidades [82].

En la **Tabla 3** se muestra un ejemplo hipotético de estimación del RCV a diez años con diversas ecuaciones inespecíficas y específicas de personas con DTI, en tres escenarios hipotéticos de bajo, moderado y alto RCV. Destacar que los datos entre las ecuaciones no son directamente comparables, dado que predicen resultados diferentes (e.g. la ecuación de la ACC/AHA y LIFE-TID predicen los clásicos eventos cardiovasculares mayores de tres puntos mientras que Steno-Risk añade EAP y la ecuación propuesta por *McGurnaghan S, et al.* incluye resultados más subjetivos, como la necesidad de revascularización coronaria). Además, sólo algunas de ellas proponen puntos de corte para clasificar el RCV, en muchos casos arbitrarios, y teniendo en cuenta que siempre se pierde información cuando se categoriza una variable continua.

Tabla 3. Ejemplo práctico del riesgo cardiovascular estimado a 10 años con diversas ecuaciones específicas y no específicas de población con diabetes tipo 1.

Ecuación	“Bajo” [*]	“Moderado” [†]	“Alto” [‡]
ACC/AHA [43]	0,8%	3%	31,2%
Steno-Risk [73]	5,2%	12,3%	50,1%
QRISK3 [69]	3,7%	12,2%	37,4%
McGurnaghan S, et al., [74]	4%	12%	38%
LIFE-TID [75]	0,9%	2,2%	26,2%

^{*} Mujer caucásica, edad 40 años, no enfermedad cardiovascular previa, duración de la diabetes 10 años, HbA1c 7% (se asume constante en el tiempo), colesterol total 180 mg/dL, cLDL 100 mg/dL, cHDL 55 mg/dL, presión arterial 120/70 mmHg, nunca tabaquismo, cociente albúmina/creatinina en orina 10 mg/g, índice de masa corporal 22 kg/m², razón cintura/cadera 0,7, actividad física > 30 minutos diarios, filtrado glomerular estimado 95 mL/min/1,73 m².

[†] Mujer caucásica, edad 50 años, no enfermedad cardiovascular previa, duración de la diabetes 15 años, HbA1c 7.3% (se asume constante en el tiempo), colesterol total 200 mg/dL, cLDL 120 mg/dL, cHDL 50 mg/dL, presión arterial 135/80 mmHg, exfumadora desde hace 1 año, cociente albúmina/creatinina en orina 15 mg/g, índice de masa corporal 25 kg/m², razón cintura/cadera 0,9, actividad física < 30 minutos diarios, filtrado glomerular estimado 90 mL/min/1,73 m².

[‡] Hombre caucásico, edad 55 años, no enfermedad cardiovascular previa, duración de la diabetes 30 años, HbA1c 8% (se asume constante en el tiempo), colesterol total 230 mg/dL, cLDL 154 mg/dL, cHDL 38 mg/dL, presión arterial 140/90 mmHg, tabaquismo 20 paquetes/año, cociente albúmina/creatinina en orina 32 mg/g, índice de masa corporal 30 kg/m², razón cintura/cadera 1,1, no actividad física, filtrado glomerular estimado 60 mL/min/1,73 m².

El resto de parámetros incluidos en algunas ecuaciones se han considerado no presentes/negativos (e.g. presencia de otras comorbilidades o complicaciones microvasculares, historia familiar de enfermedad cardiovascular prematura, tratamientos concomitantes) o en términos de igualdad (e.g. quintil de deprivación), en todos los escenarios.

Se ha ensombrecido en verde el riesgo cardiovascular considerado bajo, naranja el moderado y rojo el alto, en base a los puntos de corte propuestos por cada una de las ecuaciones o, en caso de no haberlos, se ha considerado una aproximación: <10%: bajo; 11-19%: moderado; ≥20%: alto.

Como se puede observar, en el caso hipotético de “RCV bajo” (mujer de 40 años, sin complicaciones microvasculares, control glucémico óptimo, normopeso...) y en el de “riesgo alto” (varón de 55 años, presencia de ERD, mal control glucémico, obesidad...), las predicciones se correlacionan correctamente

otorgando porcentajes “bajos” y “altos”, respectivamente. Sin embargo, la verdadera utilidad del uso de estas predicciones no es clasificar los extremos, los cuales no suelen plantear tantas dudas en práctica clínica habitual, si no escalar o desescalar a aquellos que forman el grueso intermedio de la población. En este sentido, se observa la disparidad del riesgo estimado en el caso del “riesgo moderado” (mujer de 50 años, sobrepeso, control glucémico y lipídico subóptimos...), un perfil muy habitual en el día a día asistencial.

Pese a los recientes esfuerzos en la elaboración de estas ecuaciones, las dudas persisten. ¿Qué ecuación se debe utilizar?, ¿qué complicaciones deben predecir? ¿se están evaluando todas las variables predictoras relevantes o sólo los FRCV más reconocidos y de fácil acceso y determinación?, ¿se pueden aplicar las ecuaciones en personas de raza/etnia no caucásica? Ante este trasfondo, se expondrá en el siguiente apartado la necesidad de disponer de nuevos biomarcadores de RCV, que puedan resultar ser predictores de ECVA, y abran la posibilidad a ser incluidos en futuras herramientas predictoras.

4. La necesidad de disponer de nuevos marcadores de riesgo

Como se ha comentado anteriormente, las personas con DTI presentan un elevado RCV, incluso en los casos con un buen control glucémico y ausencia de ERD [5,7]. La fundación *Residual Risk Reduction Initiative* (R3i), formada por varios nombres muy reconocidos en el campo del RCV, define el riesgo residual como “el riesgo de eventos cardiovasculares que persiste en las personas a pesar de la consecución de los objetivos de tratamiento para el cLDL, la presión arterial y la glucemia, según las normas de atención actuales” [83]. Que exista una fundación con este fin, muestra que el riesgo residual es un tema candente, también en las personas sin diabetes. De esta definición derivan tres cuestiones:

1) ¿El riesgo residual viene condicionado por el efecto acumulativo de los FRCV, y el control tardío de los mismos no consigue revertir las alteraciones estructurales ya instauradas? Por ejemplo, una aproximación basada en datos

epidemiológicos de EEUU, estimó que para desarrollar lesiones ateroscleróticas suficientes como para desencadenar un IAM, una persona debe acumular una exposición al cLDL promedio de 5000 mg-años, o lo que es lo mismo, unos niveles de cLDL medios de 125 mg/dL, desde el nacimiento hasta los 40 años, o de 100 mg/dL hasta los 50 años [84]. Bajo este supuesto, la mayoría de sujetos de los principales ECA con estatinas en prevención primaria, ya albergarían placas significativas de ateroma [85–87]. Otro estudio muestra como el manejo temprano y agresivo de la HTA es crucial para revertir la hipertrofia del ventrículo izquierdo, una de las principales lesiones de órgano diana [88]. Ante la dificultad metodológica y económica de llevar a cabo estudios de intervención a muy largo plazo, se debe considerar el cribado y tratamiento precoz de estos FRCV.

2) ¿Los objetivos “según las normas de atención actuales” son correctos o deberían reajustarse? El grueso de la evidencia parece empujar en la dirección del reajuste a la baja de los objetivos lipídicos, de presión arterial e incluso de glucemia. Por ejemplo, el estudio FOURIER (*Further Cardiovascular Outcomes Research With PCSK9 Inhibition in Subjects With Elevated Risk*) demostró que el tratamiento con evolocumab, un inhibidor de la proproteína convertasa subtilisina/kexina tipo 9 (PCSK9), en prevención secundaria, fue beneficioso de manera independiente al nivel basal de cLDL [89]. Con todo, tras 2,2 años de seguimiento, el 10% de los participantes sufrió un evento cardiovascular a pesar de alcanzar niveles medianos de cLDL de 30 mg/dL, lo cual lleva a hipotetizar que, pese al papel crucial de los lípidos en la aterosclerosis, se deben tener en cuenta otros factores. Por otro lado, datos del estudio *Multi-Ethnic Study of Atherosclerosis* (MESA) objetivaron que los principales predictores de este “riesgo residual” son, en su mayoría, debidos a un incorrecto control de los FRCV clásicos, como el tabaquismo o el cLDL, además de la presencia de marcadores de imagen (mayor grosor de la íntima-media [GIM] carotídea) o inflamatorios (niveles de proteína C reactiva ultrasensible [PCRus]) [90]. Sin restar importancia al manejo precoz y agresivo de los FRCV clásicos que, en magnitud, pueden condicionar la mayor parte del RCV global, no parece que por sí solos expliquen todo el riesgo residual observado. Sería ideal disponer de

datos de ECVA derivados de cohortes modernas con un excelso control de dichos FRCV.

3) La existencia de otros FRCV no clásicos (**Figura 1**). En personas con DTI, más allá del impacto de los FRCV clásicos o tradicionales (edad, sexo, HTA, tabaquismo, cLDL, colesterol HDL [cHDL]), se han propuesto múltiples posibles FRCV relacionados con la inflamación, disautonomía cardíaca, la variabilidad glucémica, patología de la pared arterial, alteraciones cualitativas del metabolismo lipoproteico o factores obstétricos, entre otros [91,92]. En su mayoría, estos posibles factores han mostrado asociación con la ECVA. Sin embargo, para conocer el impacto de un posible nuevo FRCV no solo debe haber sido identificado, también se debe disponer de un biomarcador fiable del mismo y, finalmente, evaluar su contribución a la incidencia de ECVA en estudios prospectivos.

Figura 1. Representación gráfica de diversos determinantes de la enfermedad cardiovascular en personas con DTI.

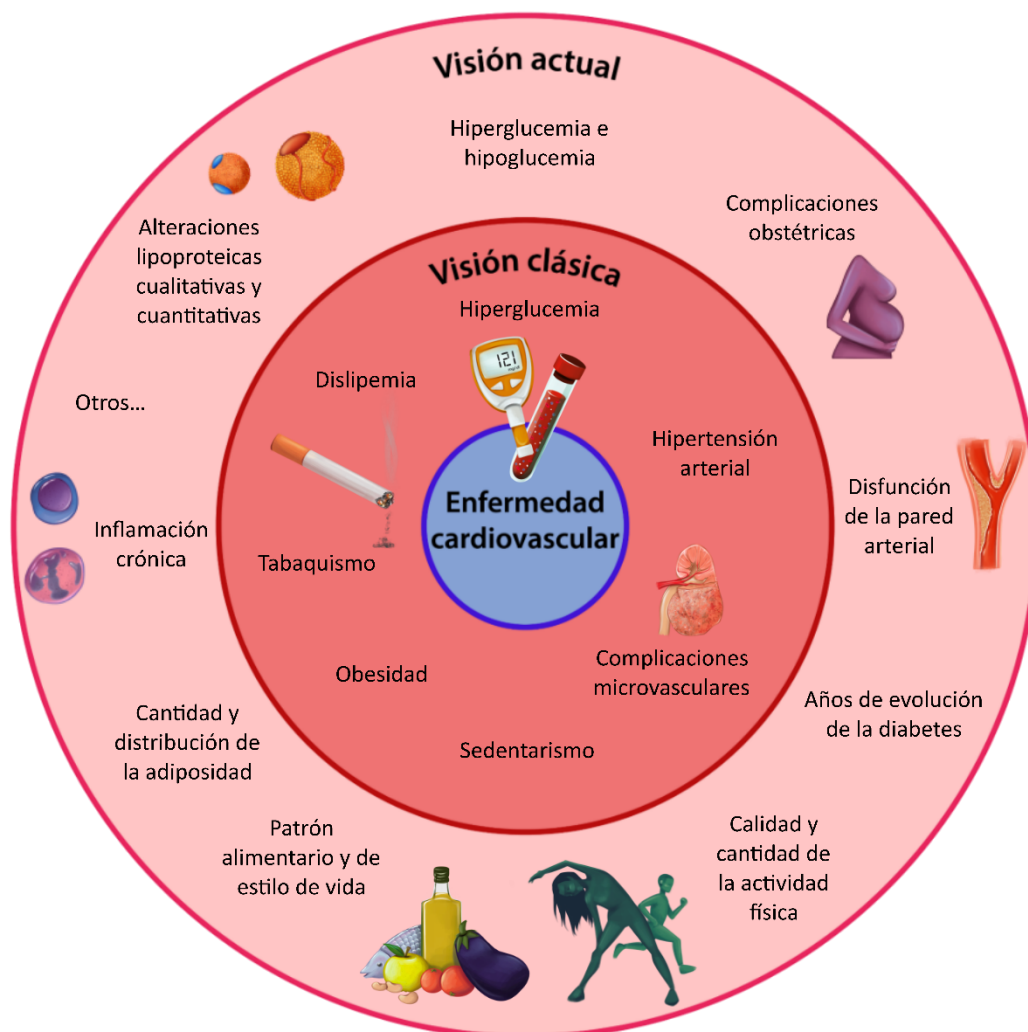


Figura original. Ilustraciones de @llaui.setart y edición por el autor de la tesis.

Tal y como se ha expuesto, caracterizar el RCV para poder predecir de forma precisa la probabilidad de ECVA en cada individuo y así poder ajustar el manejo terapéutico, sigue generando dudas en la población general y se encuentra en constante investigación. En personas con DTI, una población especialmente vulnerable y en la que apenas se dispone de evidencia científica, cobra especial importancia aportar herramientas y marcadores que ayuden con esta finalidad.

5. Marcadores de riesgo no clásicos

Tras lo expuesto en las páginas previas, se enmarcan los objetivos de la presente tesis, centrada en analizar el rendimiento de la estrategia actual de prevención cardiovascular en personas con DTI y valorar la utilidad de diversas herramientas y marcadores de riesgo no clásicos o no tradicionales, que no están incluidos en las ecuaciones de riesgo específicas actuales. En los apartados subsiguientes se introducen los principales, siguiendo el orden de los objetivos del manuscrito.

Se entiende por marcador o factor de riesgo no clásico o no tradicional (**Tabla 4**) principalmente a aquellos no descritos en la cohorte del estudio *Framingham Heart Study*: edad, HTA, presencia de diabetes, tabaquismo, hipercolesterolemia (elevación de colesterol total, cLDL o cHDL bajo) y obesidad [93–96]. En personas con DTI, a pesar de no estar tan claramente separados, por su fuerte asociación con el desarrollo de ECVA, se pueden considerar como clásicos la presencia de complicaciones microvasculares (principalmente ERD), la hiperglucemia crónica y los años de evolución de la diabetes [26–31,51–53].

Tabla 4. Principales marcadores de riesgo no clásicos en la población con diabetes tipo 1.

Marcadores de imagen o relacionados con el daño vascular
Aterosclerosis subclínica
Funcionalidad cardíaca
Rigidez arterial
Técnicas moleculares (e.g. radiotrazadores dirigidos a moléculas inflamatorias)
Marcadores relacionados con el estilo de vida
Dietéticos
Actividad física
Calidad del sueño
Estrés psicosocial
Marcadores reproductivos y obstétricos
Trastornos hipertensivos (preeclampsia, eclampsia y HTA gestacional)
Muerte intrauterina
Parto pretérmino
Desprendimiento de placenta
Multiparidad
Marcadores relacionados con el metabolismo lipídico
Apolipoproteínas y lipoproteínas
Lipidómica
Lipoproteína (a)
Marcadores inflamatorios
Convencionales (e.g. PCRus o leucocitos circulantes)
Perfil avanzado de glicoproteínas inflamatorias
Otros (e.g. interleucinas circulantes, quimioquinas o anticuerpos miocárdicos)
Otros marcadores de riesgo
Sociodemográficos (etnia, raza, privación)
Clínicos (e.g. resistencia a la insulina o neuropatía autonómica cardíaca)
Genéticos (e.g. haptoglobina 2-2)
Otros parámetros relacionados con el control glucémico (e.g. variabilidad glucémica o hipoglucemias)
Otros biomarcadores circulantes (e.g. daño miocárdico)

HTA: hipertensión arterial; PCRus: proteína C reactiva ultrasensible

De cara a facilitar la comprensión del resto del manuscrito, debe ser comentado que en la literatura se utilizan los términos de “marcador o biomarcador de riesgo” y “factor de riesgo” de forma indistinta. En ambos casos, el “factor” o “marcador” se correlaciona con el desarrollo de la patología. No obstante, se considera como “factor de riesgo” aquel marcador que ha demostrado un

beneficio clínico al ser modificado en estudios de intervención [97]. Por ejemplo, la HTA no tan solo identifica pacientes con riesgo de ECVA, sino que numerosos estudios de intervención han demostrado un beneficio clínico al reducirla. Ciñéndose a esta definición, la mayoría de marcadores propuestos a continuación deben seguir considerándose “marcadores” en la población con DTI, a la espera de más evidencias.

5.1. Marcadores de imagen o relacionados con el daño vascular

La aterosclerosis es la antesala de cualquier evento de ECVA. Diversas técnicas de imagen permiten visualizar las placas ateroscleróticas de diversos lechos vasculares o bien, determinar otros parámetros involucrados en su fisiopatología. Ante la imprecisión de las ecuaciones de riesgo, no tan solo en personas con DTI [98,99], las guías de prevención cardiovascular de la ESC [38] y de la ACC/AHA [44], recomiendan el uso de las técnicas de imagen, sobre todo en los casos de riesgo intermedio, donde pueden ayudar a reclasificar el RCV al alza o a la baja.

Las herramientas no invasivas más ampliamente utilizadas son la ecografía carotídea y femoral, el calcio aorto-coronario (CAC score), la angiografía coronaria por tomografía computarizada (ACTC) y la resonancia magnética de grandes vasos. Otras técnicas, como el índice tobillo-brazo (ITB) o la estimación de la rigidez arterial mediante velocidad de onda de pulso carotídeo-femoral, si bien no objetivan la aterosclerosis per se, aportan información complementaria y con impacto pronóstico [100–104]. También cogen fuerza las técnicas que evalúan la funcionalidad cardíaca o las técnicas moleculares, estas últimas dirigidas a detectar procesos muy tempranos en la formación de las placas de ateroma, relacionados, por ejemplo, con la inflamación [105–108].

Es importante destacar que cada herramienta objetiva un momento cronológico en el continuo que va desde las alteraciones tempranas, hasta la rotura o erosión de la lesión aterosclerótica. Por ello, el uso de unas no excluye el de otras

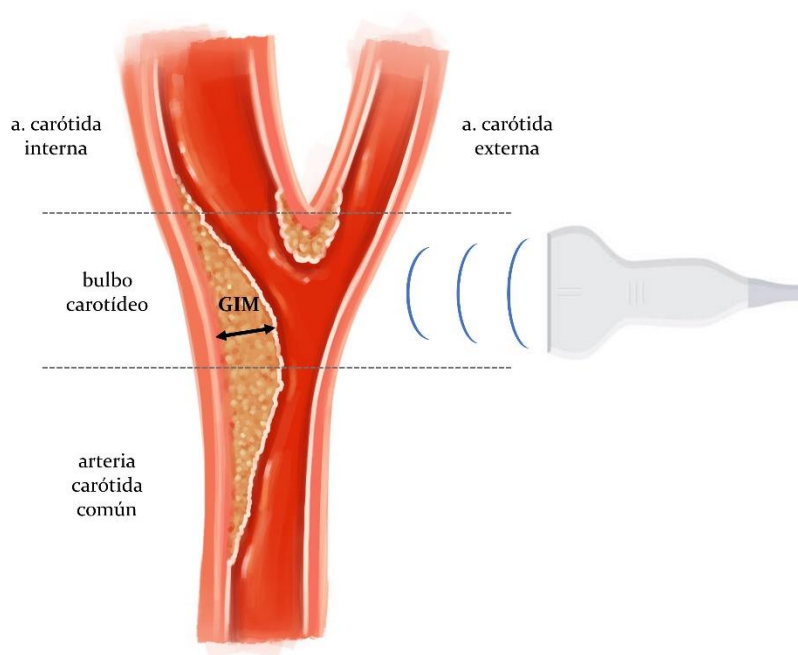
y deben escogerse de manera individualizada en función de la información que se desea obtener y según criterios de eficiencia. En este sentido, datos de la cohorte MESA muestran que el uso conjunto de múltiples variables representativas de distintas manifestaciones de la ECV subclínica, como de la aterosclerosis, por un lado, o de la arteriosclerosis por el otro, predicen el desarrollo de ECV y demencia, de manera independiente entre ellos, y de otros FRCV clásicos [109].

Las personas con diabetes presentan una aterosclerosis acelerada y una mayor predisposición a desarrollar características de placas vulnerables [110–114]. En este sentido, el uso de estas herramientas debe ir dirigido a la detección temprana de dichas lesiones y, a poder ser, que permita distinguir rasgos de vulnerabilidad de la placa. La ecografía, la ACTC y la resonancia magnética son las mejores candidatas para tal fin. Sin embargo, si el escenario clínico es más tardío (e.g. mayor edad, más años de exposición a diversos FRCV y evolución de la DTI), el rendimiento predictivo de ECVA con el uso de CAC score resulta imbatible [115,116], obedeciendo a su capacidad para la detección de placas coronarias avanzadas. A este respecto, un CAC score = 0 permite descartar de forma fiable la presencia de lesiones coronarias obstructivas en pacientes sintomáticos [117]. No obstante, entre un 4 - 32% de las personas con un CAC score = 0, presentan aterosclerosis no calcificada, la cual también se asocia a eventos futuros de ECVA [118–120].

La ecografía carotídea permite visualizar en detalle la pared arterial, determinar el grosor de la capa íntima-media, lugar en el que penetran las lipoproteínas con potencial aterogénico, y caracterizar las placas y los trastornos hemodinámicos que de su presencia se hayan podido derivar (**Figura 2**). La detección de las lesiones ateroscleróticas más tempranas ha demostrado ser un predictor de ECVA en adultos jóvenes y sin diabetes, independientemente del riesgo estimado por la ecuación SCORE2 [121]. Es una herramienta versátil, de fácil acceso en clínicas y hospitales, segura y aplicable en los estadios precoces de la aterosclerosis.

Dado que forman parte del Objetivo 1.a, los detalles de cada una de las técnicas y su utilidad en la predicción de ECVA en población con DTI, se exponen en el apartado de metodología correspondiente.

Figura 2. Representación del uso de la ecografía carotídea para la detección de aterosclerosis subclínica.



GIM: grosor íntima-media

Figura original. Ilustración de @llaui.setart y edición por el autor de la tesis.

5.2. Marcadores relacionados con el estilo de vida

La AHA propuso en 2022 los *Life's Essential 8*, una lista de los ocho marcadores/factores con mayor impacto en la salud cardiovascular [122]. La mitad de ellos se relacionan directamente con el estilo de vida (dieta, actividad física, exposición al tabaco y salud del sueño) y la otra mitad dependen en gran medida de ellos (IMC, lípidos plasmáticos, glucemia, HTA). En la población general, los patrones de alimentación que disponen de una mayor evidencia en cuanto a su beneficio cardiovascular son la dieta mediterránea y la *Dietary Approaches to Stop*

Hypertension (DASH), inspirada en la dieta mediterránea [123–129]. El RCV derivado de otros FRCV no clásicos, como el sedentarismo [130–132], o los patrones de sueño no saludables [133], así como el beneficio clínico de la adopción de hábitos de vida saludable, ha sido ampliamente reportado en personas sin diabetes [134–136].

Actualmente, no se dispone de ECA de intervenciones dietéticas con objetivos cardiovasculares en población con DTI, por lo que no es posible recomendar con firmeza un abordaje determinado [137–139]. No obstante, algunas terapias nutricionales, como el conteo de carbohidratos [140], la mayor ingesta de fibra alimentaria [141], la elección de alimentos considerados saludables [142] o las dietas bajas en carbohidratos [143–146], podrían ser de utilidad con respecto a la mejoría del control glucémico. Sin embargo, la calidad metodológica y/o limitado tamaño de las muestras no permite esclarecer el efecto de los cambios dietéticos en otros FRCV (e.g. cLDL o HTA), o poder discernir cuál es la mejor estrategia, como, por ejemplo, el uso de distintas distribuciones de macronutrientes o de los tipos de proteína y grasas [147].

En los ECA “Prevención con Dieta Mediterránea” (PREDIMED), en prevención primaria y el *CORonary Diet Intervention With Olive Oil and Cardiovascular PREvention trial* (CORDIOPREV), en prevención secundaria, se demostró una notable reducción en la incidencia de ECVA en población con y sin DT2 (aproximadamente la mitad de los participantes) [125,129]. En personas con DTI, pese a haber sido poco estudiado, resultados preliminares apuntan a que una mayor adherencia a la dieta mediterránea se asocia a mejoras del control glucémico [148–150], un mejor perfil cardiovascular [150–152], menores niveles séricos de PCRus [153] y una menor incidencia de complicaciones macro y microvasculares [154].

Por otro lado, la creciente prevalencia de sobrepeso y obesidad entre las personas con DTI ha exacerbado los efectos deletéreos de la resistencia a la insulina en esta población [155–158]. Además, las comorbilidades asociadas a la adiposidad, como la enfermedad hepática esteatósica asociada a disfunción metabólica

(MASLD, por sus siglas en inglés) o el desarrollo de “diabetes doble” (desarrollo de DT2 en contexto de DT1 preexistente), se pueden ver involucradas en una aterosclerosis acelerada y mayor incidencia de ECVA [159,160]. Las intervenciones dirigidas a reducir el peso corporal, como el uso de análogos de *glucagón-like peptide-1* (GLP-1) [161,162] o la cirugía metabólica [163,164], podrían ser herramientas seguras y beneficiosas en cuanto a la optimización del control glucémico y la reducción de las necesidades de insulina exógena. En este sentido, la implementación de cambios dietéticos encarados a la reducción ponderal en personas con DT1 pareciera ser beneficiosa, no obstante, los resultados de los estudios disponibles hasta la fecha no resultan del todo convincentes [147].

Con respecto a la actividad física, recientemente, datos del Swedish NDR objetivaron una reducción del 53 - 73% en la mortalidad por cualquier causa, mayoritariamente por ECVA, entre aquellos que realizaban más actividad física, en contraposición a los que mantenían bajos índices de la misma [165]. Los análisis llevados a cabo en las cohortes de los estudios FinnDiane [166] y EURODIAB [167] apuntan en la misma dirección. Además, la actividad física se incluye como variable predictora en la ecuación Steno-Risk [73]. Estos resultados no son de extrañar teniendo en cuenta el impacto positivo de cualquier tipo de actividad física en FRCV como la hiperglucemia [168,169], el desarrollo y progresión de la ERD [170] o en parámetros de inflamación vascular [171], entre otros.

En un contexto de creciente popularidad de herramientas dietéticas como el ayuno intermitente, el interés por investigar los ritmos circadianos de diversos procesos biológicos se ha visto reflejado también en el manejo de la diabetes. La guía clínica de la ADA, introdujo este 2024 el concepto de la crononutrición y de las interrupciones circadianas (e.g. la que padecen los trabajadores a turnos), un campo prometedor y pendiente de explorar en mayor profundidad su papel en la salud cardiometabólica [138]. A tal efecto, un estudio mostró que aquellos sujetos con DT1 que dormían menos de 7 horas diarias presentaban un peor perfil cardiometabólico, como un mayor IMC, perímetro de cintura y prevalencia de HTA, entre otros [172]. Adicionalmente, una peor calidad del sueño autopercibida

se asoció con un peor control glucémico y de calidad de la dieta, mayor IMC y menor actividad física en jóvenes adultos con DTI [173].

Finalmente, el estrés psicosocial, tanto agudo [174] como crónico [175], se ha relacionado de forma consistente con el desarrollo de ECV en personas sin diabetes. El estrés psicosocial crónico se ha asociado en múltiples estudios con un peor control glucémico en personas con DTI [176], por lo que es previsible que pudiera jugar también un papel en el desarrollo de ECVA.

5.3. Marcadores reproductivos y obstétricos

Como se ha comentado en apartados previos, las mujeres con DTI presentan un mayor incremento del RCV en comparación al que experimentan los hombres [6]. Además, un estudio llevado a cabo en nuestro entorno, mostró diferencias en la presentación del primer evento de ECV en función del sexo, siendo la insuficiencia cardíaca la manifestación más frecuente en las mujeres [78]. Estas discrepancias podrían deberse, en parte, a la exposición a FRCV reproductivos y/u obstétricos.

En las personas sin diabetes, la preeclampsia, sobre todo la que se desarrolla pretérmino, se erige como un potente predictor de ECVA [177-179]. De hecho, las principales guías clínicas de prevención cardiovascular remarcan el tener en cuenta este antecedente en la fenotipación del RCV [38,44]. Otros factores también se han visto asociados a un mayor RCV, como el parto pretérmino [180,181], la multiparidad [182,183], la muerte intrauterina [178,184,185], la HTA gestacional [178,186], el desprendimiento de placenta [178,187] o la diabetes gestacional [188].

Las mujeres con DTI presentan una mayor incidencia de complicaciones obstétricas, entre ellas, los trastornos hipertensivos, como la preeclampsia [189,190]. Recientemente, un estudio prospectivo en más de 9000 mujeres con DTI, objetivó una mayor incidencia de ECV entre aquellas pacientes con un antecedente de trastorno hipertensivo del embarazo, como la preeclampsia, la

eclampsia o la HTA gestacional [191]. Por otro lado, nuestro grupo halló un efecto de asociación aditivo de la preeclampsia y la DTI con la presencia de aterosclerosis carotídea [192]. Otro estudio prospectivo encontró también una asociación entre la preeclampsia y el desarrollo de retinopatía subsidiaria de fotocoagulación [193]. Datos del mismo grupo y cohorte también asociaron la preeclampsia con un mayor riesgo de ERD [194]. Pese a que la etiopatogenia de la preeclampsia no es del todo conocida, su estrecha relación con múltiples FRCV, orienta hacia la presencia de mecanismos comunes [195]. A la luz de las pocas evidencias disponibles en DTI, parece razonable considerar estas entidades de cara a individualizar el manejo cardiovascular.

5.4. Marcadores relacionados con el metabolismo lipídico

Los niveles séricos de cLDL juegan un papel central en el desarrollo de las placas de ateroma. La evidencia de la teoría lipídica resulta abrumadora, desde los estudios experimentales de *Nikolay Anitschkow*, hace más de cien años [196,197], demostrando la inducción de la aterosclerosis en conejos alimentados con dietas ricas en colesterol, hasta los estudios epidemiológicos, clínicos y genéticos, como los de aleatorización mendeliana [198–202].

En personas con DTI, según datos del Swedish NDR, por cada aumento de 1 mmol/L (38,67 mg/dL) de cLDL sérico aumenta el riesgo relativo de IAM en cerca del 50% [51]. Por otro lado, un aumento equiparable (1 mmol/L) de cLDL en el estudio DCCT, se asoció con un incremento del riesgo relativo de ECVA de cerca el 30% [52]. No obstante, por sí mismo no explica el elevado RCV residual de esta población. Además, muchas personas con DTI y buen control glucémico suelen presentar niveles “normales” de cLDL y triglicéridos, con niveles también normales o elevados de cHDL, lo cual puede dar una falsa apariencia de “perfil beneficioso” desde el punto de vista cardiovascular. Sin embargo, se han descrito múltiples alteraciones cualitativas y cuantitativas del metabolismo lipídico, potencialmente aterogénicas, relacionadas mayoritariamente con la hiperglucemia o con el uso de insulina exógena [203].

Por otro lado, al analizar parámetros lipídicos en la DTI, se debe tener en cuenta la heterogeneidad de la muestra, pues la influencia de ciertos factores puede ser significativa. Por ejemplo, en el estudio CACTI, cada incremento del 1% de la HbA1c se asoció con un aumento de los niveles de cLDL y triglicéridos séricos de 4 mg/dL y una disminución del cHDL cerca de 1 mg/dL [204]. Adicionalmente, un estudio prospectivo llevado a cabo en adolescentes con DTI, objetivó variaciones significativas de los lípidos convencionales en función de la edad, el tiempo de evolución de la diabetes, el IMC, el control glucémico y la albuminuria [205].

La adición de complejidad a la ya de por sí intrincada fisiopatología lipídica, invita a explorar la utilidad clínica de otros marcadores lipídicos más allá del perfil convencional.

5.4.1. Marcadores lipoproteicos

Por su tiempo de estancia sérica, las partículas de LDL constituyen la gran mayoría de las lipoproteínas aterogénicas (aquellas que contienen apolipoproteína B y tienen un diámetro inferior a 70 nanómetros) y es por ello que los niveles de cLDL, aunque se trate de una variable subrogada, se correlacionan fuertemente con los niveles de partículas, que son las que se internalizan en la pared arterial, iniciando el proceso de la aterosclerosis. Sin embargo, hasta en un 10-20% de los casos, existen discrepancias entre el total de partículas aterogénicas, medidas mediante la concentración sérica de apolipoproteína B, y el colesterol contenido en las mismas [206]. Estas diferencias son especialmente relevantes en los casos en los que existe un aumento de las lipoproteínas circulantes ricas en triglicéridos, como cuando coexiste la hiperglucemia y/o la obesidad, o en los pacientes bajo tratamiento con estatinas, en los cuales la determinación de apolipoproteína B o su estimación mediante el c-no-HDL, parecen ser mejores predictores de ECVA que los niveles de cLDL [207–210].

En la misma dirección, un reciente estudio llevado a cabo en cerca de 300 000 personas en prevención primaria del *UK Biobank*, demuestra la superioridad

de la determinación de apolipoproteína B en la predicción de ECVA, incluso tras ajustar por otros parámetros lipídicos convencionales (cLDL, cHDL y c-no-HDL) [211]. No obstante, el coste y la disponibilidad de determinar la apolipoproteína B limitan su universalización y las guías clínicas contemplan también el uso del c-no-HDL como una estimación sencilla y económica [38,44,212].

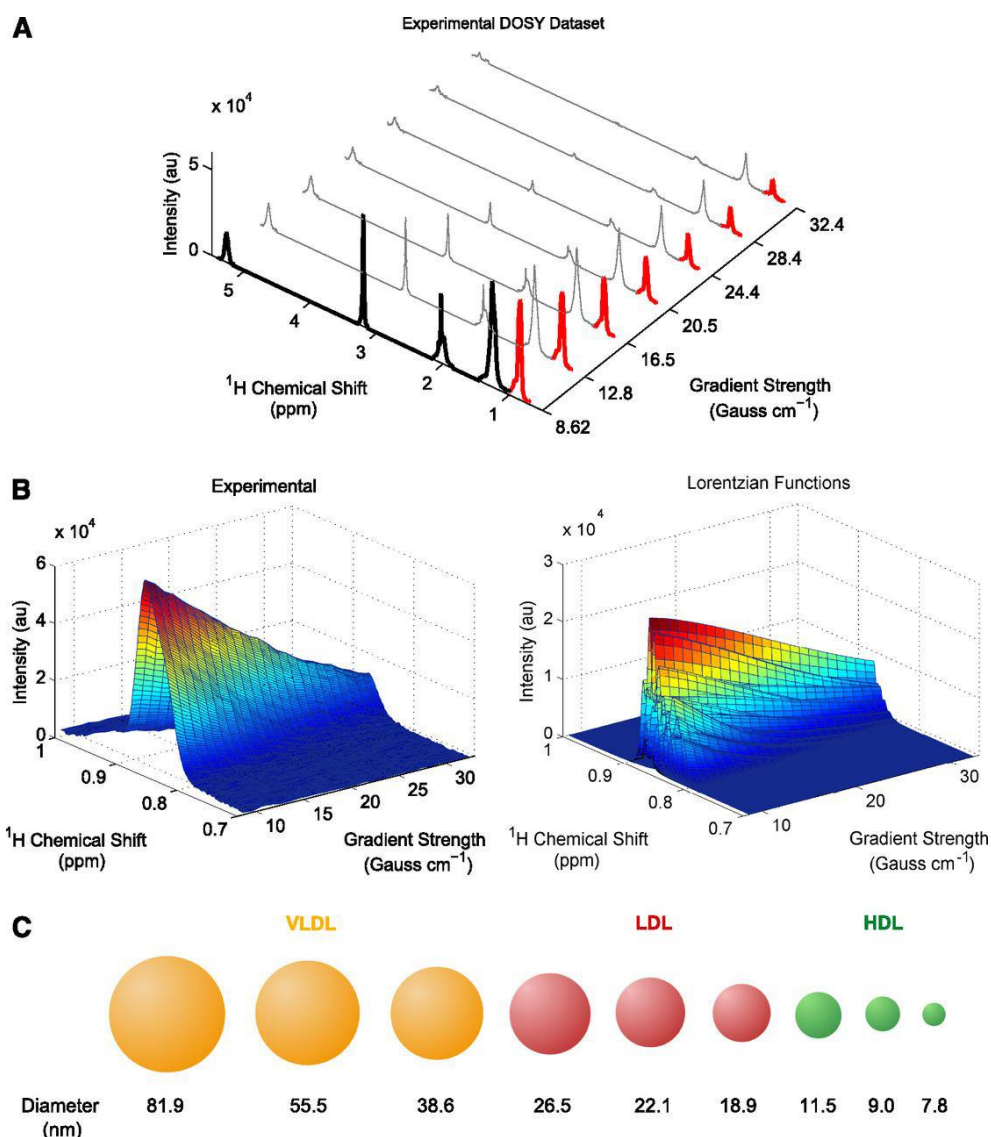
Bajo este enfoque, en el estudio FinnDiane [213], se demostró que los niveles de apolipoproteína B, en contraposición a los de cLDL, predijeron mejor los eventos coronarios en diversos subgrupos de pacientes con DTI, como en aquellos con un mejor control glucémico y los que presentaban albuminuria grados A1 y A3 (< 30 mg/g y ≥ 300 mg/g, respectivamente). La razón entre lipoproteínas aterogénicas y “anti-aterogénicas” (apolipoproteína B / apolipoproteína A1), también demostró su mayor utilidad en estos subgrupos [213]. El papel de las partículas “anti-aterogénicas”, y su subrogada (cHDL), aún está pendiente de esclarecer. Pese a su relación inversa con el desarrollo de ECVA, los niveles muy elevados de cHDL (> 70 - 90 mg/dL) se han asociado paradójicamente con un mayor RCV [214–217]. Además, las intervenciones farmacológicas que logran incrementar los niveles de apolipoproteína A1 y/o cHDL, han fracasado consecutivamente en demostrar algún beneficio cardiovascular [218–220], lo cual deja más dudas que certezas y subraya la complejidad funcional de estas lipoproteínas.

Otra molécula a tener en cuenta es la apolipoproteína C3, la cual está presente en las lipoproteínas ricas en triglicéridos (e.g. quilomicrones, VLDL), y su función principal es la de inhibir la hidrólisis periférica y la captación hepática de estas partículas [221]. Un estudio de la cohorte DCCT, analizó múltiples apolipoproteínas, siendo la apolipoproteína C3 y sus subclases, las únicas que se asociaron al desarrollo de ECV futura [222]. Los tratamientos dirigidos a esta diana terapéutica podrían ser de utilidad en esta población [223,224].

Más allá de la determinación de apolipoproteínas, se ha profundizado en el estudio avanzado de lipoproteínas mediante diversas técnicas (e.g. ultracentrifugación, cromatografía líquida, espectrometría de masas o resonancia

magnética espectroscópica). Con ellas se pueden determinar características como el número, el tamaño y el contenido (e.g. colesterol y triglicéridos) de las lipoproteínas. De las diversas técnicas disponibles, cabe destacar la espectroscopia de resonancia magnética nuclear (^1H -RMN), la cual se basa en el hecho de que el colesterol esterificado y los triglicéridos contenidos en las lipoproteínas, contienen grupos metilo en su estructura molecular que, mediante el uso de pulsos de radiofrecuencia, resuenan a diferentes frecuencias según el tamaño de la lipoproteína, generando así una serie de señales detectables [225]. De esta manera, cuanto menor es el tamaño de la lipoproteína, menor es la frecuencia de resonancia de los lípidos contenidos (**Figura 3**). Variaciones de la misma técnica permiten también determinar múltiples parámetros metabólicos, como se comentará más adelante.

Figura 3. Reporte del estudio avanzado de lipoproteínas mediante espectroscopia por resonancia nuclear magnética (^1H -RMN).



A: Ejemplo de espectros experimentales de DOSY (*2D diffusion-ordered ^1H nuclear magnetic resonance spectroscopy*) para la estimación de la cantidad de colesterol y triglicéridos de las partículas, así como para la caracterización las diferentes subclases. B: Proceso de ajuste de superficie para un determinado sujeto. C: Como resultado de todos los cálculos, se obtienen la información de las nueve subclases de lipoproteínas, concretamente las subclases pequeñas, medianas y grandes de VLDL, LDL y HDL.

Figura original de Mallol R et al. J Lipid Res. 2015 [225].

Varios estudios prospectivos han hallado relaciones positivas del número de partículas LDL y VLDL, y negativas de HDL, con la incidencia de ECV [226,227].

No obstante, una pregunta recurrente es si merece la pena implementar el estudio avanzado de lipoproteínas mediante ^1H -RMN disponiendo de otros potentes predictores de ECV, como los niveles de apolipoproteína B sérica. La respuesta se empieza a vislumbrar fruto de dos destacables estudios. En el estudio JUPITER, los niveles de las lipoproteínas de muy baja densidad (VLDL) de menor diámetro y con mayor contenido en colesterol, en contraposición al número total de partículas de LDL y los niveles de apolipoproteína B, consiguieron detectar a aquellos sujetos con un elevado riesgo residual, pese a tratamiento con estatinas y concentraciones bajas de cLDL (cLDL mediano de 55 mg/dL) [228]. Asimismo, un reciente estudio en una muestra de aproximadamente 90 000 personas del *UK Biobank*, objetivó que las concentraciones de las partículas de VLDL y de HDL, se mantuvieron asociadas a una mayor incidencia de ECV coronaria, de modo independiente a las concentraciones de apolipoproteína B [229]. Estos resultados pueden explicarse, en parte, por el papel que juegan en el desarrollo de la ECVA las lipoproteínas remanentes, es decir, aquellas que permanecen en sangre tras ser parcialmente hidrolizadas (quilomicrones y VLDL). Hasta un tercio del colesterol postprandial circulante es vehiculizado por estas lipoproteínas [230]. Además, diversos estudios epidemiológicos, clínicos y de aleatorización mendeliana asocian las partículas remanentes con el riesgo y riesgo residual de ECVA [207,231–233]. También, parece que, al comparar, partícula por partícula, las remanentes con las de LDL, las primeras poseen un mayor potencial aterogénico [234,235].

Por otro lado, también se han descrito asociaciones diferenciales entre las subclases de partículas de HDL y el riesgo de ECVA en distintas poblaciones, aunque los resultados han sido conflictivos y deben considerarse generadores de hipótesis [236–240]. Con respecto a las partículas de HDL, quizá los esfuerzos deban dirigirse a desentrañar su funcionalidad más que su composición y tamaño. Pese a las evidencias expuestas, todavía se desconoce si puede resultar eficiente la incorporación de este tipo de técnicas en la práctica clínica.

El perfil avanzado de lipoproteínas en las personas con DTI muestra características diferenciales en contraposición a la población sin diabetes. Sin embargo, los resultados han sido conflictivos e incluso contradictorios [203,241–

246]. Un estudio representativo de nuestro entorno, en adultos con DTI y relativo buen control glucémico, demostró que, al comparar con el grupo sin diabetes, el primero presentó un perfil lipídico convencional y medido mediante ¹H-RMN que se podría considerar más “beneficioso”, con un menor número de todas las partículas de VLDL y LDL y su contenido en colesterol y triglicéridos, y mayor número de todas las partículas de HDL y su contenido en colesterol y triglicéridos [245]. Además, se observó una mayor discrepancia entre los niveles de cLDL convencional y el número de partículas de LDL en el grupo con DTI, independientemente del uso de tratamiento hipolipemiante, lo cual deja entrever la utilidad de esta técnica, al poder desenmascarar a aquellos con un mayor número de partículas aterogénicas [245].

Ahora bien, la implicación de estas alteraciones lipoproteicas en el desarrollo de ECVA es todavía un objeto de debate. Las evidencias en cuanto a su utilidad clínica en DTI, provienen eminentemente de cohortes antiguas. En sujetos con debut de DTI en la infancia del estudio EDC, varios parámetros lipoproteicos medidos mediante ¹H-RMN demostraron predecir mejor que los lípidos convencionales, los eventos de ECVA coronaria, destacando especialmente el número de partículas VLDL, el de HDL de tamaño medio (asociación positiva) y el de HDL grandes (asociación negativa) [247]. En contraposición, un estudio multicéntrico no halló asociaciones significativas entre el número o tamaño de las partículas de LDL, VLDL o HDL, y el CAC score [248]. Varios estudios han mostrado resultados conflictivos en cuanto a las relaciones del perfil avanzado de lipoproteínas y otros marcadores de RCV, como la retinopatía diabética [249], disfunción renal [250–252], la preeclampsia [253,254], la rigidez arterial [246,255] o el GIM carotídeo [256,257]. La evidencia es aún escasa y se requieren estudios prospectivos para dilucidar la verdadera utilidad de su determinación en esta población.

5.4.2. Marcadores lipidómicos

La lipidómica se define como la caracterización completa de las especies moleculares lipídicas y de sus funciones biológicas con respecto a la expresión de las proteínas implicadas en el metabolismo y la función de los lípidos, incluida la regulación génica [258]. Existen entre diez y cientos de miles de diferentes especies de lípidos en el organismo, además, sus niveles varían enormemente según las condiciones fisiológicas, patológicas y del entorno, lo cual dificulta la metodología de su estudio [259]. Los lípidos cumplen una plétora de funciones metabólicas, estructurales y de señalización celular, habiéndose relacionado múltiples de ellos con propiedades pro y anti-aterogénicas [260–265]. Su análisis puede realizarse a partir de distintas muestras, como la sangre, células u otros fluidos.

La extracción de los lípidos de las muestras biológicas y su posterior análisis, se lleva a cabo mediante el uso de diversas técnicas, como la espectrometría de masas, combinada o no con métodos cromatográficos o bien de imagen [266]. Una técnica novedosa, reproducible y que proporciona información cuantitativa y directa, es el análisis lipidómico mediante ^1H -RMN. Su funcionamiento es similar al explicado en el apartado previo. Los distintos lípidos resuenan de manera característica tras ser sometidos a pulsos de radiofrecuencia, pudiendo captar esas señales para caracterizar las proporciones de cada uno en una muestra determinada (**Figura 4**) [267].

Figura 4. Ejemplo del espectro de ^1H -RMN de un extracto lipídico humano y su pico molecular correspondiente.

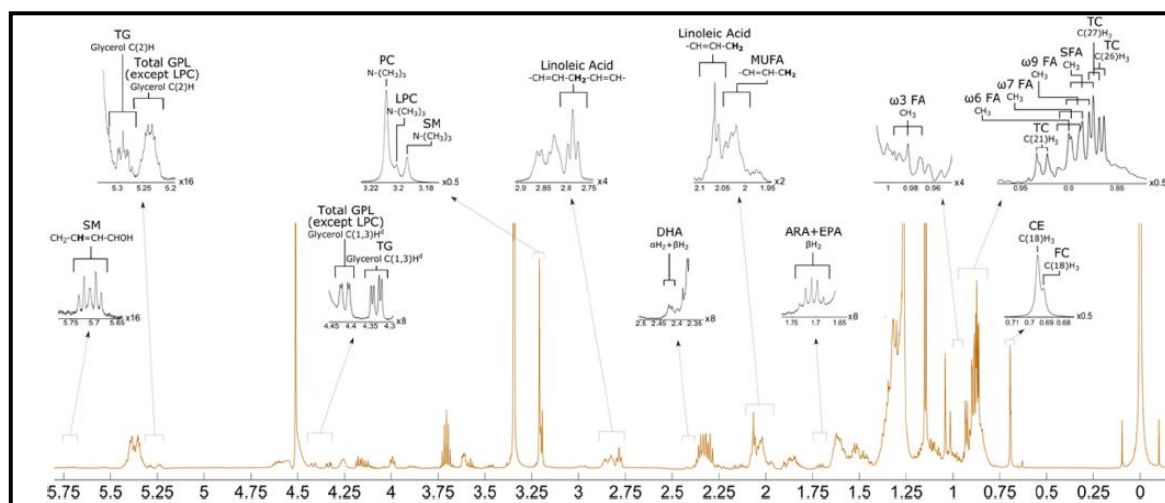


Figura original de Moreno-Vedia J et al. Euro J Clin Invest [267].

En población general y con DT2, múltiples especies lipídicas se han relacionado con el riesgo de ECVA, como diversos glicerolípidos, los ésteres de colesterol, los fosfolípidos como la fosfatidilcolina, la esfingomieline o las ceramidas [268–277]. Un estudio destacable es el que definió la “firma metabólica” (parámetros metabolómicos) asociada a la adherencia a la dieta mediterránea, mostrando un patrón característico que se asoció a los eventos de ECV futura, tras ajustar por múltiples variables [278]. Es posible que los diferentes lípidos se comporten de manera distintiva en función del momento evolutivo de la ECVA, por lo que se requiere de estudios en poblaciones muy bien seleccionadas y con un método de análisis estandarizado para poder dirigir los esfuerzos hacia una posible implementación clínica.

Recientemente se ha intentado caracterizar la “firma lipídica” de las personas con DTI en nuestro entorno [279]. Mediante técnica combinada de espectrometría de masas en muestras sanguíneas, los participantes con DTI sin ECV previa ($n = 91$), mostraron niveles inferiores de esfingomieline, fosfatidilcolina, diacilgliceroles y ceramidas, y superiores de fosfatidiletanolaminas, lisofosfatidiletanolaminas y lisofosfatidilcolina, en

contraposición a las personas sin diabetes [279]. Algunas de estas diferencias se vieron influenciadas en función del sexo. Otro estudio, también de nuestro entorno y llevado a cabo en una pequeña cantidad de pacientes, mediante ^1H -RMN en muestras de eritrocitos, objetivó que las personas con DTI presentaban niveles reducidos de ácidos grasos insaturados, poliinsaturados y de fosfolípidos, así como una disminución de la longitud de las cadenas lipídicas [280]. La significación clínica de estas alteraciones, no obstante, sigue siendo meramente especulativa.

La utilidad de las determinaciones lipidómicas en cuanto a la predicción de ECVA en DTI ha sido poco estudiada. Un estudio danés en más de 600 sujetos adultos mostró asociaciones positivas de la esfingomielina (34:1) y negativas de tres diferentes subespecies de fosfatidilcolinas con la incidencia de ECVA [281]. Por otro lado, niveles superiores de la esfingomielina (40:1) y (41:1) se asociaron a menor mortalidad cardiovascular [281]. Datos previos del mismo grupo también demostraron asociaciones del perfil lipidómico con peores resultados renales. Asimismo, diversas subespecies de esfingomielina (40:1 y 41:1) y de fosfatidilcolina se asociaron negativamente con la mortalidad por cualquier causa, sin embargo, la inclusión de estos parámetros a las variables clínicas, no consiguió mejorar de forma significativa la predicción de la misma [282]. Un análisis del estudio FinnDiane, asoció el desarrollo de ECV coronaria y progresión de ERD con los niveles de esfingomielina medida con ^1H -RMN [283]. Finalmente, se han descrito también diversas alteraciones del lipidoma en relación a la presencia de retinopatía [284], preeclampsia [285] y aterosclerosis subclínica [286]. Las distintas poblaciones y métodos de determinar el perfil lipidómico dificultan la comparación de los estudios comentados y, por lo tanto, de poder extraer conclusiones sólidas.

Para concluir, la complejidad de la interpretación de los estudios ómicos (lipidómica, proteómica, metabolómica y transcriptómica), ha llevado a plantear la utilidad del aprendizaje automatizado en este tipo de análisis. En una primera aproximación se ha intentado fenotipar el perfil lipidómico de la DTI con la ayuda del aprendizaje automatizado y con un análisis detallado mediante espectrometría de masas, objetivando diversas alteraciones en varias subclases de triacilglicerol,

esfingomielinas y ésteres de colesterol, al comparar con las personas sin diabetes [287]. En un estudio llevado a cabo en nuestro medio, destaca la asociación de algunas subespecies de esfingomielina, fosfatidilcolinas y ésteres de colesterol con la presencia de aterosclerosis subclínica [288]. La aplicación de la inteligencia artificial en muestras de mayor tamaño y en el campo del RCV, podría ayudar a simplificar la enorme cantidad de datos que este tipo de análisis ofrece, y esclarecer su posible utilidad clínica.

5.4.3. Lipoproteína (a)

Es importante destacar la importancia de la lipoproteína (a) por su implicación directa, causal e independiente en la ECVA, a partir de estudios epidemiológicos y genéticos [289–298]. Se trata de una partícula de LDL unida a una apolipoproteína (a), esta última fuertemente asociada a la aterosclerosis y calcificación de la válvula aórtica. Sus niveles están determinados genéticamente y varían muy poco en función de otros factores (e.g. estilo de vida). Pese a no conocer su función fisiológica, se le confieren propiedades proaterogénicas, proinflamatorias y protrombóticas [299].

Conviene realizar un pequeño recuerdo histórico para entender la investigación acerca de esta molécula. Aunque parezca una determinación, novedosa, conocemos su relación con la aterosclerosis desde los años 1970' [300]. Desde aquel entonces se publicaron múltiples artículos al respecto. Sin embargo, *Ridker PM* et al, publicaron un gran estudio en cerca de 15 000 adultos en el que no hallaron ninguna asociación entre la lipoproteína (a) y la incidencia de IAM [301]. No fue hasta veinte años después, cuando se reavivó el interés tras los resultados cardiovasculares positivos del estudio Copenhagen [302,303]. Ello se puede observar claramente en la curva bimodal de artículos publicados acerca de la lipoproteína (a) y la ECV. De hecho, las guías europeas no empezaron a recomendar el cribado poblacional de los niveles de lipoproteína (a) hasta 2019 [304].

En el campo de la DTI, hubo un mayor interés por la lipoproteína (a) hace veinte años y todavía no parece haberse retomado. Por aquel entonces, se establecieron asociaciones con diversas entidades, como la ERD [305–307], la EAP [308] o la retinopatía diabética [309]. Un estudio en nuestro ámbito mostró que los niveles de lipoproteína (a) no dependían de la mejoría del control glucémico con terapia intensiva con insulina [310]. En cuanto a su relación con la ECVA, disponemos de dos estudios. El primero, con un diseño prospectivo y proveniente de una cohorte europea antigua, demostró que niveles de más de 30 mg/dL duplicaron el riesgo de ECVA, incluso tras ajustar por niveles de lípidos convencionales [311]. El segundo, más reciente, con una mayor muestra (n = 1860), aunque de diseño transversal, objetivó asociaciones positivas de la lipoproteína (a) con diversas complicaciones micro y macrovasculares, además de la calcificación de la válvula aórtica [312].

5.5. Marcadores inflamatorios

La inflamación juega un papel crucial en el inicio y la progresión de la ECVA, teniendo en cuenta que la disfunción endotelial y la inflamación crónica de bajo grado son procesos condicionantes de la aterosclerosis, además del efecto causal de las lipoproteínas aterogénicas [313,314]. A modo de resumen, en contexto de daño endotelial, condicionado por múltiples factores (e.g. HTA, hiperglucemia), las lipoproteínas aterogénicas penetran la pared íntima arterial. Una vez quedan atrapadas padecen modificaciones, como la oxidación, que fomentan la migración de monocitos circulantes hasta su localización, transformándose en macrófagos. Estos últimos fagocitan estas lipoproteínas, dando, en consecuencia, células espumosas. Otros leucocitos, como los linfocitos T, también migran al interior de la pared íntima arterial y se propicia, a través de diversos mediadores, la migración de células musculares lisas desde la capa media arterial, las cuales alteran la composición de la placa aterosclerótica naciente y perpetúan la inflamación [315]. Se trata de un proceso ampliamente estudiado, pero con muchas incógnitas aún

por resolver. En el mismo intervienen múltiples células y moléculas inflamatorias, relacionadas tanto con la inmunidad innata como la adaptativa.

Existe una amplia evidencia del RCV incrementado que presentan las personas con diferentes enfermedades inflamatorias, como las enfermedades reumatológicas [316–318] o la enfermedad inflamatoria intestinal [319]. Un gran estudio realizado en base a los registros de más de 22 millones de personas, objetivó un riesgo incrementado de ECV en las diecinueve enfermedades autoinmunes analizadas, entre ellas la DTI [320]. Además, el efecto de estas enfermedades parece ser aditivo [320]. Adicionalmente, varios estudios de intervención, dirigidos a reducir la inflamación sistémica, han demostrado reducir eventos cardiovasculares en prevención secundaria. A destacar, el estudio CANTOS (*Canakinumab Anti-Inflammatory Thrombosis Outcomes Study*) con inhibidor de la interleucina-1 β [321], y los estudios COLCOT (*Colchicine Cardiovascular Outcomes Trial*) y LoDoCo2 (*Low Dose Colchicine 2*), con dosis bajas de colchicina [322,323].

Dentro de este marco, se han propuesto varios parámetros inflamatorios como posibles predictores de ECVA en prevención primaria. Algunos estudios indican una mejor predicción de eventos al incorporar los niveles séricos de PCRus [324–330] o de interleucina-6 (IL-6) a las variables clínicas [331]. Un estudio llevado a cabo con datos individuales de más de 160 000 sujetos de estudios prospectivos en prevención primaria, apuntó hacia la utilidad de algunos marcadores inflamatorios, entre ellos los niveles séricos de PCRus, en la mejoría de la capacidad predictora de ECV, al ser añadidos junto a otros FRCV [332]. Otro estudio apoya la determinación de la PCRus en sujetos en tratamiento con estatinas, erigiéndolo como un mejor predictor del riesgo residual que los niveles de cLDL [333]. Sin embargo, una reciente revisión sistemática pone en duda la utilidad de los mismos, o de otros, como el factor de necrosis tumoral alfa (TNF- α) [334]. Es por ello que las guías clínicas de prevención cardiovascular, si bien contemplan su uso como posibles modificadores del RCV, no recomiendan su determinación sistemática, ni tampoco se incorporan en las escalas de riesgo convencionales [38,44].

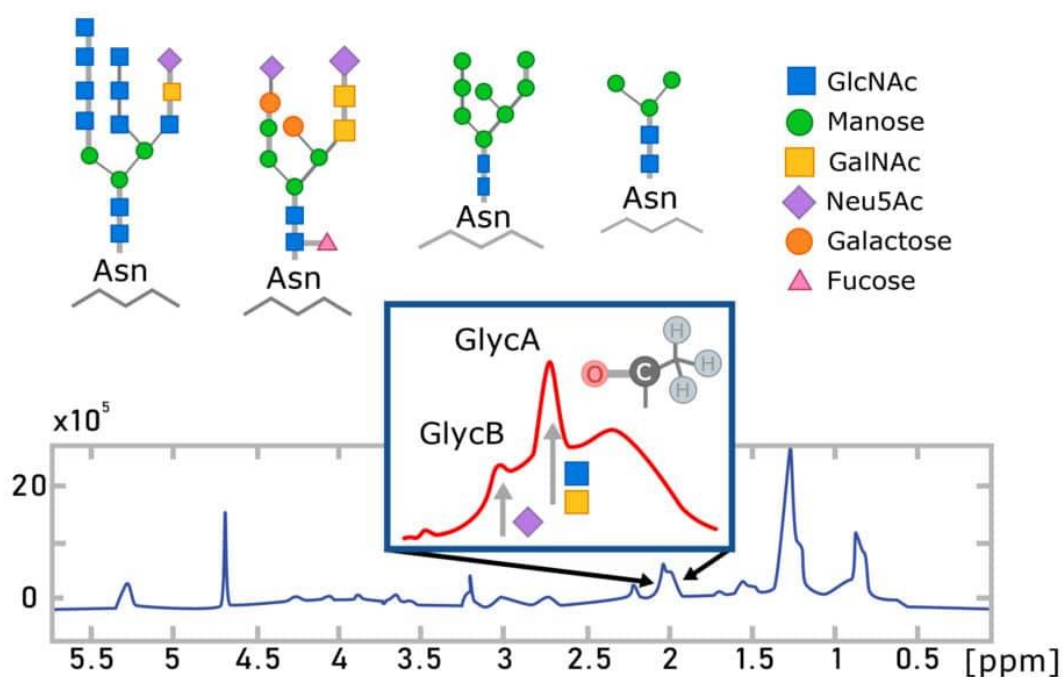
Tanto la DTI como la ECVA son enfermedades estrechamente relacionadas con la inflamación. De hecho, se han reportado niveles superiores de múltiples parámetros inflamatorios (e.g. PCRus, fibrinógeno o leucocitos circulantes) en comparación a población sin diabetes [335–337]. En cuanto a su implicación clínica, un estudio efectuado en personas afroamericanas estadounidenses, analizó múltiples parámetros inflamatorios y de disfunción endotelial mediante técnicas moleculares e identificó varias alteraciones asociadas al desarrollo de ECV [338], retinopatía diabética [339] y ERD [340]. Adicionalmente, los niveles de PCR, IL-6 y TNF- α también mostraron relacionarse positivamente con las complicaciones micro y macrovasculares en los análisis transversales de la cohorte EURODIAB [341]. En el estudio DCCT/EDIC, ningún parámetro individual se asoció con la aterosclerosis carotídea, sin embargo, al usar la puntuación conjunta de varios determinantes (e.g. reactantes de fase aguda como PCR y fibrinógeno), si se objetivó una asociación significativa y positiva con el GIM en los años finales del estudio [342]. Otros estudios que han evaluado el perfil inflamatorio en relación a distintos marcadores de rigidez arterial han mostrado resultados conflictivos [343,344]. Escasean estudios prospectivos en diferentes ámbitos y/o que analicen otros posibles biomarcadores.

En este sentido, otro candidato a destacar sería la razón entre neutrófilos y linfocitos (RNL), cuyo aumento se ha mostrado como fuerte predictor de ECVA en la población general y con DT2 [345–349]. Su alteración indica un desbalance entre la inmunidad innata y adaptativa y su utilidad se basa en las observaciones previas del mayor RCV observado en personas con niveles elevados de neutrófilos o bajos de linfocitos [350,351]. Su principal ventaja es su disponibilidad y bajo coste, pues solo requiere de la determinación de un hemograma convencional. En poblaciones con DTI, su incremento se ha asociado con la presencia de complicaciones microvasculares, como la retinopatía y la neuropatía [352,353] así como con la rigidez arterial [354].

Como se ha comentado en apartados previos, la ^1H -RMN permite analizar, en una sola muestra, múltiples parámetros metabólicos y lipoproteicos. Otra de sus aplicaciones es la determinación de glicoproteínas inflamatorias siguiendo un

método similar al ya expuesto. La señal del espectro emitida por las glicoproteínas inflamatorias está producida por los grupos acetilo -COCH₃ de la N-acetilglucosamina y la N-acetilgalactosamina (GlycA) y el ácido N-acetilneuramínico (GlycB), entre otros (**Figura 5**) [355,356]. La virtud de estos parámetros es que recogen las señales de un conjunto de moléculas inflamatorias, ofreciendo una visión más amplia de la inflamación. Por ejemplo, GlycA integra las concentraciones y los estados de glicosilación de varios de los más abundantes reactantes de fase aguda (α 1-glicoproteína ácida, haptoglobina, α 1-antitripsina, α 1-antiquimotripsina y transferrina) [356]. El parámetro más estudiado ha sido GlycA, el cual se ha asociado, en distintas poblaciones y en algunos casos de manera independiente y/o aditiva a los niveles de PCRus, con una mayor incidencia de ECV [357–360], mortalidad por cualquier causa [358,361], cáncer [358,362], DT2 [355,363,364], dislipemia aterogénica [355], aterosclerosis subclínica [365], HTA [366], MASLD y obesidad [367]. También ha se ha reportado su utilidad como herramienta pronóstica en la artritis reumatoide [368] y el VIH [369].

Figura 5. Representación pictórica del análisis de las glicoproteínas inflamatorias mediante ¹H-RMN.



En la parte superior se muestran ejemplos de N-glicanos con diferentes residuos unidos a

la cadena proteica por asparagina (Asn). En la parte inferior se muestra el espectro ¹H-RMN producido por la muestra en la que está marcada la región de las glicoproteínas. Debajo del mismo se indica el grupo químico que produce la señal.

Figura original propiedad de Biosfer Teslab SL. Disponible en su web corporativa y editada en Fuertes-Martín et al, J Clin Med, 2020 [370]

En la población con DTI existen muy pocos estudios que hayan evaluado esta novedosa técnica. *Puig-Jové C et al* demostró la asociación de GlycA con la disfunción miocárdica subclínica (signos de disfunción sistólica y/o diastólica en ecocardiografía), en sujetos con DTI en prevención primaria, provenientes de una cohorte danesa [371]. Otro estudio, realizado en nuestro entorno, no detectó asociaciones entre ninguno de los parámetros inflamatorios determinados mediante ¹H-RMN y la presencia o gravedad de la retinopatía diabética [372]. También se han hallado asociaciones de algunos parámetros con la rigidez arterial [373]. Finalmente, en mujeres con y sin DTI con antecedente de preeclampsia, nuestro grupo descubrió asociaciones positivas de varios parámetros (GlycA, GlycF y la razón entre altura y anchura del espectro de GlycB), con la aterosclerosis carotídea preclínica, mientras que los parámetros convencionales PCRus y niveles de leucocitos circulantes, no se asociaron [374]. El diseño transversal de los estudios mencionados, no permite todavía establecer conclusiones clínicamente relevantes.

Más allá de los principales biomarcadores expuestos, se han propuesto multiplicidad de distintos parámetros inflamatorios y/o relacionados con la disfunción de la pared endotelial en personas con DTI. Desde citoquinas (e.g. interleucina 1, proteína inducida por interferón γ o el receptor soluble de la interleucina 2), moléculas de la familia de los factores de necrosis tumoral (e.g. TNF- α o ligando soluble del cluster de diferenciación 40), quimioquinas (e.g. quimiquina atractiva de monocitos), factores relacionados con el estrés oxidativo (e.g. superóxido dismutasa), la fibrosis (e.g. fibrinógeno), u otras moléculas inflamatorias, como el amiloide sérico A o la proteína p65 [375]. De entre todos,

los anticuerpos relacionados con la autoinmunidad cardíaca, parecen prometedores [376–378]. No obstante, la mayoría de estos parámetros no disponen de estudios clínicos que avalen su utilidad en la predicción de ECVA en DTI.

5.6. Otros marcadores de riesgo

La lista de posibles marcadores de RCV es prácticamente inabarcable. A continuación, se intenta destacar aquellos que posiblemente tengan una mayor repercusión en la DTI.

5.6.1. Factores sociodemográficos

Los factores sociodemográficos, como la deprivación social, el lugar donde se vive o las diferencias económicas, se relacionan con una mayor inequidad del acceso a la salud, pudiendo derivar en peores resultados clínicos. La metodología de este tipo de estudios es muy compleja, dada la gran diversidad de variables a tener en cuenta. Por ejemplo, la deprivación social puede analizarse en grupos minoritarios excluidos, o bien, puede ser subjetiva en función de la autopercepción de cada persona.

Además de la disparidad del acceso a la salud, se ha descrito que los diversos condicionantes sociales pueden activar múltiples vías inflamatorias y fomentar el desarrollo y progresión de la ECV [379]. En este sentido, una revisión sistemática y metaanálisis, demostró un riesgo estandarizado de mortalidad de hasta nueve veces superior en aquellas personas sin hogar, los que habían estado en prisión, profesión sexual o historia de uso de sustancias de abuso [380]. Tan solo se reportaron resultados de la prevalencia de ECV en el 13% de los estudios, aunque está fue tres veces superior entre las poblaciones mencionadas [380]. En la misma dirección, los bajos niveles de educación e ingresos económicos, en contraposición a los altos, se asocian a un riesgo de mortalidad cardiovascular del 39% y 76% superiores, respectivamente [381]. Estas magnitudes parecen diferir en función de

la raza o etnia. Un estudio realizado en EEUU en 26 316 participantes, objetivó un mayor impacto en la salud cardiovascular de los factores sociodemográficos en las personas afroamericanas [382]. De hecho, la adición de los mismos a la escala de riesgo PREVENT, mejoró la capacidad de predicción únicamente en este subgrupo [382]. En la misma línea, el perfil cardiometabólico parece mejorar en los estudios que han evaluado algunas intervenciones dirigidas a cubrir las necesidades básicas de las personas más desfavorecidas [383,384].

La gran repercusión de los determinantes sociales en la salud, ha propiciado un creciente interés en el ámbito de la DTI. En el Swedish NDR, se objetivaron asociaciones de multiplicidad de determinantes sociales con la incidencia de mortalidad cardiovascular en modelos multivariable: estar casado vs soltero (64% menos riesgo) e ingresos económicos en el quintil inferior vs superior (228% mayor riesgo) [385]. A este respecto, en el *Type 1 diabetes exchange clinic registry* (TID-ECR), un registro de más de 18 000 adolescentes y adultos con DTI de EEUU, aquellos que vivían en las áreas con mayor deprivación social, manifestaron un 78% mayor probabilidad de exhibir, de modo concomitante, peor control glucémico, dislipidemia e HTA [386]. Otros estudios apuntan en la misma dirección, identificando asociaciones entre la deprivación social y la rigidez arterial [387] o la baja educación sanitaria y un peor control glucémico [388].

Otro factor que está en relación al lugar en que se vive, es la polución aérea. La exposición a múltiples partículas se ha asociado consistentemente con una mortalidad y ECV incrementadas [389–393]. Un reciente estudio realizado en base al registro del *Medicare* estadounidense, reveló una mayor mortalidad relacionada con la DTI en aquellos con una exposición incrementada a las partículas finas de diámetro inferior a 2,5 micrómetros (PM_{2.5}) y a dióxido de nitrógeno (NO₂), dos contaminantes aéreos [394]. Estos dos mismos agentes se han vinculado también con la HTA en el estudio TID-ECR [395].

5.6.2. Factores clínicos

Como se ha comentado en apartados previos, el sobrepeso, la obesidad y las complicaciones metabólicas derivadas de las mismas, forman parte de la consulta habitual en DTI [155-160]. La génesis del MASLD está directamente condicionada por el grado de adiposidad, sobre todo en los compartimentos viscerales. Dependiendo del método diagnóstico, la prevalencia de MASLD en personas con DTI se encuentra entre el 0 – 64,7%, con una media del 19,3% [396]. El MASLD diagnosticado mediante ecografía, se ha asociado con un riesgo notablemente incrementado de ECV en la DTI [397,398]. Adicionalmente, el *Fatty Liver Index* (FLI) es una prueba sencilla (se calcula a partir del IMC, perímetro de cintura, niveles séricos de triglicéridos y de gamma glutamil transferasa [GGT]), validada y no invasiva, capaz de predecir la presencia de MASLD [399,400]. Con ello, un reciente estudio prospectivo en adultos jóvenes europeos con DTI, demuestra la relación positiva, progresiva e independiente del FLI, con la incidencia de eventos cardiovasculares y la mortalidad por cualquier causa [159]. A destacar que, tras ajustar por el valor de Steno-Risk y la ocurrencia de eventos cardiovasculares previos (~5% de la muestra), un FLI de más de 60 condicionó un riesgo de mortalidad por cualquier causa tres veces superior al del grupo de referencia (FLI inferior a 30) [159].

Enlazando con lo anterior, la resistencia a la insulina es una de las entidades centrales en el inicio y desarrollo de múltiples FRCV y de la ECVA [401,402]. La estimación de la misma se realiza en práctica clínica mediante el uso de variables subrogadas (e.g. cHDL, triglicéridos séricos) y/o fórmulas que estiman la resistencia o sensibilidad a la insulina (e.g. *Homeostasis Model Assessment of Insulin Resistance* [HOMA], índice de Matsuda o *Quantitative Insulin Sensitivity Check Index* [QUICKI]). Las limitaciones de las determinaciones lipídicas convencionales, como se ha expuesto en apartados previos, y el hecho de que todas las fórmulas requieren de la glucosa e insulina séricas para su cálculo, descarta directamente su uso en DTI. En este sentido, el *estimated Glucose Disposal Rate* (eGDR), un indicador de sensibilidad a la insulina validado en DTI, se calcula en base a la HbA1c, la presencia de HTA y el perímetro de cintura o la razón

cintura/cadera [403]. Datos del Swedish NDR, muestran la fuerte asociación de este parámetro con la mortalidad cardiovascular y por cualquier causa [404]. Estos hallazgos se han replicado, con diversos resultados cardiovasculares, en las cohortes del estudio DCCT/EDIC [405], FinnDiane [406] y EDC [407], lo cual erige el eGDR como un importante marcador de RCV en esta población. Por último, en nuestro territorio, el eGDR se asoció, de forma independiente, con la carga aterosclerótica carotídea en adultos con DTI [408]. La presencia de síndrome metabólico, en cambio, no se relacionó con la aterosclerosis carotídea en ninguno de los modelos estadísticos, evidenciando las limitaciones de los FRCV tradicionales utilizados en la población sin DTI [408].

Finalmente, comentar que una de las complicaciones microvasculares que menos se tienen en cuenta, también puede jugar un papel en el RCV de las personas con DTI. La neuropatía autonómica cardíaca (NAC) se ha relacionado con un mayor riesgo de muerte súbita, arritmias cardíacas e isquemia miocárdica silente [409]. Su diagnóstico se basa en la clínica compatible (e.g. hipotensión ortostática o taquicardia en reposo) y varias pruebas de valoración de los reflejos autonómicos cardiovasculares, como la respuesta de la presión arterial y la frecuencia cardíaca a estímulos como la bipedestación o la maniobra de Valsalva. Un metaanálisis, realizado hace más de veinte años, expuso que la presencia de NAC se asociaba con un riesgo de mortalidad del 20 – 245% superior en personas con diabetes [410]. También se ha asociado a un mayor GIM carotídeo [411] y a otras complicaciones microvasculares, como la retinopatía y la ERD [412]. Sin embargo, su implicación en la ECVA no ha sido consistentemente demostrada.

5.6.3. Factores genéticos

La utilidad de los estudios genéticos en la fenotipación del RCV en la DTI ha sido muy poco estudiado. La variante genética más ampliamente reportada ha sido la haptoglobina 2-2. La haptoglobina es una proteína plasmática cuya principal función es la de formar complejos con la hemoglobina liberada de los eritrocitos cuando estos se destruyen. Los macrófagos reconocen estos complejos

y se facilita su eliminación por fagocitosis. Su función es crucial dado que el exceso de hierro de la hemoglobina es tóxico para células y tejidos, secundario a su capacidad de generar especies reactivas de oxígeno [413]. Las personas con el polimorfismo de la haptoglobina 2-2, presente en el 26 – 43% de la población europea, muestran niveles inferiores de haptoglobina sérica, así como una reducida afinidad por la hemoglobina [414–416]. La reducida capacidad antioxidante de los que presentan este genotipo en homocigosis, se ha visto relacionada con un mayor riesgo de ECVA en población con DT2 [417]. No obstante, en personas con DT1 los resultados han sido poco esclarecedores en cuanto a la relación de la haptoglobina 2-2 con la ECV [418–423] o la ERD [424–429].

Por otro lado, un estudio de aleatorización mendeliana indicó la posible relación causal de la DT1 con el desarrollo de EAP y aterosclerosis coronaria [55]. Un efecto que se mantuvo tras ajustar por múltiples variables de confusión y que estuvo parcialmente mediado por la HTA [55]. Otro estudio demostró que, en poblaciones caucásicas, el haplotipo 12q24, el cual contiene loci de riesgo para DT1, se asoció también con la ECV coronaria, la HTA y la celiaquía [430]. Un haplotipo es una combinación de variantes génicas que tienden a heredarse conjuntamente debido a su proximidad dentro de los cromosomas, por lo que, el estudio mencionado, deja entrever una posible relación genética entre las patologías [430].

No cabe duda de que los avances en genética y el abaratamiento del coste de sus estudios, permitirán explorar la implicación de muchos otros genes potencialmente implicados en la ECV en las personas con DT1.

5.6.4. Otros parámetros relacionados con el control glucémico

Al margen del impacto de la hiperglucemia crónica en la aterosclerosis, otros parámetros glucémicos se han visto implicados en la ECVA. En este sentido, la hipoglucemia se ha relacionado con múltiples alteraciones deletéreas con potencial repercusión en la salud cardiovascular. Entre sus efectos se encuentran el incremento de parámetros inflamatorios y protrombóticos, cambios

hemodinámicos y de funcionalidad cardíaca e incremento del estrés oxidativo [431]. En voluntarios con DT1, la inducción artificial de hipoglucemia aguda, produjo incrementos de diversas moléculas proaterogénicas, proinflamatorias y protrombóticas [432]. Las alteraciones derivadas de las hipoglucemias pueden manifestarse de diversas formas.

En primer lugar, las hipoglucemias graves o de nivel 3 se definen, según la ADA, como aquellas cuya alteración clínica requiere de la asistencia de una tercera persona para recuperarse, independientemente del nivel de glucosa [433]. En personas con DT2, los datos de estudios prospectivos muestran una sólida asociación con la ECV [434,435]. Sin embargo, la evidencia no es tan clara en la DT1. Por ejemplo, el estudio EURODIAB, no halló relaciones entre las hipoglucemias graves autodeclaradas y la incidencia de ECV [436]. Aunque si hubo diferencias significativas en la incidencia de ECV no fatal entre los que no presentaron hipoglucemias graves el último año y los que presentaron más de tres episodios [436]. Un análisis retrospectivo en más de 10 000 participantes con DT1 de Taiwán, apuntó que el haber presentado hipoglucemias graves el año anterior, se vinculó a una probabilidad de aproximadamente dos y tres veces superior de ECV y mortalidad por cualquier causa, respectivamente [437]. En el mismo sentido, otro estudio retrospectivo realizado en base al registro catalán de terapia con infusión subcutánea continua de insulina (ISCI), mostró una mayor prevalencia de ECV entre los que presentaban historia de hipoglucemias graves en los doce meses anteriores [438]. No obstante, estas dejaron de verse asociadas tras el ajuste multivariable [438]. Por otro lado, en el estudio DCCT, no se llegó a demostrar ninguna asociación entre la exposición a las hipoglucemias graves y las complicaciones microvasculares [439]. Sin embargo, datos posteriores de la cohorte DCCT/EDIC, con un mayor tiempo de seguimiento, si demostraron que las hipoglucemias graves se relacionaron con la ECVA cardíaca en los adultos jóvenes [440]. Por último, nuestro grupo demostró que el tiempo por debajo de rango < 54 mg/dL determinado mediante MCG, se asoció a la presencia de placas carotídeas de manera independiente al control glucémico [441].

En segundo lugar, las hipoglucemias de repetición pueden producir adaptaciones neuroendocrinas que llevan a reducir la respuesta sintomática ante estas y derivar en un aumento de las hipoglucemias inadvertidas [442,443]. En un estudio en nuestro entorno, se compararon 25 sujetos con DTI e hipoglucemias recurrentes con 20 controles con DTI, emparejados por edad y sexo. Los principales hallazgos fueron que los primeros mostraron una mayor frecuencia de hipoglucemias inadvertidas, mayor tiempo en hipoglucemia (valorado mediante MCG), mayor aterosclerosis preclínica y niveles superiores de parámetros tanto inflamatorios como de disfunción endotelial [444].

En tercer lugar, las hipoglucemias inadvertidas, también conocidas como desapercibidas, percepción alterada a las hipoglucemias o fallo autonómico asociado a la hipoglucemia, son aquellas que se presentan con síntomas neuroglucopénicos (e.g. visión borrosa, disminución del nivel de conciencia) sin que previamente se hayan presentado los adrenérgicos (e.g. temblor, sudoración) [445]. Su evaluación puede llevarse a cabo mediante cuestionarios validados como el de Gold o el Clarke [446,447], este último validado en nuestra región [448]. Un estudio prospectivo en dos cohortes norte-europeas de adultos con DTI, fueron seguidas durante 6,5 – 12 años, sin objetivar que las hipoglucemias graves o desapercibidas se asociaran a la mortalidad cardiovascular o por cualquier causa [449]. Cabe destacar que, como bien indican los autores en las limitaciones, con el bajo número de muertes observadas, se haya podido incurrir en un error de tipo II [449]. Por último, un reciente estudio de nuestro grupo, demostró que la historia de hipoglucemias graves y las hipoglucemias inadvertidas se asociaron con la presencia de aterosclerosis carotídea en adultos con DTI y RCV bajo/moderado según la ecuación Steno-Risk [450]. Adicionalmente, la inclusión de estos dos parámetros clínicos a Steno-Risk, mejoró significativamente la identificación de la aterosclerosis entre los de bajo RCV, apoyando el uso de estos marcadores en este subgrupo [450].

Las hipoglucemias cuentan con la suficiente evidencia experimental en cuanto a su implicación en procesos considerados condicionantes de la ECVA. Si bien su repercusión parece clara en la DT2 [434,435], los estudios clínicos en

personas con DTI no acaban de dictaminar su papel a este respecto. Los mecanismos que entrañan estas disonancias son aún objeto de controversia.

Finalmente, la elevada variabilidad glucémica (HbA1c entre visitas), parece ser otra métrica a tener en cuenta, al haberse asociado en múltiples estudios a las complicaciones microvasculares (ERD y retinopatía) y eventos de ECV en DTI [29,451,452]. Se han propuesto una variedad de mecanismos potencialmente implicados, entre ellos los producidos por la hipoglucemia, anteriormente expuestos [453]. El papel en la ECV de otras métricas de variabilidad glucémica, como las derivadas de la MCG (e.g. coeficiente de variación, desviación estándar de la glucosa media), está todavía pendiente de dilucidar [441,454–457].

5.6.5. Otros biomarcadores circulantes

Para terminar, y teniendo en cuenta que resulta imposible enumerar todos los posibles biomarcadores de riesgo que se han ido proponiendo, cabe mencionar algunos biomarcadores cardíacos, por su creciente importancia.

Los niveles de troponina T de alta sensibilidad (TnTas), un marcador de daño miocárdico, y los de la fracción N-terminal del péptido natriurético de tipo B (NT-proBNP), un parámetro relacionado con el estrés miocárdico, se consideran potentes predictores de ECV en la población general [458,459]. En personas con DTI, la TnTas se ha visto asociada a una mayor incidencia de mortalidad, de ECV y al empeoramiento de la función renal, únicamente en los sujetos con DTI que ya presentaban ERD [460]. No obstante, la inclusión de la misma a los FRCV convencionales, demostró mejorar la predicción de la mortalidad por cualquier causa en el global de la muestra ($n = 900$) [460]. En cuanto al NT-proBNP, un estudio prospectivo encontró una asociación positiva con el riesgo de ECV y mortalidad por cualquier causa [461]. Además, en combinación con E/e' , un parámetro ecocardiográfico indicativo de disfunción diastólica, mejoró la capacidad de predicción de la ecuación Steno-Risk, tanto para eventos de ECV como para la mortalidad [461]. Otro estudio danés mostró

resultados similares en cuanto a mortalidad [462]. Con todo, los análisis del estudio EDC, no han conseguido replicar resultados favorables, indicando que ninguno de los dos candidatos a biomarcadores (TnTas y NT-pro-BNP) mejoraron la predicción de ECV por encima de otros FRCV tradicionales [463]. A pesar de ello, en los modelos ajustados, los dos marcadores se asociaron a la incidencia de ECV en el subgrupo masculino [463].

V. Hipótesis

La estrategia de prevención cardiovascular actual, basada en escalas de riesgo utilizadas en población general, no identifica correctamente a los sujetos con DTI susceptibles de presentar futuros eventos cardiovasculares. El uso de distintos factores y biomarcadores podría permitir la reclasificación de los pacientes y, con ello, guiar de forma más precisa las medidas preventivas.

VI. Objetivos

Objetivo 1

Evaluar la concordancia entre la escala de riesgo genérica propuesta por las guías ESC/EASD-2019, con la ecuación, específica de población con diabetes tipo 1, Steno-Risk, y evaluar su utilidad para identificar al paciente con mayor carga aterosclerótica.

a) Revisar las principales herramientas para el diagnóstico no invasivo de la aterosclerosis subclínica en personas con diabetes tipo 1 y escoger la técnica más idónea y plausible para evaluar nuestra cohorte.

b) Comparar la concordancia entre la escala ESC/EASD-2019 y la ecuación Steno-Risk. Analizar posteriormente las relaciones de dichas herramientas con la aterosclerosis preclínica medida mediante ecografía carotídea.

Objetivo 2

Analizar la asociación de varios factores de riesgo no clásicos y la aterosclerosis carotídea en pacientes con DTI en prevención primaria.

a) Analizar la relación entre una mayor adherencia a la dieta mediterránea hipocalórica, a través de los datos individuales y agrupados del cuestionario de 17 ítems del ensayo PREDIMED-Plus, y la presencia y número de placas carotídeas en personas adultas con diabetes tipo 1.

b) Examinar las asociaciones entre la preeclampsia, la paridad y la aterosclerosis carotídea subclínica. Asimismo, analizar el rendimiento diagnóstico que puedan aportar los factores obstétricos a la ecuación Steno-Risk.

Objetivo 3

Evaluar las relaciones del perfil avanzado de lipoproteínas con la presencia y número de placas carotídeas en esta población.

Objetivo 4

Explorar la asociación de los diversos parámetros lipidómicos, determinados mediante ^1H -RMN, y la aterosclerosis carotídea preclínica en adultos con diabetes tipo 1.

Objetivo 5

Evaluar la asociación entre diversos marcadores inflamatorios clásicos y no clásicos con la presencia y número de placas carotídeas en personas con diabetes tipo 1 sin enfermedad cardiovascular.

a) Examinar la relación de múltiples determinantes inflamatorios circulantes convencionales, con la aterosclerosis subclínica.

b) Analizar las asociaciones entre múltiples parámetros de glicoproteínas inflamatorias medidas por ^1H -RMN y la aterosclerosis carotídea subclínica.

VII. Material, métodos y resultados

ARTÍCULO ORIGINAL 1: DETECCIÓN DE LA ATEROSCLEROSIS SUBCLÍNICA Y PREDICCIÓN DE EVENTOS CARDIOVASCULARES EN PERSONAS CON DIABETES TIPO 1

Resumen: A pesar del mejor control de varios FRCV clásicos, especialmente el mejor control glucémico, la morbilidad y mortalidad cardiovascular continúa siendo superior en personas con DTI, en comparación a la población general. Dado que la DTI aparece a una edad temprana, la exposición prolongada a la enfermedad y sus consecuencias (por ejemplo, hiperglucemia, cambios en el metabolismo lipídico o inflamación) tiene un impacto perjudicial en la salud cardiovascular. Por lo tanto, es fundamental contar con herramientas que permitan la identificación temprana de aquellos individuos con mayor RCV y, así, poder tomar las decisiones de manejo más apropiadas en cada caso. En este sentido, la aterosclerosis es el preludio de la mayoría de los eventos cardiovasculares. Las personas con diabetes presentan alteraciones fisiopatológicas que facilitan el desarrollo de aterosclerosis y que pueden implicar una mayor vulnerabilidad de las placas ateromatosas. El cribado de la aterosclerosis subclínica mediante diversas técnicas, principalmente de imagen, ha demostrado ser valioso para predecir eventos cardiovasculares. Su uso permite la reclasificación del RCV y, por lo tanto, un ajuste individualizado del manejo terapéutico. Sin embargo, la evidencia disponible en personas con DTI es escasa. Esta revisión narrativa proporciona una visión actualizada de las principales pruebas no invasivas para detectar placas de aterosclerosis y su asociación con la ECV en personas con DTI.



Review

Screening for Subclinical Atherosclerosis and the Prediction of Cardiovascular Events in People with Type 1 Diabetes

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Abstract: People with type 1 diabetes (T1D) have a high cardiovascular disease (CVD) risk, which remains the leading cause of death in this population. Despite the improved control of several classic risk factors, particularly better glycaemic control, cardiovascular morbidity and mortality continue to be significantly higher than in the general population. In routine clinical practice, estimating cardiovascular risk (CVR) in people with T1D using scales or equations is often imprecise because much of the evidence comes from pooled samples of people with type 2 diabetes (T2D) and T1D or from extrapolations of studies performed on people with T2D. Given that T1D onsets at a young age, prolonged exposure to the disease and its consequences (e.g., hyperglycaemia, changes in lipid metabolism or inflammation) have a detrimental impact on cardiovascular health. Therefore, it is critical to have tools that allow for the early identification of those individuals with a higher CVR and thus be able to make the most appropriate management decisions in each case. In this sense, atherosclerosis is the prelude to most cardiovascular events. People with diabetes present pathophysiological alterations that facilitate atherosclerosis development and that may imply a greater vulnerability of atheromatous plaques. Screening for subclinical atherosclerosis using various techniques, mainly imaging, has proven valuable in predicting cardiovascular events. Its use enables the reclassification of CVR and, therefore, an individualised adjustment of therapeutic management. However, the available evidence in people with T1D is scarce. This narrative review provides and updated overview of the main non-invasive tests for detecting atherosclerosis plaques and their association with CVD in people with T1D.



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Keywords: type 1 diabetes; cardiovascular risk; atherosclerosis; subclinical atherosclerosis; plaque; carotid plaque; coronary calcium score; coronary computed tomography angiography; magnetic resonance; ankle–brachial index

1. Introduction

Cardiovascular disease is the leading cause of mortality and morbidity worldwide. Although medical advances have reduced the incidence of death over the past decade, global prevalence and mortality have continued to rise [1]. Atherosclerotic cardiovascular disease (ASCVD), mainly coronary artery disease (CAD) and atherothrombotic stroke, represents the leading cause within this group, accounting for over 13 million deaths in 2021 [2].

CVR is often assessed using various equations that estimate the 10-year probability of suffering an event. This strategy has several disadvantages: (1) most of them are not applicable in people aged <40 years, and age greatly influences the estimated risk, which makes it difficult to identify young people at high risk; (2) they are designed to use cross-sectional data, although the impact of the main risk factors occurs cumulatively; and (3) they consider only a few classical risk factors (e.g., sex, age, smoking habit, cholesterol levels or systolic blood pressure) and leave it to the clinician to decide how to weight the risk indicated by several other variables that modify CVR (e.g., social deprivation, the presence

of obstetric factors or autoinflammatory diseases). Furthermore, there is no clear consensus between the different strategies. Several studies show heterogeneous recommendations depending on the equation used, and a lower than desired discriminatory power [3–5]. All this underlines the need for additional tools to better assess CVR in each individual.

The prelude to an acute event is the formation of atheromatous plaques in large and medium-sized arteries, which begins early in life and progresses silently over several years. The detection of subclinical atherosclerosis, which can be easily assessed using mostly non-invasive imaging tests, is one of the main strategies employed to individualise this risk. The presence of atheromatous plaques is associated with incident cardiovascular events in studies on large population cohorts without diabetes [6–8]. It allows us to identify young individuals at high CVR who may benefit from a long-term preventive strategy, knowing that they could benefit most from CVR control such as low-density lipoprotein cholesterol (LDLc) levels or blood pressure [9,10]. This is important because statins [11] and PCSK9 inhibitors [12,13] can delay the process of atherosclerosis, stabilise plaques already formed and reduce the likelihood of cardiovascular events. In this sense, the main clinical guidelines for cardiovascular prevention consider subclinical atherosclerosis detection as a risk-modifying factor, primarily in intermediate or borderline risk patients, both up- and down-regulating, with the consequent changes in treatment and follow-up that this entails [14,15]. In addition, the visualisation of atherosclerotic plaques by patients themselves is not only useful for the clinician but can also improve adherence to lifestyle measures and treatments with proven cardioprotective effects (e.g., lipid-lowering, antihypertensive and antiplatelet therapy) [16].

People with T1D have a four to eight times higher risk of CVD than the general population [17,18]. The physiopathology of T1D is characterised by the rapid and early autoimmune destruction of pancreatic beta cells, resulting in hyperglycaemia and the requirement for lifelong insulin replacement therapy. Hyperglycaemia is one of the most important CVR factors; however, even those with optimal glycaemic control (time-updated haemoglobin A1c (HbA1c) $\leq 6.9\%$ or 51.9 mmol/mol) have a three-fold increased risk of CVD death compared with their counterparts without diabetes [19]. This fact suggests the existence of other factors involved in the pathogenesis of CVD in T1D such as exposure to hypoglycaemia, glycaemic variability, quantitative and qualitative abnormalities of lipoproteins, immune dysfunction, inflammation or cardiac autoimmunity, among others [20,21]. Furthermore, a recent Mendelian randomisation study supports the hypothesis of the presence of T1D as a CVD causal factor [22]. Previously, we showed how equations for estimating CVR that are not specific to T1D can have poor diagnostic performance [23]. Although several methods have been developed for this purpose [24,25], most assessments of CVR and cardiovascular management are based on extrapolations from studies in people with T2D. The two entities have different pathophysiologies. They share little beyond hyperglycaemia. These differences highlight the many mechanisms in the mechanisms leading to CVD. It is essential to develop reliable strategies to classify CVR and implement preventive strategies early in this vulnerable population.

Against this background, this narrative review aims to evaluate the clinical utility of using non-invasive techniques to detect subclinical atherosclerosis in people with T1D. Due to their invasiveness, cost and lower applicability for screening in clinical practice, diagnostic tests such as angiography, intravascular ultrasound and intravascular optical coherence tomography will not be reviewed in this manuscript.

2. Methods of Searching

In the present study, a narrative review of the literature was carried out focused on the different tools for the detection of atherosclerosis and its association with CVD in people with T1D. A comprehensive search of international the PubMed and Embase (Elsevier, Amsterdam, The Netherlands) databases was conducted for all articles available up to 12 January 2024. The suitability of articles collected from the electronic search was reviewed based on the abstracts. The search criteria were from lowest to highest specificity

depending on the number of results available. We took all the results into account. We prioritised articles that evaluated clinical variables and were published in high-impact journals. Articles that were not related to the objective of the manuscript, conference abstracts, and duplicate articles were excluded from the review process. Only articles published in English were considered.

3. Physiopathology of Atherosclerosis in Diabetes

Atherosclerosis is a complex and not fully understood process. In brief, the evidence seems to state that it begins with the penetration and accumulation of apolipoprotein B-100-containing particles, mainly LDL particles, into the intimal layer of the arterial wall. In this new environment, they are oxidised and modified, leading to an inflammatory and immunogenic activation. Although the exact mechanisms are not fully understood, several processes, such as increased oxidative stress [26] and the degeneration of the endothelial glycocalyx [27], have been implicated. Subsequently, circulating T lymphocytes and monocytes enter the intimal layer through a dysfunctional endothelium. The latter mature into macrophages expressing scavenger receptors that recognise these modified lipoprotein particles and internalise them, notably increasing the cholesterol content of macrophages, turning them into foam cells. These foam cells release a plethora of proinflammatory cytokines that promote the process of atherosclerosis [28].

These leukocytes produce various mediators. The mediators cause smooth muscle cells to move from the media layer to the intima. There, the smooth muscle cells can grow and produce extracellular matrix molecules that increase the size of the plaque. From here, inflammation is perpetuated, and multiple processes occur that influence the progression of atherosclerosis. Finally, there are mainly two plaque complications: intraluminal growth with vascular stenosis and rupture or erosion with intravascular thrombus formation [29].

Various factors enhance and/or accelerate several of the above processes in people with diabetes. For example, hyperglycaemia leads to the glycation of various proteins, which undergo multiple reactions culminating in the formation of advanced glycation end products (AGEs). AGEs have been implicated in several steps in the development of atheromatous plaques, including accelerated monocyte migration, the glycation of lipoproteins facilitating the recognition by macrophages, an increased production of inflammatory cytokines and procoagulant effects, among others [30]. In addition, the increased productions of sorbitol and fructose (polyol pathway) also increase the production of AGEs. Further, AGEs are hardly degradable and may persist over time, which may explain why those who have had poor glycaemic control are at increased risk of vascular complications despite a better current control; this is known as the legacy effect [31,32].

Notwithstanding, through various mechanisms, mainly intracellular hyperglycaemia, there is an increase in oxidative stress with an increased production of reactive oxygen species, which react with various structures such as nucleic acids and proteins, increasing the expression of adhesion and inflammatory factors and affect genes involved in the pathogenesis of atherosclerosis [33,34]. There is an increased synthesis of pro-inflammatory cytokines in people with diabetes and several inflammatory markers have been associated with atherosclerosis in T1D [35–37]. In addition, elevated glucose levels and other processes increase the production of diacylglycerol, which, together with calcium, activates protein kinase C. Its activation has been implicated in several steps of atheroma plaque formation [30,34,38]. Furthermore, people with diabetes have qualitative and quantitative changes in the lipoprotein metabolism that have been implicated in the process of atherogenesis [39–41]. Most of the above processes are interrelated and promote each other. This perpetuates and accelerates the process of atherosclerosis.

Finally, people with diabetes not only have a greater atherosclerotic burden than the general population [42], but also have a greater inflammatory infiltrate, necrotic core and calcification that have been linked with increased plaque vulnerability [43]. Several of these changes are due to hyperglycaemia, but the high residual risk indicates that other agents are also involved. Even a recent Mendelian randomisation study suggests a

possible causal role of T1D in peripheral and coronary atherosclerosis after adjusting for confounders (comorbidities, classic CVR factors, lipid and inflammatory variables), with a partial mediation of the effect through hypertension [22].

4. Screening Methods for Subclinical Atherosclerosis

4.1. Carotid Ultrasound

The carotid territories, and especially the carotid bifurcation, are prone to atherosclerosis in predisposed individuals with CVR factors due to physiological changes in blood flow. As this is a shallow area, ultrasound using high-frequency probes (usually > 9 MHz) allows for a detailed visualisation of the arterial wall in its entire extracranial extent. The thickness of the intima and media layers (intima-media thickness or IMT) is measured and, although various cut-off points have been proposed, the consensus is that plaque is defined as a focal structure that encroaches into the arterial lumen by at least 0.5 mm or 50% of the surrounding IMT value or has an IMT thicker than 1.5 mm [44]. If the plaque is of significant size, the degree of stenosis is estimated using a variety of methods, most notably through the use of flow velocities in the stenotic area using pulsed-wave Doppler mode. In addition to conventional ultrasound, other related tools include contrast-enhanced ultrasound (CEUS), which allows the characterisation of features associated with plaque vulnerability such as neovascularisation [45], and 3D volumetric ultrasound (3DVUS), which helps in the spatial interpretation and calculation of the total area of atheromatous lesions [46].

Unlike other methods, carotid ultrasound does not emit radiation, allows for the visualisation of plaques in the early stages (Figure 1), is inexpensive and quick to perform, and it is also common to have an ultrasound scanner in the office, enabling an easy assessment and the diagnosis and initiation of treatment at the same time. Focusing on the arterial wall rather than the lumen makes it easier to characterise plaques and identify those with features associated with a higher risk of complications such as echolucency or surface ulceration [47,48]. However, its main disadvantages are its operator dependency, which requires trained personnel, and the lack of standardisation of IMT measurements.

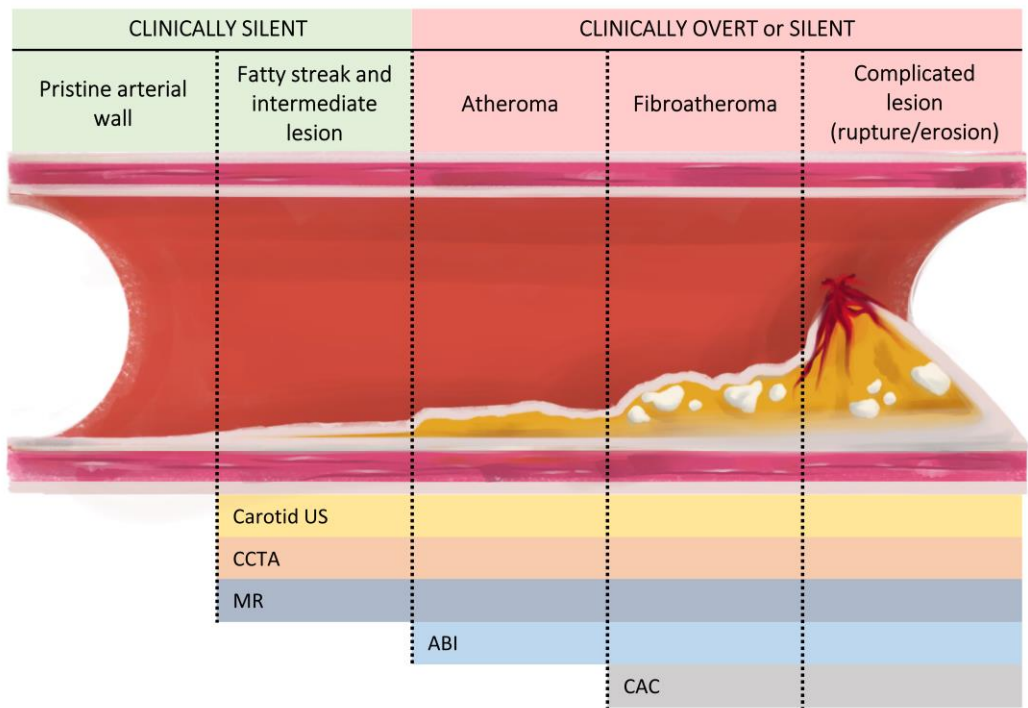


Figure 1. Main imaging screening methods according to atherosclerosis progression. The progression of atherosclerosis begins with the formation of lesions with fatty streaks that progress to intermediate lesions that are clinically asymptomatic but already detectable by various imaging methods. It then

progresses to atheroma plaque, which, depending on the degree of stenosis and haemodynamic impact, may yield a pathological ABI result. Over time, the plaques increase their fibrotic content and may present calcifications. At this point, the use of CAC is sufficiently sensitive for their detection. Finally, atherosclerotic lesions may become complicated through rupture or erosion and lead to acute cardiovascular events. ABI: ankle–brachial index; CAC: coronary artery calcium; CCTA: coronary computed tomography angiography; MR: magnetic resonance; US: ultrasound.

The usefulness of IMT measurement and the detection of carotid plaque in T1D has been poorly studied, and the results are controversial. Data from the Diabetes Control and Complications Trial/Epidemiology of Diabetes Interventions and Complications (DCCT/EDIC) cohort are consistent in that various CVRs such as age, male sex, systolic blood pressure, smoking, HbA1c and albuminuria are predictors of IMT progression and that intensive diabetic treatment slows such progression [49]. Subsequently, the association between measures of IMT and CAD events was analysed after 17 years of follow-up with more than 1300 subjects [50]. Increased common carotid artery IMT was consistently associated with CVD events incidence in models adjusted individually for age, sex, HbA1c, systolic blood pressure, HDL cholesterol, total cholesterol and smoking. However, despite a trend, this association did not remain significant after adjusting for all variables, nor did internal carotid artery IMT. It should be noted that they analysed baseline ultrasound data from a young sample (mean age 35 years) without information on atherosclerotic progression, which is, if anything, a more important measure than the mere presence of plaque [51]. In addition, the number of events was low ($n = 135$), which may affect the power of the study. However, carotid atherosclerosis in T1D is associated with many pathologies and alterations linked to a higher risk of CVD. These include high systolic blood pressure [52], preeclampsia [53,54], retinopathy [55,56], insulin resistance [57,58], excess weight gain [59], enlarged left ventricular mass [60], cerebral microbleeds [61], cognitive impairment [62], inflammation and endothelial dysfunction [36,63]. The aforementioned, in addition to the proven predictive capacity of CVD events in the general population [7,64], lead us to believe that, pending more properly designed prospective studies, there is no reason not to consider carotid ultrasound as a useful tool in the evaluation of these patients. Finally, the use of this technique is not limited to the phenotyping of CVD risk but may serve as a marker of treatment response [65–67] and may even lead the clinician to intensify cardioprotective treatment and the patient to adopt a healthier lifestyle and be more compliant with treatment [68].

4.2. Coronary Artery Calcium (CAC) Scan

When using non-contrast computed tomography (CT), the CAC of the epicardial arteries is measured, with an area of $\geq 1 \text{ mm}^2$ and > 130 Hounsfield units being considered a lesion [69]. The product of the calcified area and the score of the lesion itself (ranging from 1 to 4) are known as the calcium score. The total CAC score is the sum of all calcium score units and is reported in Agatston units (AU), considering no CAC (0), minimal CAC (1–10), mild CAC (11–100), moderate CAC (101–400) and severe CAC (>400).

CAC scanning is a relatively fast, economical, reproducible and easy-to-interpret method. One of its limitations is that it emits radiation, albeit a small amount. In addition, it measures coronary calcification, an advanced stage of atherosclerosis, and misses obstructive and non-calcified lesions, the latter generally representing an earlier stage that may be present in young people with high CVR that might go unnoticed through this method. Also, statin treatment, despite its proven cardiovascular benefits, may lead to an increase in CAC due to the regression of non-calcified areas and promotion of intraplaque calcification [70,71], so it is unclear whether the CAC score may be useful as an additional tool for monitoring treatment. Calcifications are associated with higher plaque stability and lower event probability [72]. However, a higher CAC volume is positively associated with multiple CVR factors [73]. As a product that takes into account calcification and lesion

area, an increased CAC score usually indicates a higher total atherosclerotic burden [74]. This is why it now stands as a powerful predictor of cardiovascular events [75].

In people with T1D, its usefulness as a predictor of CVD has been studied more than with other methods. In the DCCT/EDIC cohort [76], a consistent and proportional association between CAC score and CVD events was demonstrated after 10–13 years of follow-up. After adjusting for HbA1c and other CVR factors, a CAC score of >100 AU was associated with subsequent CVD and major adverse cardiovascular events (MACE). However, it did not show a significant incremental value in ROC analyses after adding it to the model that already took into account the variables mentioned above. In the Pittsburgh Epidemiology of Diabetes Complications (EDC) study [77], similar results were observed. The baseline CAC score and CAC score progression 4–8 years later were independently associated with CVD events in childhood-onset T1D. The former showed increased predictive ability when associated with other established CVR factors. It should be noted that CAC score increases with age, with a positive CAC occurring in >80% of the general population aged 65 years or more [78]. The previously reported cohorts are from studies initiated more than 30 years ago that subscribe only to the USA, and the prevalence of a positive CAC score was 30–40% [76,77].

Data from different demographics, geographies and periods show widely differing prevalences, ranging from 18 to 94% [79–82], so studies assessing its predictive utility for CVD events in different settings are needed. In addition, it appears that people with T1D tend to have a greater progression of CAC compared to people without diabetes [83,84]. Data from the EDC study show that CAC progression increases with BMI, non-high-density lipoprotein cholesterol levels, the duration of diabetes and albuminuria [85]. From the same sample, it was published years later that baseline CAC and, above all, CAC progression were associated with cognitive impairment [86]. Similar results are also available from the Coronary Artery Calcification in Type 1 Diabetes (CACTI) study. CAC progression was associated with insulin sensitivity [87], nephropathy [88], menopause [84], markers of inflammation [89] and obesity [90]. CAC progression has also been associated with suboptimal glycaemic control [91]. Finally, intensive diabetes treatment in a DCCT trial was associated with lower CAC scores, an effect mainly mediated by the reduction in HbA1c during the study and the legacy effect seen 7–9 years later, when CAC was determined [92].

4.3. Coronary Computed Tomography Angiography (CCTA)

CCTA is a non-invasive technique using intravenous iodinated contrast. It allows the detection of CAD and the characterisation of atherosclerotic plaques in the epicardial arterial tree. After contrast administration, CT images are acquired during inspiratory breath hold to avoid artefacts resulting from chest motion [93]. To optimise the quality of the images, it is necessary not to exceed a certain pulsation threshold (usually < 60–65 beats/min), which sometimes requires the use of negative chronotropic drugs such as beta blockers, and makes their application difficult in the case of a high heart rate and/or non-sinus rhythm [94].

CCTA can be used as a prognostic tool to characterise calcified and non-calcified plaques, the latter of which may be present in young people and may even indicate greater plaque vulnerability and risk of complications [95,96]. It is also indicated in symptomatic patients, as one of its main advantages is that it detects obstructive lesions and allows the measurement of the stenosis degree. It is a reliable and reproducible technique, easy to interpret, and its results can be used to decide whether an invasive or therapeutic approach is required. Among its drawbacks is being a more costly, laborious technique, with radiation and contrast exposure. The use of iodinated contrast may limit its use in cases of nephropathy and/or allergy. Further, calcified lesions may incur false positives due to blooming artefacts. Also, image quality may be suboptimal in cases of obesity [94].

The FACTOR-64 clinical trial [97] evaluated the usefulness of screening with CCTA versus standard national guidelines-based optimal diabetes care in 900 people with diabetes (12% with T1D) and no symptoms of CAD. Based on the screening results, the decision

was made to use standard, aggressive therapy with more adjusted control targets for various CVR factors or aggressive therapy associated with invasive coronary angiography, subsequently assessing the incidence of MACE. After 4 years of follow-up, although there was a trend in favour of the use of CCTA, it was not significant compared to the control group. It should be noted that (1) the number of events in both groups was lower than expected and (2) the control group had a good management of several CVR factors (baseline mean LDLc 87.7 mg/dL and mean systolic blood pressure 130.5 mmHg). Both of these points could have compromised the power to detect differences between the groups. There are no other studies available that assess CCTA as a screening test in this population; however, some indirect information is available. For example, a prospective study on over 2000 patients showed an association between atherosclerotic lesions detected by CCTA and several specific CVR factors such as glycaemic control, diabetic neuropathy or repeated hypoglycaemias [98]. In individuals with long-standing T1D, it has also been associated with LDLc levels [99,100] and glycaemic control [100]. Finally, it should be emphasised that in young adults, the identification of subclinical atherosclerosis is significantly more likely using CCTA compared to the CAC score, enabling earlier detection and intervention [101].

4.4. Ankle–Brachial Index (ABI)

The ankle–brachial index (ABI) estimates the presence of ischaemia in the lower extremities. It is a screening method for peripheral arterial disease (PAD) that provides indirect evidence of the presence of systemic atherosclerosis. According to the European Society of Cardiology (ESC) Peripheral Arterial Disease guidelines [102], it is performed in the supine position, with a cuff placed just above the ankle. After a 5–10-min rest, the systolic blood pressure is measured with a Doppler probe (5–10 MHz) in the posterior and the anterior tibial (or dorsalis pedis) arteries of each foot and on the brachial artery of each arm. The ABI of each leg is calculated by dividing the highest ankle SBP by the highest arm SBP. A value < 0.9 indicates ischaemia; between 0.9 and 1 is considered borderline; between 1 and 1.4, normal; and >1.4 is indicative of arterial stiffness or calcification.

Its main advantages are that it is inexpensive, standardised, easy to interpret and quick to perform. Yet, its results are operator-dependent and, as it does not directly visualise atherosclerosis, it does not allow the characterisation and quantification of plaques. In addition, it detects disease with haemodynamic repercussions and therefore cannot have sufficient sensitivity for early subclinical stages. A low performance has also been described in the case of arterial calcification, a fairly common occurrence in people with diabetes [103], and in haemodialysis patients in whom other alternatives such as the toe–brachial index could offer better results, as the measurements are performed on digital vessels that rarely suffer calcifications [104,105].

It should be noted that PAD is one of the most frequently occurring CVDs in people with T1D [18,106]. In screening for this complication, the use of ABI is recommended in the presence of consistent signs and symptoms [107]. ABI's sensitivity is questionable in an asymptomatic population and/or at early stages of the disease [108,109]. Several meta-analyses associate high and low ABI values with CVD in the general population [110–112] and in people with T2D [113,114], although studies on T1D are scarce. In a cross-sectional study on 289 adults with T1D without PAD symptoms, an ABI < 0.9 was detected in 6% and ABI > 1.2 in 26%. Of those with abnormal ABI, 15% had ultrasound-assessed carotid atherosclerosis, and 40% had silent PAD confirmed through lower extremity arterial Doppler ultrasound and/or the toe–brachial index [115]. Another cross-sectional study conducted in 185 adults with T1D from the EDC study analysed the association between ABI scores and medial arterial calcification (MAC) assessed using lower extremity radiographs, as a proxy CVD. In total, 57% had MAC, 8% had ABI > 1.3 and another 8% had an ankle–brachial difference (ABD) >75 mmHg. The predictive ability of MAC using the ABI or ABD was modest, suggesting a higher diagnostic yield of ABD as opposed to ABI. However, in using these cut-off points, more than 40–50% of cases with MAC remained undiagnosed [116]. In summary, the designs of the above studies yielded results that

should be considered exploratory, and studies analysing the incidence of CVD based on ABI results in the asymptomatic T1D population are required.

4.5. Magnetic Resonance Imaging (MRI)

Magnetic resonance imaging (MRI) includes many techniques used in different vascular beds and provide information on both morphology and functionality (mainly cardiac). Its excellent resolution in soft tissues enables the distinction of plaque features (Table 1). The use of intravenous contrast with gadolinium is common and permits the assessment of neovascularisation and other vulnerability traits such as intra-plaque haemorrhage or fibrous cap thickness. One of its main drawbacks is that it is susceptible to motion-related artefacts (e.g., cardiac and respiratory movements). Therefore, its main application is in large vessels (e.g., carotid arteries or the aorta), which are relatively immobile. Its use in smaller vessels such as coronary arteries is complex, although advances in motion artefact correction, image acceleration and reconstruction techniques have improved [117]. It is also useful as a myocardial perfusion study as it has a higher spatial resolution than classical radionuclide studies [118]. In general, despite the versatility of MRI, its widespread clinical application is hampered by extended acquisition times, intricate scan planning and cost.

Table 1. Characteristics of the main non-invasive imaging methods for detecting subclinical atherosclerosis.

Imaging Modality	Strengths	Limitations
Carotid US	Identifies atherosclerosis in early stages Enables the differentiation of some plaque characteristics (better characterisation if CEUS or 3DVUS is used) Low cost No radiation exposure	Operator-dependent Lack of methodological standardisation of IMT measurements
CAC	Reproducible and standardised Strong CVD predictor Low cost	Radiation exposure (low) Does not identify plaque in early stages (only detects calcified plaques) Doubtful usefulness for monitoring treatment (promotion of plaque calcification with statin use)
CCTA	Reproducible and standardised Detects obstructive coronary lesions and degree of stenosis (direct detection of CAD) Plaque characterisation	Expensive Radiation exposure Iodinated contrast exposure (allergy, nephrotoxicity) Imaging is limited if arrhythmias and/or obesity Blooming artefact in calcified lesions
ABI	Standardised Easy-to-interpret results Inexpensive No radiation exposure Quick to perform	Does not visualise atherosclerosis Primarily picks up hemodynamically significant lesions and therefore may miss subclinical disease Limited interpretation in certain patient populations (high calcification burden, haemodialysis)
MR	Excellent soft tissue resolution for plaque characterisation Multiparametric (morphology, functionality) No radiation exposure	Motion artefacts (mainly limited to large-calibre vessels) Expensive Time consuming

ABI: ankle-brachial index; CAC: coronary artery calcium; CAD: coronary artery disease; CCTA: coronary computed tomography angiography; CEUS: contrast-enhanced ultrasound; CVD: cardiovascular disease; IMT: intima-media thickness; MR: magnetic resonance; US: ultrasound; 3DVUS: 3D vascular ultrasound.

In this regard, MRI's use as a screening method in the T1D population is infrequent. Weckbach et al. performed whole-body MRI and MR-angiography on 65 patients with diabetes duration >10 years (31% with T1D) and 200 healthy individuals and observed a markedly higher prevalence of atherosclerosis in almost all territories studied in those

with diabetes. Of note, >50% of clinically significant changes (e.g., carotid, vertebral or renal artery stenosis, pattern compatible with myocardial infarction or cerebral ischaemic infarction) were previously unknown, suggesting the usefulness of using their screening protocol in this population [119]. Further, a cross-sectional study in 136 adult patients with long-standing T1D suggested a higher plaque burden in the right coronary artery in those with albuminuria >300 mg/24 h. There were no differences when assessing the abdominal and thoracic aorta [120]. The results of the above studies are in line with expectations and evidence from other screening tests for subclinical atherosclerotic disease.

4.6. Other Screening Methods

The main non-invasive tools used in clinical practice for the detection of atherosclerotic plaques have been described. However, there are other available techniques that are either novel or do not directly study atherosclerosis itself. For example, increased arterial stiffness or arteriosclerosis, a different process but often associated with atherosclerosis, has been associated with micro- and macrovascular complications in T1D [121]. Furthermore, molecular imaging using positron emission tomography (PET), PET-CT, PET-MRI or nuclear MRI are promising tests that allow the study of multiple early processes involved in atherosclerosis using radiotracers (e.g., targeting different markers of inflammation, extracellular matrix components or macrophages) [37,122–124]. Finally, perfusion and functional cardiac imaging could enhance the accuracy of detecting CAD in subjects with high CVR, such as people with T1D. Resting global longitudinal strain has also been highlighted as an early marker of myocardial damage in people with chronic coronary syndromes, and stress echocardiography has been used to detect coronary artery disease burden [125,126].

5. Future Directions

The current healthcare system is mainly focused on the advanced stages of atherosclerosis, showing that we act “too late and too little” in the early stages. Atheroma plaque formation starts in childhood and adolescence and is consistently associated with the presence of multiple modifiable CVRs [127]. The Progression of Early Subclinical Atherosclerosis (PESA) study showed that the prevalence of subclinical atherosclerosis was >60% in middle-aged men, lagging behind the disease (atherosclerosis) by that time [128]. In people with T1D, the need for biomarkers to identify those most likely to benefit from pharmacological and non-pharmacological treatments with proven cardiovascular benefits becomes even more apparent.

The ideal screening method should detect atherosclerotic lesions in their earliest stages. It should also reliably predict cardiovascular events. It should also be efficient and applicable regularly to assess CVR longitudinally and dynamically. A tool that meets all these requirements is not yet available or it does not have the scientific evidence to support its usefulness with certainty. Anyway, given the pathophysiological complexity of CVD and the multiple factors involved, the optimal approach probably lies in the combined use of imaging techniques, the assessment of classical risk factors (e.g., conventional lipid profile, high blood pressure), polygenic scoring and tests aimed at detecting other alterations associated with early atherosclerosis (e.g., endothelial dysfunction, inflammatory markers [e.g., C-Reactive Protein, IL-1, IL-6, IL-18], proteomic or transcriptomic analyses and leptin and ghrelin dysregulations) (Figure 2). Advances must be aimed not only at detecting the earliest alterations of atherosclerotic lesions but also at identifying subjects at greater risk of plaque development, progression and acute complications. Longitudinal studies are needed to assess an appropriate screening strategy in these cases. Pending further and better evidence, in light of what is available, we advocate for early and systematic screening for subclinical atherosclerosis in this vulnerable population.

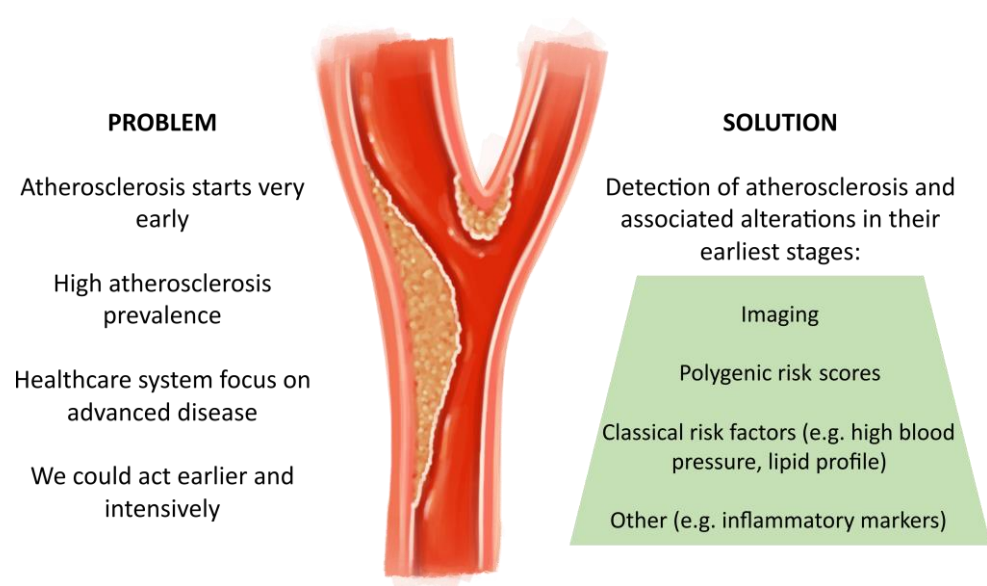


Figure 2. Future directions.

6. Conclusions

Little evidence is available on the usefulness of screening for subclinical atherosclerosis in the T1D population. So far, studies suggest similar results to those found in the general population. The use of the CAC score is probably the best predictor of future cardiovascular events, especially CAD. However, since it detects lesions at a more advanced stage, it is not useful in the younger population. The use of other techniques such as carotid ultrasound, CCTA and MRI may be useful in these earlier stages of atherosclerosis; however, more studies are needed to recommend their routine use as a screening test in T1D. Finally, ABI is useful as a first diagnostic test for PAD, but its ability to detect lesions without haemodynamic impact is low.

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ARTÍCULO ORIGINAL 2: EL USO DE *STENO TI RISK ENGINE* IDENTIFICA LA ATEROSCLEROSIS PRECLÍNICA MEJOR QUE EL USO DE ESC/EASD-2019 EN SUJETOS ADULTOS CON DIABETES TIPO 1 Y ALTO RIESGO CARDIOVASCULAR

Objetivos: Evaluar la concordancia entre la escala de estimación del RCV genérica ESC/EASD-2019 y la ecuación, específica de DTI, Steno-Risk en pacientes adultos con DTI en prevención primaria: así como analizar las asociaciones de ambas con la aterosclerosis carotídea.

Métodos: Seleccionamos consecutivamente pacientes con edad ≥ 40 años, ERD y/o con ≥ 10 años de evolución de DTI con otro FRCV. La aterosclerosis carotídea se evaluó mediante ecografía. El riesgo cardiovascular se estimó de acuerdo con los grupos de riesgo de ESC/EASD-2019 (moderado/alto/muy alto) y de Steno-Risk ($<10\%$, bajo; $10-20\%$, moderado; $\geq 20\%$, alto). Como análisis exploratorio, también se evaluó la ecuación genérica *pooled cohort equation* de la ACC/AHA 2013.

Resultados: 501 pacientes (53% hombres, edad media 48,8 años, mediana de duración de DTI 26,5 años, 41,3% con placas). La concordancia entre las escalas fue pobre ($k = 0.19$). Hubo un aumento gradual en la presencia de placas según la categoría de Steno-Risk (18,4%, 38,2% y 64,1%, respectivamente; p de tendencia $<0,001$), sin diferencias según ESC/EASD-2019 ($p = 0,130$). Steno-Risk identificó a los individuos con placas, a diferencia de ESC/EASD-2019 (área bajo la curva [AUC] 0,691, $p < 0.001$, vs. AUC 0,538, $p = 0,149$). En los modelos ajustados, un riesgo alto según Steno-Risk, independientemente de la estimación por ESC/EASD-2019, se asoció con la aterosclerosis (riesgo moderado/alto según ESC/EASD-2019, OR 2,91 [IC 95% 1,27-6,72] y 4,94 [2,35-10,4] para la presencia de placa y ≥ 2 placas). Se obtuvieron resultados similares con un mayor riesgo discordante según Steno-Risk frente a ACC/AHA-2013 ($p < 0,001$).

Conclusiones: En personas con DTI sin ECV previa, Steno-Risk parece reconocer mejor a los individuos con aterosclerosis, en comparación a ESC/EASD-2019. El uso de la ecografía carotídea podría mejorar la categorización del riesgo cardiovascular en esta población.



Use of the Steno T1 Risk Engine Identifies Preclinical Atherosclerosis Better Than Use of ESC/EASD-2019 in Adult Subjects With Type 1 Diabetes at High Risk

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OBJECTIVE

To evaluate the concordance between the 2019 ESC Guidelines on diabetes, pre-diabetes, and cardiovascular diseases developed in collaboration with the EASD (ESC/EASD-2019) and the Steno T1 Risk Engine (Steno-Risk) cardiovascular risk scales for individuals with type 1 diabetes (T1D) without cardiovascular disease (CVD) and to analyze the relationships of their use with identification of preclinical atherosclerosis.

RESEARCH DESIGN AND METHODS

We consecutively selected patients with T1D, without CVD, age ≥ 40 years, with nephropathy, and/or with ≥ 10 years of T1D evolution with another risk factor. The presence of plaque at different carotid segments was determined by ultrasonography. Cardiovascular risk was estimated in accord with ESC/EASD-2019 risk groups (moderate/high/very high) and the Steno-Risk ($<10\%$, low; $10\text{--}20\%$, moderate; $\geq 20\%$, high), as T1D-specific scores. In an exploratory analysis, we also evaluated the non-T1D-specific 2013 ACC/AHA Guideline on the Assessment of Cardiovascular Risk (ACC/AHA-2013) pooled cohort equation for individuals between 40 and 79 years of age.

RESULTS

We included 501 patients (53% men, mean age 48.8 years, median T1D duration 26.5 years, 41.3% harboring plaques). Concordance between T1D-specific scales was poor ($\kappa = 0.19$). A stepped increase in the presence of plaques according to Steno-Risk category was seen (18.4%, 38.2%, and 64.1%, for low, moderate, and high risk, respectively; P for trend <0.001), with no differences according to ESC/EASD-2019 ($P = 0.130$). Steno-Risk identified individuals with plaques, unlike ESC/EASD-2019 (area under the curve [AUC] 0.691, $P < 0.001$, vs. AUC 0.538, $P = 0.149$). Finally, in polynomial regression models (with adjustment for lipid parameters and cardioprotective treatment), irrespective of the ESC/EASD-2019 category, high risk by Steno-Risk was directly associated with atherosclerosis (in moderate/high-risk by ESC/EASD-2019 odds ratio 2.91 [95% CI 1.27–6.72] and 4.94 [2.35–10.40] for the presence of plaque and two or more plaques). Similar results were obtained with discordant higher Steno-Risk versus ACC/AHA-2013 ($P < 0.001$).

CONCLUSIONS

Among T1D patients undergoing primary prevention, use of Steno-Risk seems to result in better recognition of individuals with atherosclerosis in comparison with ESC/EASD-2019. Notwithstanding, carotid ultrasound could improve the categorization of cardiovascular risk.

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Cardiovascular disease (CVD) is the leading cause of death in the general population (1). Similarly, it is also the leading cause of death among patients with type 1 diabetes (T1D) (2). Large studies have shown a reduction in cardiovascular morbidity and mortality either under intensive insulin therapy (3) or with improved adherence to lipid-lowering drugs (4). Intensive treatment of these and other cardiovascular risk factors (CVRF), optimization of therapeutic education, and advances in the management of chronic disease have led to a significant trend toward a lower rate of cardiovascular events in this population (5,6). Despite the aforementioned, the long-term risk of CVD among T1D patients is four- to eightfold higher than in the general European population (7,8).

Given the importance of CVD in persons with T1D, tools to predict cardiovascular events are essential to tailor cardiovascular risk management according to each individual's risk. Classically, cardiovascular risk scales, which estimate the risk of events according to clinical and laboratory variables, have been used for this purpose (e.g., Systematic Coronary Risk Evaluation [SCORE] or the Framingham-REGICOR equation) (9). These equations are intended to be applied to the general population, although they have been extrapolated to the T1D group. These approaches usually show little predictive value in the case of T1D (10), given the different pathophysiology and the fact that CVRF specific to this entity, such as albuminuria or duration of diabetes, are not taken into account. Subsequently, specific T1D scales with a higher performance have emerged, such as the Steno T1 Risk Engine (Steno-Risk) (11), use of which has been shown to have an independent association with identification of several markers of subclinical atherosclerosis (arterial stiffness [12] or carotid plaques visualized by ultrasonography [13]) and which may also be useful for prediction of CVD events in other T1D populations (14). Similarly, the recently published 2019 ESC Guidelines on diabetes, prediabetes, and cardiovascular diseases developed in collaboration with the EASD (ESC/EASD-2019) specifically defined differentiated subgroups of patients with T1D at high/very high cardiovascular risk (15). However, agreement between these two strategies has been scarcely

studied (16), and in no previous study has their utility been assessed in identifying individuals more prone to presenting CVD.

With this background, in this study we aimed to assess the concordance between the cardiovascular risk estimation scales ESC/EASD-2019 and Steno-Risk and analyze the relationship of their use with identification of preclinical atherosclerosis in subjects with T1D without CVD but at high risk.

RESEARCH DESIGN AND METHODS

Subjects

This was a cross-sectional investigation conducted in participants with T1D without CVD (coronary artery disease, ischemic stroke, peripheral artery disease, or heart failure) enrolled from a specialized Diabetes Unit of the Hospital Clinic in Barcelona, Spain. Hospital Clinic is a tertiary university hospital, with a reference population of 540,000 individuals, and actively follows 2,000–2,500 patients with T1D. All subjects met the subsequent inclusion criteria: age ≥ 40 years; the presence of diabetic nephropathy, regardless of age or diabetes duration; and/or duration of T1D ≥ 10 years with one or more CVRF.

We considered the following as additional CVRF: active smoking habit (excluding former smokers), diabetic retinopathy, hypertension (defined as systolic blood pressure ≥ 140 mmHg and diastolic blood pressure ≥ 90 mmHg or treatment with antihypertensive drugs), triglycerides > 150 mg/dL, low HDL cholesterol (< 40 mg/dL in men, < 45 mg/dL in women), family history of premature CVD in first-degree relatives (defined as CVD occurring at < 55 years of age in men and < 65 years in women), severe hypoglycemia (defined as an episode of confirmed hypoglycemia requiring external assistance for recovery) or hypoglycemia unawareness (defined as a score > 3 on the Clarke test, with the validated Spanish version [17]), and prior pre-eclampsia/eclampsia in women.

The study protocol was conducted according to the Declaration of Helsinki. All patients provided informed consent, and the study was approved by the local ethics committee (Barcelona, Spain).

Clinical and Laboratory Measures

Both demographic and clinical data including duration of T1D, family history

of premature CVD in first-degree relatives, history of microvascular diabetes complications, and medical treatment (multiple-dose insulin, continuous subcutaneous insulin infusion, lipid-lowering agents, and antihypertensive and antiplatelet drugs) were obtained from medical records. The term “high-intensity statin therapy” was defined as statin doses that reduced LDL cholesterol by $\geq 50\%$.

Diabetic nephropathy was assessed according to the albumin-to-creatinine ratio, with < 30 mg/g considered as normal and ≥ 30 mg/g as diabetic nephropathy (confirmed on at least two of three consecutive determinations). The use of ACE inhibitors (ACEi) or angiotensin II receptor blockers (ARB), without a history of hypertension or CVD, was also considered as diabetic nephropathy. Fundus oculi was used for diagnosis of diabetic retinopathy, which was always confirmed by an ophthalmologist.

Anthropometric measurements (weight, height, and waist circumference) were also obtained. Patients were weighed wearing light clothing and barefoot, with use of a calibrated electronic scale. BMI was calculated as weight in kilograms divided by the square of height in meters. The midpoint between the lowest rib and the iliac crest was used to measure waist circumference.

Laboratory parameters were measured in fasting blood and first-morning urine spot samples. Lipid profile (including total cholesterol, triglycerides, and HDL cholesterol), glucose, creatinine, and albumin-to-creatinine ratio were assessed with standardized assays. LDL cholesterol was determined with the Friedewald formula. Non-HDL cholesterol was calculated as the deduction of HDL cholesterol from total cholesterol. Estimated glomerular filtration rate (eGFR) was assessed with the Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI) equation. Glycated hemoglobin (HbA_{1c}) values (Tosoh G8 Automated HPLC Analyzer; Tosoh Bioscience, South San Francisco, CA) (Diabetes Control and Complications Trial [DCCT] aligned, normal range 4–6% [20–42 mmol/mol]) were also recorded.

Cardiovascular Risk Estimation

Cardiovascular risk was assessed with two different strategies, specific to the T1D population: classification according

to Steno-Risk (11) and the cardiovascular risk groups proposed by ESC/EASD-2019 (15). Briefly, Steno-Risk estimates the 10-year risk of fatal or nonfatal CVD (ischemic heart disease, ischemic stroke, heart failure, and peripheral artery disease) based on 10 variables (age, sex, diabetes duration, HbA_{1c}, systolic blood pressure, LDL cholesterol, albuminuria, eGFR, smoking habit, and regular exercise [≥ 3.5 h/week]). Individuals were classified as having low ($<10\%$), moderate (10–19.9%), or high ($\geq 20\%$) risk, accordingly (11). ESC/EASD-2019 defined the following cardiovascular risk groups: moderate risk, those with T1D duration <10 years and without any additional CVRF or chronic diabetes complications; high risk, those with T1D duration ≥ 10 years and any additional CVRF (but without target organ damage [TOD]); and very high risk, those with TOD, three or more major CVRF (hypertension, dyslipidemia, smoking habit, obesity), or early T1D onset (<10 years old) and T1D duration >20 years.

An exploratory analysis was also performed with use of the non-diabetes-specific, broadly used, pooled cohort equation risk estimation of the American College of Cardiology/American Heart Association (2013 ACC/AHA Guideline on the Assessment of Cardiovascular Risk [ACC/AHA-2013]) (18), as recommended by the American Diabetes Association (19). The equation estimates the 10-year risk of atherosclerotic CVD (coronary death or nonfatal myocardial infarction or fatal or nonfatal stroke) from several variables (age, sex, race, systolic and diastolic blood pressure, total cholesterol, HDL cholesterol, history of diabetes, hypertension treatment, and smoking status). Subjects were classified according to the categories low-borderline ($<7.4\%$), intermediate (7.5–19.9%), and high ($\geq 20\%$) risk, as appropriate.

Carotid B-Mode Ultrasound Imaging

Carotid plaques were evaluated with high-resolution B-mode ultrasound (ACUSON X700 [Siemens Healthineers] or Aplio 450 [Canon]) with the same electric linear array 5–10 MHz transducer. Predefined and standardized imaging protocols to measure carotid intima-media thickness (IMT) were used, and the presence of plaque was determined as previously described (20,21). Carotid images

were visualized with B-mode and color Doppler in longitudinal and transverse planes to appraise circumferential asymmetry. Carotid plaques were defined according to focal echo structures trespassing into the arterial lumen by at least 50% of the surrounding IMT value or when IMT was at least 1.5 mm as measured from the media-adventitia interface to the intima-lumen surface (22). Two experienced endocrinologists performed all the procedures, and IMT and plaque ascertainment and measurements were made by the same researcher (A.J.A.) using semiautomatic software. The mean and mean maximum IMT of all carotid segments (common carotid, bulb, and internal carotid) were documented, in addition to the maximum height of carotid plaque. If carotid plaques were present, the maximum IMT was equal to the highest carotid plaque height. Peak systolic and end-diastolic velocities were used to determine carotid stenosis, and when significant plaques (IMT >2.5 mm) were found, the planimetric area was measured in a transversal view.

Statistical Analysis

The data were subjected to descriptive analysis. The Kolmogorov-Smirnov test was used to evaluate the normal distribution of continuous variables. Results for qualitative variables are stated as frequency and percentage, quantitative normal variables as mean $\pm$ SD, and nonnormal quantitative variables as median (first quartile–third quartile). Comparisons between binomial qualitative or quantitative variables were performed with parametric or nonparametric tests as appropriate. Cohen κ coefficient was used to assess concordance between scales (Steno-Risk vs. ESC/EASD-2019), either in the whole cohort or after division by sex. Furthermore, the characteristics of the individuals discordant with both strategies were also assessed.

The prevalence of preclinical atherosclerosis (no plaque, one plaque, or two or two or more plaques) according to risk strata with both strategies was assessed by means of the χ^2 test. The sensitivity and specificity of the scales for identifying individuals harboring carotid plaques (receiver operating characteristic curves) were further assessed, providing the Youden J statistic, accordingly. Finally, polynomial regression models were constructed

for assessing the independent associations of the discordance in estimated risk (independent variable) with preclinical atherosclerosis (both presence of plaques or two or more 2 plaques [dependent variables]), with adjustment for variables not included in these strategies (HDL cholesterol, triglycerides, and cardioprotective treatment [i.e., statins, ACEi/ARB, and antiplatelet drugs]).

As an exploratory analysis, in participants between 40 and 79 years old ($n = 419$), all of the previous comparisons between T1D-specific strategies were additionally compared with the ACC/AHA-2013 risk equation. Polynomial regression models were also constructed for the discordance in the estimated risk between Steno-Risk and ACC/AHA-2013 (with adjustment for triglyceride levels, statins, and antiplatelet drugs).

All of the statistical analyses were performed with IBM SPSS Statistics, version 26 (IBM Corporation, Armonk, NY), statistical software. The significance level was set as a P value <0.05 .

RESULTS

Subject Characteristics

A total of 501 subjects were included (53% men, mean $\pm$ SD), age 48.8 ± 10.25 years, median duration of diabetes 26.5 years (interquartile range 20.9–34.0). Overall, 29.5% were smokers, 25% had hypertension, 11% had nephropathy, and 38.3% had retinopathy (34.9% proliferative). There were 42.9% on statin treatment and 9.4% taking antiplatelet drugs. In the whole cohort, 41.3% had at least one carotid plaque (23.6% with two or more plaques), with no between-sex differences ($P > 0.350$ for both). As the number of plaques increased, there was a stepped increase in age, smokers, the presence of hypertension, diabetic nephropathy, and triglyceride values and a decrease in eGFR ($P < 0.05$ for all). The remaining characteristics are shown in Table 1.

Estimation of Cardiovascular Risk

With use of the Steno-Risk strategy, we classified one of four as having low cardiovascular risk and 43.9% and 31.1% as moderate or high risk, respectively. In contrast, in using the ESC/EASD-2019, we classified almost all individuals as at high or very high risk (51.3% and 48.1%), with virtually no individuals classified as

Table 1—Characteristics of the study participants according to the presence of carotid plaque

	No plaque (n = 294)	Presence of one plaque (n = 89)	Presence of two or more plaques (n = 118)	P
Clinical characteristics				
Male	151 (51)	49 (55)	66 (56)	0.646
Age (years)	45.64 ± 9.56	50.36 ± 9.87	55.61 ± 8.57	<0.001
Premature CVD in first-degree relatives*	36 (12)	8 (9)	19 (16)	0.300
Current smokers	68 (27)	19 (21)	51 (43)	0.001
Cumulative smoking (pack-years)	0 (0–10)	0 (0–11)	11 (0–30.25)	<0.001
Hypertension	56 (19)	20 (23)	49 (42)	<0.001
SBP (mmHg)	125 (118–135)	130 (120–137.5)	134 (122.75–145)	<0.001
DBP (mmHg)	82 (75–86.25)	80 (74–85)	81 (74–86)	0.223
BMI (kg/m ²)	25.98 (23.95–28.47)	26.26 (23.43–28.32)	26.47 (23.86–29.20)	0.583
Waist circumference (cm)				
Women	87.08 ± 11.71	85.29 ± 10.82	87.82 ± 12.48	0.663
Men	94.99 ± 10.24	96.99 ± 9.35	97.51 ± 10.69	0.223
T1D duration (years)	25.46 (20.71–31.90)	27.57 (22.21–33.62)	29.27 (20.56–37.54)	0.015
Diabetic nephropathy	33 (11)	3 (3)	19 (16)	0.015
Diabetic retinopathy	104 (35)	36 (40)	52 (44)	0.235
CSII therapy	106 (36)	27 (30)	33 (28)	0.238
Laboratory characteristics				
Fasting plasma glucose (mg/dL)	144 (107–194)	164 (118–207)	148.50 (121.50–207.50)	0.066
HbA _{1c} [%; mmol/mol]	7.5 (7.0–8), (58)	7.7 (7.2–8.2), (61)	7.8 (7.1–8.2), (62)	0.087
Serum creatinine (mg/dL)	0.84 (0.73–0.95)	0.82 (0.73–0.94)	0.83 (0.74–0.98)	0.540
eGFR, CKD-EPI (mL/min/1.73 m ²)	98 (87–107)	95 (84–105)	89 (80–99)	<0.001
ALT (IU/L)	20 (15–27)	21 (16–27)	19 (15–26)	0.349
Total cholesterol (mg/dL)	188 (170–210)	188 (169–214)	186 (168–206)	0.841
HDL cholesterol (mg/dL)	60 (50–71)	58 (50–70)	58 (50–67)	0.511
LDL cholesterol (mg/dL)	109 (96–128)	114 (97–131)	109 (93–123)	0.345
Triglycerides (mg/dL)	72 (58–97)	79 (64–100)	83 (65–113)	0.007
Non-HDL cholesterol (mg/dL)	126 (111–146)	130 (114–151)	128 (113–141)	0.520
Pharmacological treatment				
Statins	109 (37)	40 (45)	66 (56)	0.002
ACEi/ARB	67 (23)	21 (24)	49 (42)	<0.001
Antiplatelet drugs	19 (7)	10 (11)	18 (15)	0.017

Data are n (% in each column), mean ± SD, or median (interquartile range). P values for group comparisons are reported. CSII, continuous subcutaneous insulin infusion; DBP, diastolic blood pressure; SBP, systolic blood pressure. *Defined as <55 years of age in men and <65 years in women. Significant P values (<0.05) are shown in bold.

moderate risk (Fig. 1). No differences in sex were found for any of the scales ($P = 0.638$ and $P = 0.574$) (Supplementary Fig. 1).

Clinical and laboratory characteristics according to estimated risk were further assessed. Regarding the Steno-Risk, a progressive increase in most of the CVRF was found as the estimated risk increased (namely, age, smoking status, hypertension, duration of diabetes, nephropathy, HbA_{1c}, waist circumference, retinopathy, and triglyceride levels), as well as in the use of the cardioprotective treatments ($P < 0.05$ for all comparisons) (Supplementary Table 1). Differences according to ESC/EASD-2019 categories (very high vs. moderate/high risk) were also assessed. Although some CVRF were increased for individuals at higher risk (i.e., BMI, hypertension, chronic complications, and HbA_{1c}; $P < 0.05$ for

all), neither age, smoking habit, nor most of the cardioprotective drugs were statistically different (Supplementary Table 2).

Cardiovascular Risk Strategies' Agreement (Steno-Risk Versus ESC/EASD-2019)

The concordance between the two strategies was poor ($\kappa = -0.079$, $P < 0.001$) (Supplementary Table 3), even after we performed the analysis for the highest category of risk for both strategies ($\kappa = 0.186$ for all the cohort) (Supplementary Table 4), with no sex differences ($\kappa = 0.173$ and 0.195 for women and men; respectively) (Supplementary Table 4). In analyses of patients with discrepant results between the two strategies, we found that those with a higher estimated risk with Steno-Risk (12%) were older and had longer diabetes duration and worse glycemic control, higher prevalence of

hypertension and higher triglyceride levels, worse renal function, and greater use of cardioprotective drugs. Conversely, those with discrepant lower risk in Steno-Risk (28%) were younger, with a better overall cardiovascular risk profile as well as lower use of cardioprotective drugs (Supplementary Table 5).

Relationships Between Estimated Risk and Preclinical Carotid Atherosclerosis (Steno-Risk Versus ESC/EASD-2019)

According to Steno-Risk, most of the patients at low risk were free of plaques (82%) and only 4% harbored two or more plaques. Conversely, two of three of the patients in the high-risk category showed plaques (46% with two or more plaques; $P < 0.001$ for linear trend) (Fig. 2). Regarding the ESC/EASD-2019 strategy, irrespective of the allocation of

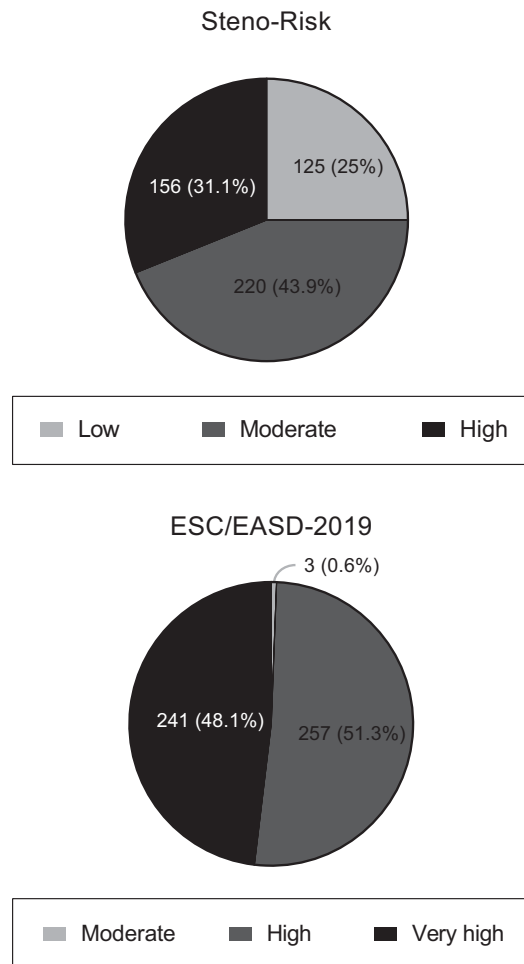


Figure 1— Distribution of the study subjects according to Steno-Risk and ESC/EASD-2019 categories. Data are shown as n (%).

high or very high risk, most showed no plaques (63% and 55% for high and very high risk, respectively) (Fig. 2). Accordingly, no differences were found in preclinical atherosclerosis status, as the estimated risk increased with this strategy (P for trend = 0.127) (Fig. 2). Furthermore, no statistical differences were found in plaque presence in comparing some of the very-high-risk subgroups with the whole high-risk group according to ESC/EASD-2019 (T1D + TOD, $n = 118$; 45.4% of plaque presence; early-onset T1D + ≥ 20 years of diabetes duration, $n = 89$; 40.4% of plaques; $P = 0.077$ and $P = 0.604$).

Whereas the area under the curve (AUC) in receiver operating characteristic curves was statistically significant for Steno-Risk (AUC 0.691; Youden J statistic 0.236; $P < 0.001$), those harboring plaques were not identified with use of ESC/EASD-2019 (AUC 0.538; $P = 0.149$) (Supplementary Fig. 2). Accordingly,

diagnostic performance regarding plaque identification was better with Steno-Risk, both for those at moderate-high risk (Steno-Risk $\geq 10\%$; sensitivity = 0.889 and specificity = 0.348; Youden J statistic = 0.236) and high risk (Steno-Risk $\geq 20\%$; sensitivity = 0.483 and specificity = 0.809; Youden J statistic = 0.292), vs. ESC/EASD-2019 for very high risk (sensitivity = 0.527 and specificity = 0.553; Youden J statistic = 0.080). Similar results were found for two or more plaques, with AUC 0.736 ($P < 0.001$) for Steno-Risk (sensitivity = 0.958 and specificity = 0.314 and sensitivity = 0.602 and specificity = 0.777 for moderate-high and high risk, respectively) and AUC 0.537 ($P = 0.226$) for ESC/EASD-2019 (sensitivity = 0.542 and specificity = 0.539 for very-high risk) (Supplementary Fig. 2). We also analyzed performance using the absolute risk values estimated with the Steno-Risk equation, showing a slight improvement compared with the classification into three groups

(AUC 0.725 for the presence of plaque [Youden J statistic = 0.378] and AUC 0.772 for two or more plaques [Youden J statistic = 0.457]; $P < 0.001$ for both) (Supplementary Fig. 3).

Finally, concordance in cardiovascular risk assessment concerning preclinical atherosclerosis was also studied. Irrespective of the estimated risk with ESC/EASD-2019, individuals classified as high risk with Steno-Risk showed a significantly higher prevalence of plaques ($P < 0.001$) (Supplementary Table 5). Even after adjustment for several variables not included in any of the strategies (HDL cholesterol, triglycerides, and cardioprotective treatment), discordance and higher risk according to Steno-Risk showed a similar or even greater association with presence of preclinical atherosclerosis than concordance and high/very high risk according to both strategies (presence of plaques odds ratio [OR] 2.914 [95% CI 1.268–6.716] and 2.152 [0.991–4.673] and two or more plaques 4.938 [2.346–10.395] and 5.29 [2.746–10.189] for discordance with higher Steno-Risk and concordance with high/very high risk; respectively) (Fig. 3). Conversely, even in individuals classified as at very high risk with ESC/EASD-2019, if Steno-Risk was low/moderate, the presence of plaques was similar to that of those identified as non-high risk according to both strategies ($P > 0.254$) (Fig. 3).

Performance of General Cardiovascular Risk Scores in the T1D Population: The ACC/AHA-2013 Pooled Cohort Equation

Of the $n = 419$ individuals for whom the risk equation could be applied, almost two-thirds (62%) showed low or borderline risk, 27.7% intermediate risk, and only 10.3% high risk with ACC/AHA-2013, with a higher estimated risk in men than women ($P < 0.001$) (Supplementary Fig. 4). Regarding patient characteristics according to risk strata, a stepped increase in the proportion of men, age, and presence of active smoking habit, hypertension, and diabetic nephropathy was observed as the estimated risk increased (Supplementary Table 6). Furthermore, triglycerides, creatinine, and cardioprotective treatment increased and HDL cholesterol decreased as the ACC/AHA-2013 risk categories increased (Supplementary Table 6).

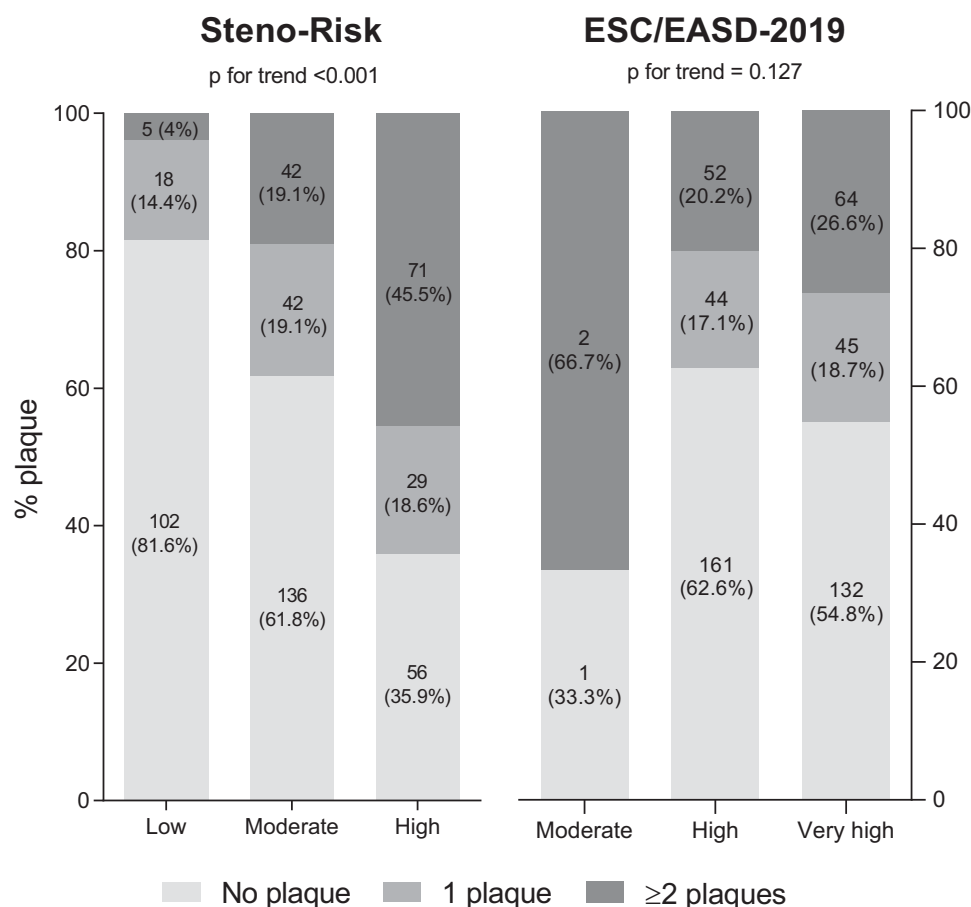


Figure 2—Relationships between cardiovascular risk categories and preclinical atherosclerosis for both risk scales. Data are shown as *n* (%). *P* = 0.164 for comparison between high vs. very high risk category according to ESC/EASD-2019 classification strategy.

There was a gradual increase in the number of plaques as the estimated risk increased, with at least one plaque in 32.7%, 62.9%, and 79.1% of those at low-borderline, intermediate, and high risk, respectively (*P* for lineal trend <0.001) (Supplementary Fig. 5). However, most of the patients harboring carotid plaques were classified as non-high risk (82.3% of patients with plaques) and almost half (44.3%) fell into the low-borderline category. The discriminatory diagnostic capacity of the ACC/AHA-2013 equation was also compared with T1D-specific scores in these 419 individuals. The ability to detect preclinical atherosclerosis was similar to that shown by Steno-Risk (presence of plaque AUC 0.673 and 0.664, and two or more plaques AUC 0.688 and 0.693 for ACC/AHA-2013 and Steno-Risk; *P* < 0.001 for both) (Supplementary Fig. 6A–D) and higher than that by ESC/EASD-2019 (AUC 0.558 and 0.556 for the presence of plaque and two or more plaques) (Supplementary Fig. 6A and B). Furthermore, in evaluating only those in the highest risk

category with the three scales, Steno-Risk better discriminated individuals with the most advanced atherosclerosis (two or more plaques AUC 0.669, *P* < 0.001, AUC 0.579, *P* = 0.012, and AUC 0.560, *P* = 0.058, for Steno-Risk, ACC/AHA-2013 and ESC/EASD-2019) (Supplementary Fig. 6E and F). The performance of the three strategies in identifying individuals with preclinical carotid atherosclerosis is summarized in Supplementary Table 7.

Finally, risk classification agreement between Steno-Risk and ACC/AHA-2013 was also analyzed. Individuals at higher risk by Steno-Risk showed a significantly higher prevalence of plaques, irrespective of ACC/AHA-2013 risk category allocation (*P* < 0.001) (Supplementary Table 8). And, after adjustment for possible confounders not included in both equations, participants with discordance in risk assessment with higher risk as estimated with Steno-Risk showed a greater atherosclerotic burden (OR 3.333 [95% CI 1.930–5.756], *P* < 0.001, for two or more plaques) (Supplementary Fig. 7).

CONCLUSIONS

In our sample of T1D adults at prespecified high risk undergoing primary prevention, there was poor concordance between cardiovascular risk scales, with an overall higher estimated risk with ESC/EASD-2019 compared with Steno-Risk. Furthermore, this discrepancy also translated into identification of individuals with more advanced atherosclerosis. Whereas ESC/EASD-2019 classification failed to detect individuals harboring more carotid plaques, the Steno-Risk was directly associated with the presence and number of plaques in a stepped manner, even after adjustment for other CVRF. Moreover, the performance of ESC/EASD-2019 guidelines in identifying individuals with higher plaque burden was worse than that of cardiovascular risk scores specifically designed for the general population. As far as we know, this is the first study assessing the differences between these strategies and the relationship of their use with identification of preclinical atherosclerosis as detected by ultrasound in

Steno-Risk vs ESC/EASD-2019 concordance

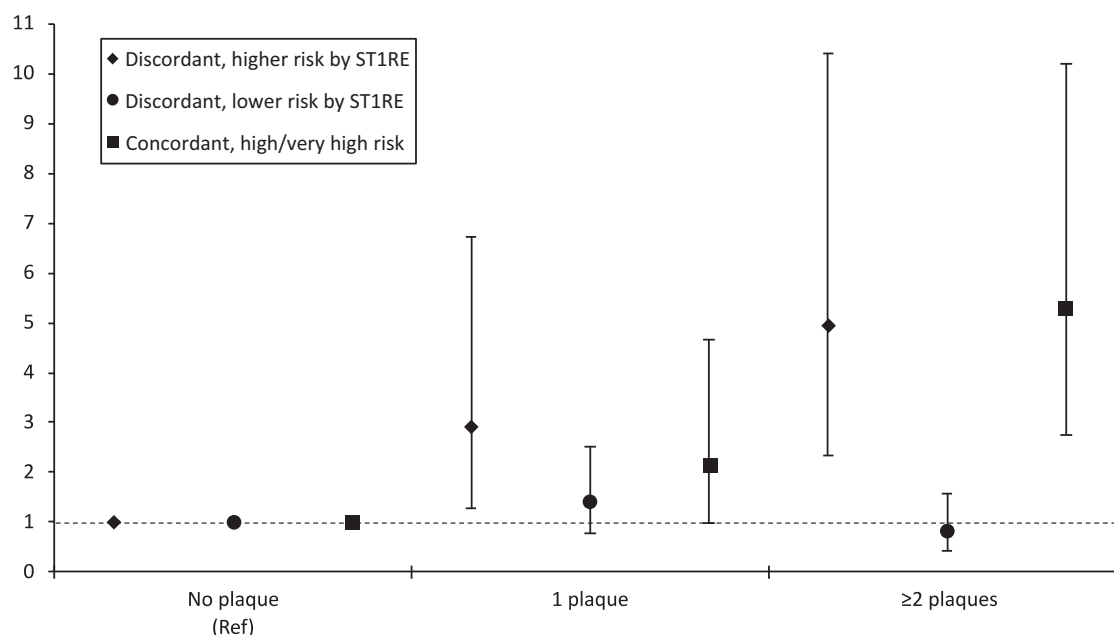


Figure 3—Associations of the discordance in estimated risk between Steno-Risk and ESC/EASD-2019 for cardiovascular risk classification in relation to preclinical atherosclerosis by polynomial regression analysis. Data are shown as OR (95% CI). Presence of one plaque: discordant, higher risk by Steno-Risk (high by Steno-Risk and moderate/high by ESC/EASD-2019) OR 2.914 (1.268–6.716, $P = 0.012$); discordant, lower risk by Steno-Risk (low/moderate by Steno-Risk and very high by ESC/EASD-2019) 1.4 (0.785–2.498, $P = 0.254$); concordant, high/very high (high risk by Steno-Risk and very high risk by ESC/EASD-2019) 2.152 (0.991–4.673, $P = 0.053$). Presence of two or more plaques: discordant, higher risk by Steno-Risk 4.938 (2.346–10.395, $P < 0.001$); discordant, lower risk by Steno-Risk 0.806 (0.417–1.558, $P = 0.521$); concordant, high/very high (ESC/EASD-2019) risk 5.29 (2.746–10.189, $P < 0.001$). Polynomial regression models adjusted for HDL cholesterol, triglycerides, and cardioprotective treatment (statins, ACEi/ARB, and antiplatelet drugs). ORs and 95% CIs are reported. Ref, reference; ST1RE, Steno T1 Risk Engine (Steno-Risk).

an adult population with T1D at high cardiovascular risk.

Estimating cardiovascular risk in people with T1D remains a challenge nowadays. Predictive tools such as the classification proposed by the ESC/EASD-2019 emphasize the increased risk associated with age, the presence of TOD, and the duration of diabetes (15), mainly based on the excess of mortality found in the Swedish National Diabetes Register among individuals with earlier disease onset (23). However, some criticism has been raised regarding classifying all the patients with early-onset and long duration of T1D as at very high risk (24). Similarly, in our cohort, among patients of this kind there was a prevalence of preclinical atherosclerosis similar to that of individuals with a lower estimated risk. This lack of effectiveness in identifying patients at risk implies that, with the exception of subjects <35 years of age, duration of diabetes <10 years, and no other CVRF, the majority of patients would be considered at high/very high risk and, therefore, with an

indication for lipid-lowering and even antiplatelet therapy. The classification of cardiovascular risk proposed in the recently published 2021 ESC Guidelines on cardiovascular disease prevention in clinical practice (ESC-2021) (25) has not undergone substantial changes in this respect. In this regard, other guidelines with use of age and diabetes duration as the main drivers for the estimation of cardiovascular risk (such as the National Institute for Health and Care Excellence [NICE] guidelines [26]), have shown poor performance for risk stratification. For instance, if these guidelines were applied to the Swedish National Diabetes Register's cohort, 81–90% and 100% of patients between 20–40 years and ≥40 years of age, respectively, would be on statins (27). Similarly, if we look at the regional and national databases of adult individuals with T1D in our country, the average age is >40 years and mean duration of diabetes between 10 and 20 years (28–30). Therefore, the vast majority would also be classified as at high or

very high risk according to ESC/EASD-2019, and intensified treatment of CVRF would be warranted as well. One of the possible explanations of this poor performance of the European guidelines is that other known predictors of mortality and CVD, such as glycemic control, are not taken into account (31). Conversely, the Steno-Risk equation includes several pathology-specific variables, which could allow greater individualization of risk and, consequently, tailored treatment. In our cohort, the concordance between these two strategies was poor and up to 60% of those at very high risk according to ESC/EASD-2019 were at low/moderate risk according to Steno-Risk (Supplementary Table 4). Pointing in the same direction, in a study conducted in an unselected Italian cohort of T1D patients (16), only 12% aged >35 years were included in the highest risk categories according to both scales and ~3% were for analyses of the entire cohort. In our study, the concordance across the highest risk categories was greater (20%), which is to be

expected considering that among our cohort patients were older (mean $\pm$ SD age 48.8 ± 10.25 vs. 36 ± 12 years) and had a longer diabetes duration (median 26.5 years [interquartile range 20.9–34.0] vs. 19 ± 11 years), with higher prevalence of other CVRF (Table 1).

A relevant finding of our study was the stepped relationship between preclinical atherosclerosis and estimated cardiovascular risk according to Steno-Risk (Fig. 2), which was also independent of that yielded by ESC/EASD-2019 (Fig. 3). In no previous study have investigators specifically evaluated the presence of preclinical atherosclerosis by validated methods or the incidence of CVD according to different risk scales among the T1D population. In a sample of $n = 302$ individuals with T1D, our group recently showed that Steno-Risk was independently associated with the presence and the number of carotid plaques, even after adjustment for other CVRF (13). Similarly, Boscari et al. (14) found that a higher risk according to this score better discriminated between subjects with a higher prevalence of carotid plaques and those who presented a cardiovascular event. Although the follow-up time was almost 5 years, it should be noted that the sample was small ($n = 223$) and the number of CVD events very low ($n = 3$); therefore, further studies are needed to ascertain its usefulness. Pending results of prospective studies with hard CVD outcomes, the findings of the current study provide the first evidence that Steno-Risk could better identify individuals with T1D at the highest risk of CVD (vs. ESC/EASD-2019).

As an exploratory analysis, we also assessed the performance of the ACC/AHA-2013 risk equation in our sample. Despite being a non-T1D-specific tool, it showed superior efficacy to detect atherosclerosis compared with the ESC/EASD-2019 classification and efficacy similar to that of Steno-Risk (Supplementary Fig. 6). However, some caveats should be noted. First, the ACC/AHA-2013 risk equation cannot be applied for individuals <40 years of age. Taking into account that T1D specially affects younger individuals (32), the use of this nonspecific score would leave out of the estimation a large number of patients who could benefit from early cardioprotective treatment. Second, in the context of a disease in which CVD is

the leading cause of early mortality (2), it seems surprising that the overall estimated cardiovascular risk could be low in the vast majority of the individuals (Supplementary Fig. 4). In the same sense, the ACC/AHA-2013 risk equation also failed to identify the patients with preclinical atherosclerosis, since $>82\%$ of patients with at least one carotid plaque were allocated as non-high risk and, thus, not requiring cardioprotective treatment. This is in contrast to Steno-Risk, according to which only 11.1% of patients with preclinical carotid atherosclerosis were classified as low risk and almost half of those with plaques were correctly classified as high risk (Fig. 2 and Supplementary Fig. 5). Finally, even though ACC/AHA-2013 performed better than other T1D-specific strategies (i.e., ESC/EASD-2019), Steno-Risk seems to better identify individuals with more advanced atherosclerosis (two or more carotid plaques), even over the estimation of this general population risk scale (Supplementary Fig. 7). Altogether, this reinforces the statement of the recent American Diabetes Association/ European Association for the Study of Diabetes consensus, in which specific risk scores (i.e., Steno-Risk) are recommended for selecting individuals for whom cardioprotective treatment should be prescribed (33).

Classically, and after the irruption of the large Diabetes Control and Complications Trial (34) and the observational Epidemiology of Diabetes Interventions and Complications (EDIC) study (3), the management of T1D was and continues to be eminently glucocentric. In this regard, in the last years, a lot of technological advances have helped with improvement of glycemic control (35). However, despite good glycemic control, and even without the presence of other chronic complications, cardiovascular risk in T1D patients is never equal to that of the general population (36). Therefore, studies focused on other aspects of the pathophysiology of CVD in T1D are mandatory. Thus, both nonspecific (i.e., age, dyslipidemia, or obstetric complications) and T1D-specific (i.e., nephropathy and retinopathy) factors have been involved in cardiovascular risk in this population (37–40). Our findings are that 1) Steno-Risk better identifies individuals with a higher prevalence of CVRF vs. ESC/EASD-2019 (Supplementary Tables 1

and 2) and 2) this scale was strongly associated with identification of preclinical atherosclerosis, which could help with overcoming this glucocentric scenario. Taking into account that the 10 variables included in the score are easily available (11), Steno-Risk should be implemented in clinical practice.

Generic CVD events prediction tools are not easily extrapolated to the T1D population, and even in using those T1D-specific tools (i.e., Steno-Risk), $>40\%$ of the individuals are classified as at moderate risk, which is an indeterminate area in CVD prevention (Fig. 1). Against this background, the use of other potential markers that can increase predictive performance becomes paramount. Imaging biomarkers, such as the detection of carotid plaques by ultrasound, have demonstrated their usefulness as predictors of CVD (41) and could serve to reclassify risk in subjects in the general population according to the recently released ESC-2021 guidelines on CVD prevention (25). In addition, the visualization of plaques by the patients themselves can increase adherence to a healthy lifestyle and cardioprotective drugs and may even reduce the risk of cardiovascular events (42). Although the usefulness of this biomarker in the setting of the T1D population is less known, our study could help to fill the gap in this promising research area. Thus, carotid ultrasonography could be especially useful in the setting of moderate risk allocation with Steno-Risk, in which case there is uncertainty in identifying individuals with atherosclerosis (Fig. 2). Furthermore, there is also some preliminary evidence that other biomarkers aimed at detecting the quality of diet (43) or systemic inflammation (44) could be useful and open the door to different approaches and therapeutic targets. The implementation of biomarkers in daily practice could help to intensify cardiovascular disease prevention in patients with greater plaque burden, although it also allows de-escalation of unnecessary treatments for those with discordance between the estimated risk and the presence of preclinical atherosclerosis.

Our study has several strengths: 1) its novelty, as to the best of our knowledge, this is the first study with analysis of the relationships of different risk scales with identification of a proven proxy of cardiovascular events, such as

the presence of carotid plaques (41); 2) the quality of the data, which were carefully collected, including for pharmacological treatments and other clinical and analytical variables, reduces the risk of bias in our results; 3) the large sample included, and the use of standardized procedures, reduces the variability and, therefore, leads to more accurate results. However, our study also has limitations. Firstly, the cross-sectional design does not allow assessment of the appearance of atherosclerotic disease or cardiovascular events. In this regard, although the detection of carotid plaques by ultrasound has demonstrated predictive capacity for future cardiovascular events, it is a surrogate variable. Further prospective studies are needed to ascertain the usefulness of CVD risk scores in identifying those more prone to hard clinical events. Secondly, given that the study was carried out in a single tertiary hospital and that only prespecified high-risk patients without CVD were included, extrapolation to other patients with a different setting or cardiovascular profile should be made with caution. However, although our sample of study was a bit older, with a longer diabetes duration, than those of other recent data sets representative of our geographical area (28–30), the other key characteristics of cardiovascular risk, such as mean BMI, prevalence of smoking habit, and hypertension, or microvascular complications, were very similar. Thirdly, due to our inclusion criteria, high/very high risk strata according to ESC/EASD-2019 classification strategy could be overrepresented. Thus, the better performance of Steno-Risk for those classified as moderate risk by this strategy may be unproven. However, in looking at other studies with recruitment of a representative sample of adults with T1D from our same area (28–30), mean age was >40 years and in the case of most patients mean diabetes duration was >10 years, which, according to ESC/EASD-2019 guidelines, would, in fact, classify most at high/very high risk. Furthermore, even in other studies, with inclusion of T1D individuals with a priori lesser risk compared with our sample (mean age 36 years and diabetes duration 19 years; <20% with diabetic retinopathy), only 4% were classified as at moderate risk according to the ESC/EASD-2019 strategy (16). Altogether, this

suggests that moderate risk according to this classification strategy would hardly be seen among nonpediatric T1D patients, allowing our results to be extrapolated to other adult populations with T1D. Finally, although regular exercise (≥ 3.5 h/week) was a variable included in Steno-Risk, more specific information about the intensity and frequency of physical activity was lacking. Since investigators of some previous studies have found a close relationships between this variable and future CVD events among the T1D population (45), it would have been interesting to assess the relationships of physical activity, estimated cardiovascular risk, and preclinical atherosclerosis.

In summary, in our sample of patients with T1D at high cardiovascular risk, the ESC/EASD-2019 generic risk scale showed poor concordance with the more T1D-specific Steno-Risk scale. The latter presented a significant and independent association with the presence and number of carotid plaques, even after adjustment for other variables strongly associated with atherosclerosis. Furthermore, and independently of estimations according to the ESC/EASD-2019 strategy, Steno-Risk better identified individuals with more advanced atherosclerosis, even after comparisons with the ACC/AHA-2013 pooled cohort equation. The availability of more accurate tools for predicting CVD events, and the advances in the knowledge of different CVRF in T1D, allows moving in the direction of individualized medicine to identify individuals who can benefit most from a more aggressive and targeted management.

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Author Contributions. All authors discussed the results and commented on the final version of the manuscript. T.S.-N., M.G., V.P., L.B., C.V., J.B., I.V., A.P., E.E., I.C., and A.J.A. acquired and processed all clinical data. A.J.A. performed the U.S. measurements. T.S.-N. and A.J.A. contributed to data analysis and interpretation and wrote, reviewed, and edited the manuscript. V.P. and A.J.A. contributed to the study concept and design. M.G., V.P., C.V., J.B., I.V., I.C., and A.J.A. supervised the study and participated in

data analysis and interpretation. T.S.-N. and A.J.A. wrote the manuscript, designed the figures, and had final responsibility for the decision to submit for publication. A.J.A. is the guarantor of this work and, as such, had full access to all the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis.

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Supplemental Material

Corresponding to

The Steno Type 1 Risk Engine identifies preclinical atherosclerosis better than the ESC/EASD-2019 Guidelines in adult subjects with type 1 diabetes at high-risk.

Short running title: Risk scales and carotid atherosclerosis in T1D

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Total Supplemental Tables: 8

Total Supplemental Figures: 7

Supplementary Table 1. Characteristics of the study participants according to the Steno-Risk categories.

	Low-risk (n=125)	Moderate-risk (n=220)	High-risk (n=156)	p-value
Clinical characteristics				
Male	62 (50)	118 (54)	86 (55)	0.638
Age (years)	37.39 (± 6.08)	48.31 (± 5.82)	58.72 (± 7.56)	<0.001
Premature CVD in first-degree relatives*	13 (10)	31 (14)	19 (12)	0.601
Current smokers	27 (22)	65 (30)	56 (36)	0.033
Cumulative smoking (Pack-years)	0 (0-6.88)	0 (0-12.50)	5 (0-25.50)	<0.001
Hypertension	9 (7)	39 (18)	77 (49)	<0.001
SBP (mmHg)	120 (113-129)	127 (119.25-135)	136 (126-148)	<0.001
DBP (mmHg)	81 (74.50-85.50)	82 (76-86)	81 (74-86)	0.495
BMI (kg/m ²)	25.73 (23.46-28.51)	26.14 (23.85-28.45)	26.49 (24.36-28.72)	0.370
Waist circumference (cm)				
Female (cm)	86.29 (± 12.39)	86.71 (± 12.16)	88.14 (± 10.52)	0.667
Male (cm)	94 (± 9.19)	95.13 (± 11.12)	98.68 (± 9.22)	0.016
T1D duration (years)	22.80 (17.49-26.26)	26.63 (21.40-33.75)	32.02 (24.33-41.02)	<0.001
Diabetic nephropathy	7 (6)	13 (6)	35 (22)	<0.001
Diabetic retinopathy	43 (34)	67 (31)	82 (53)	<0.001
CSII therapy	39 (31)	78 (36)	49 (31)	0.620
Laboratory characteristics				
Fasting plasma glucose (mg/dL)	141.50 (113-192)	153 (108-205)	151 (122-207)	0.267
Hemoglobin A1c (% (mmol/mol))	7.3 (6.9-7.9), (58)	7.6 (7.1-8), (61)	7.9 (7.3-8.4), (62)	<0.001
Serum creatinine (mg/dL)	0.82 (0.72-0.94)	0.82 (0.72-0.93)	0.86 (0.76-0.99)	0.011
eGFR (CKD-EPI; ml/min/1.73m ²)	106 (94-113)	99 (87-105)	86 (75-95)	<0.001
ALT (UI/L)	19 (14-25)	21 (16-28)	20 (16-26)	0.076
Total cholesterol (mg/dL)	190 (174-209)	189 (169-208)	186 (167-215)	0.633
HDL-cholesterol (mg/dL)	56 (50-69)	59 (50-70)	62 (51-71)	0.238
LDL-cholesterol (mg/dL)	115 (100-130)	110 (98-126)	106 (92-131)	0.075
Triglycerides (mg/dL)	75 (59-100)	71 (57-92)	83 (69-109)	<0.001
Non-HDL cholesterol (mg/dL)	131 (114-147)	126 (111-141)	127 (110-148)	0.313
Pharmacological treatment				
Statins	31 (25)	93 (42)	91 (58)	<0.001
ACEi / ARB	13 (10)	44 (20)	80 (51)	<0.001
Antiplatelet drugs	1 (1)	11 (5)	35 (22)	<0.001

Data are shown as n (percentage in each column), mean ($\pm$ SD), or median (Q1-Q3).

p values for group comparisons are reported

ACEi: angiotensin-converting enzyme inhibitor; ALT: alanine aminotransferase; ARB: angiotensin receptor blocker; BMI: body mass index; CVD: cardiovascular disease; CSII: continuous subcutaneous insulin infusion; DBP: diastolic blood pressure; eGFR: estimated glomerular filtration rate; SBP: systolic blood pressure; T1D: type 1 diabetes

* Defined as <55 years in men and <65 years in women.

Supplementary Table 2. Characteristics of the study participants according to the ESC/EASD-2019 classification.

	Moderate/High-risk (n=260)	Very-high-risk (n=241)	p-value
Clinical characteristics			
Male	133 (51)	133 (55)	0.372
Age (years)	49.43 (±9.97)	48.18 (±10.53)	0.175
Premature CVD in first-degree relatives*	35 (14)	28 (12)	0.590
Current smokers	71 (27)	77 (32)	0.281
Cumulative smoking (Pack-years)	0 (0-10)	0 (0-15)	0.238
Hypertension	51 (20)	74 (31)	0.005
SBP (mmHg)	127 (118-136)	128 (119-139)	0.273
DBP (mmHg)	82 (76-87)	80 (74-85)	0.019
BMI (kg/m ²)	25.70 (23.28-28.21)	26.59 (24.34-28.99)	0.007
Waist circumference (cm)			
Female (cm)	85.43 (±10.51)	88.60 (±12.73)	0.057
Male (cm)	95.97 (±9.90)	98.02 (±10.57)	0.970
T1D duration (years)	23.02 (17.81-27.62)	31.60 (25.76-38.42)	<0.001
Diabetic nephropathy	0 (0)	55 (23)	<0.001
Diabetic retinopathy	0 (0)	192 (80)	<0.001
CSII therapy	76 (29)	90 (37)	0.058
Laboratory characteristics			
Fasting plasma glucose (mg/dL)	148 (111-195)	153 (112-207)	0.398
Hemoglobin A1c (% (mmol/mol))	7.5 (7-8), (59)	7.7 (7.2-8.3), (61)	<0.001
Serum creatinine (mg/dL)	0.83 (0.72-0.93)	0.85 (0.73-0.97)	0.087
eGFR (CKD-EPI; ml/min/1.73m ²)	96 (84-105)	95 (84-106)	0.994
ALT (U/L)	20 (15-26)	20 (15-28)	0.566
Total cholesterol (mg/dL)	190 (171-213)	185 (167-205)	0.226
HDL-cholesterol (mg/dL)	59 (51-71)	59 (50-69)	0.425
LDL-cholesterol (mg/dL)	112 (98-128)	109 (94-126)	0.180
Triglycerides (mg/dL)	74 (59-96)	78 (62-108)	0.105
Non-HDL cholesterol (mg/dL)	129 (113-144)	126 (110-145)	0.454
Pharmacological treatment			
Statins	104 (40)	111 (46)	0.177
ACEi / ARB	47 (18)	90 (37)	<0.001
Antiplatelet drugs	18 (7)	29 (12)	0.065

Data are shown as n (percentage in each column), mean (±SD), or median (Q1-Q3).

p values for group comparisons are reported

ACEi: angiotensin-converting enzyme inhibitor; ALT: alanine aminotransferase; ARB: angiotensin receptor blocker; BMI: body mass index; CVD: cardiovascular disease; CSII: continuous subcutaneous insulin infusion; DBP: diastolic blood pressure; eGFR: estimated glomerular filtration rate; SBP: systolic blood pressure; T1D: type 1 diabetes

* Defined as <55 years in men and <65 years in women.

Supplementary Table 3. Distribution of subjects according to all risk categories.

	ESC/EASD-2019 moderate	ESC/EASD-2019 high	ESC/EASD-2019 very- high
Steno-Risk low	1 (0)	66 (13)	58 (12)
Steno-Risk moderate	1 (0)	134 (27)	85 (17)
Steno-Risk high	1 (0)	57 (11)	98 (20)

Data are shown as n (percentage of the total).

Kappa coefficient= -0.079 for comparison between Steno-Risk and ESC/EASD-2019 scales.

Supplementary Table 4. Distribution of subjects according to the highest risk categories and after dividing by sex.

TOTAL COHORT		
	ESC/EASD-2019 moderate/high	ESC/EASD-2019 very-high
Steno-Risk low/moderate	202 (40)	143 (28)
Steno-Risk high	58 (12)	98 (20)
MALES		
	ESC/EASD-2019 moderate/high	ESC/EASD-2019 very-high
Steno-Risk low/moderate	103 (39)	77 (29)
Steno-Risk high	30 (11)	56 (21)
FEMALES		
	ESC/EASD-2019 moderate/high	ESC/EASD-2019 very-high
Steno-Risk low/moderate	99 (42)	66 (28)
Steno-Risk high	28 (12)	42 (18)

Data are shown as n (percentage of the total).

Kappa coefficient=0.186, k=0.195, and k=0.173 for the total cohort, males and females, respectively, when comparing Steno-Risk high vs. ESC/EASD-2019 very-high risk categories.

Supplementary Table 5. Characteristics of the study participants with discordance in CVD risk assessment strategies (Steno Type 1 Risk Engine vs. ESC/EASD-2019).

	Concordant non-high-risk (n=202)	Higher risk with ST1RE (n=58)	p-value	Lower risk with ST1RE (n=143)	Concordant high-risk (n=98)	p-value
Clinical characteristics						
Male	103 (51)	30 (52)	0.921	77 (54)	77 (54)	0.613
Age (years)	46.01 (±8.02)	61.32 (±6.29)	<0.001	42.01 (±7.14)	42.01 (±7.14)	<0.001
Premature CVD in first-degree relatives*	30 (15)	5 (9)	0.220	14 (10)	14 (10)	0.285
Current smokers	56 (28)	15 (26)	0.779	36 (25)	36 (25)	0.006
Cumulative smoking (Pack-years)	0 (0-10)	0 (0-21)	0.158	0 (0-10)	0 (0-10)	<0.001
Hypertension	25 (12)	26 (45)	<0.001	23 (16)	23 (16)	<0.001
SBP (mmHg)	124 (116-134)	137.50 (126.75-153.25)	<0.001	125 (118-134)	125 (118-134)	<0.001
DBP (mmHg)	82 (76-86)	84.50 (74.75-88)	0.464	81 (74-85)	81 (74-85)	0.324
BMI (kg/m ²)	25.46 (23.13-28.11)	26.28 (24.39-28.42)	0.330	26.46 (24.34-29.02)	26.46 (24.34-29.02)	0.685
Waist circumference (cm)						
Female (cm)	85.36 (±10.80)	85.65 (±9.70)	0.908	87.95 (±13.71)	87.95 (±13.71)	0.501
Male (cm)	94.88 (±10.40)	99.77 (±6.80)	0.006	94.51 (±10.59)	94.51 (±10.59)	0.065
T1D duration (years)	22.41 (16.84-26.28)	25.04 (20.39-33.70)	0.001	29.74 (24.83-35.86)	29.74 (24.83-35.86)	<0.001
Diabetic nephropathy	0 (0)	0 (0)	-	20 (14)	20 (14)	<0.001
Diabetic retinopathy	0 (0)	0 (0)	-	110 (77)	110 (77)	0.201
CSII therapy	61 (30)	15 (26)	0.522	56 (39)	56 (39)	0.481
Laboratory characteristics						
Fasting plasma glucose (mg/dL)	146 (107.75-202.25)	148 (119-175.50)	0.960	152 (108.50-197)	153 (122-225)	0.074
Hemoglobin A1c (% (mmol/mol))	7.4 (6.9-7.9), (57.4)	7.7 (7.1-8.3), (60.7)	0.009	7.6 (7.2-8.2), (59.6)	7.9 (7.4-8.25), (62.8)	0.007
Serum creatinine (mg/dL)	0.82 (0.72-0.92)	0.84 (0.73-0.97)	0.276	0.83 (0.72-0.94)	0.88 (0.78-1.02)	0.009
eGFR (CKD-EPI; ml/min/1.73m ²)	99 (89-107)	84 (76-94)	<0.001	103 (92-109)	87 (74-95)	<0.001
ALT (UI/L)	19 (15-26)	20 (17-26)	0.505	20 (15-28)	20 (16-26)	0.870
Total cholesterol (mg/dL)	190 (171-210)	189 (173-217)	0.533	186 (170-204)	184 (166-211)	0.783

HDL-cholesterol (mg/dL)	59 (51-71)	60 (51-69)	0.867	56 (49-66)	63 (51-72)	0.042
LDL-cholesterol (mg/dL)	112 (99-128)	115 (96-134)	0.825	112 (98-126)	104 (92-126)	0.037
Triglycerides (mg/dL)	71 (58-94)	85 (69-105)	0.012	75 (57-99)	82 (69-111)	0.015
Non-HDL cholesterol (mg/dL)	127 (112-143)	133 (115-149)	0.293	129 (114-145)	123 (107-144)	0.143
Pharmacological treatment						
Statins	76 (38)	28 (48)	0.144	48 (34)	63 (64)	<0.001
ACEi / ARB	22 (11)	25 (43)	<0.001	35 (25)	55 (56)	<0.001
Antiplatelet drugs	5 (3)	13 (22)	<0.001	7 (5)	22 (22)	<0.001
Preclinical atherosclerosis						
Presence of plaque	61 (30)	37 (64)	<0.001	46 (32)	63 (64)	<0.001
Presence of ≥ 2 plaques	30 (15)	24 (41)	<0.001	17 (12)	47 (48)	<0.001

Data are shown as n (percentage in each column), mean ($\pm$ SD), or median (Q1-Q3).

p values for group comparisons are reported

Concordant non-high-risk: Steno-Risk low/moderate & ESC/EASD-2019 moderate/high; Higher risk with ST1RE: Steno-Risk high & ESC/EASD-2019 moderate/high; Concordant high-risk: Steno-Risk high & ESC/EASD-2019 very-high; Lower risk with ST1RE: Steno-Risk low/moderate & ESC/EASD-2019 very-high.

ACEi: angiotensin-converting enzyme inhibitor; ALT: alanine aminotransferase; ARB: angiotensin receptor blocker; BMI: body mass index; CVD: cardiovascular disease; CSII: continuous subcutaneous insulin infusion; DBP: diastolic blood pressure; eGFR: estimated glomerular filtration rate; SBP: systolic blood pressure; ST1RE: Steno Type 1 Risk Engine; T1D: type 1 diabetes

* Defined as <55 years in men and <65 years in women.

Supplementary Table 6. Characteristics of the study participants according to the risk estimation with ACC/AHA-2013 pooled cohort equation.

	Low-borderline risk (n=260)	Intermediate risk (n=116)	High risk (n=43)	p-value
Clinical characteristics				
Male	108 (42)	73 (63)	33 (77)	<0.001
Age (years)	47.60 (± 5.20)	55.92 (± 6.43)	65.87 (± 5.97)	<0.001
Premature CVD in first-degree relatives*	30 (12)	21 (18)	5 (12)	0.211
Current smokers	59 (23)	52 (45)	14 (33)	<0.001
Cumulative smoking (Pack-years)	0 (0-10)	9 (0-26)	10 (0-43.75)	<0.001
Hypertension	40 (15)	49 (42)	29 (67)	<0.001
SBP (mmHg)	124 (116-133)	135 (126-144)	143 (136-160)	<0.001
DBP (mmHg)	81 (75-86)	72 (75-86)	82 (72-87)	0.812
BMI (kg/m^2)	26.03 (23.83-28.44)	26.38 (24.13-28.86)	26.22 (23.63-28.30)	0.862
Waist circumference (cm)				
Female (cm)	87.37 (± 11.67)	87.06 (± 10.75)	83.33 (± 10.89)	0.594
Male (cm)	95.18 (± 9.80)	96.85 (± 11.42)	99.40 (± 8.06)	0.123
T1D duration (years)	27.60 (22.03-33.98)	27.34 (20.69-38.06)	30.66 (21.38-43.64)	0.336
Diabetic nephropathy	22 (9)	19 (16)	7 (16)	0.048
Diabetic retinopathy	94 (36)	43 (37)	20 (47)	0.427
CSII therapy	99 (38)	29 (25)	15 (35)	0.047
Laboratory characteristics				
Fasting plasma glucose (mg/dL)	151 (113-203)	153 (117-207)	145 (112-198)	0.856
Hemoglobin A1c (% (mmol/mol))	7.5 (7-8), (59)	7.6 (7.1-8), (60)	7.7 (7-8.2), (61)	0.648
Serum creatinine (mg/dL)	0.81 (0.70-0.93)	0.84 (0.77-0.96)	0.88 (0.77-1)	0.008
eGFR (CKD-EPI; ml/min/1.73m^2)	98 (84-105)	91 (80-99)	86 (74-93)	<0.001
ALT (UI/L)	19 (15-28)	19 (15-25)	21 (18-26)	0.447
Total cholesterol (mg/dL)	188 (169-210)	183 (166-210)	185 (165-203)	0.586
HDL-cholesterol (mg/dL)	62 (54-73)	56 (48-68)	59 (47-68)	0.006
LDL-cholesterol (mg/dL)	109 (96-126)	107 (93-125)	103 (89-123)	0.541
Triglycerides (mg/dL)	69 (56-89)	85 (65-115)	85 (73-103)	<0.001
Non-HDL cholesterol (mg/dL)	124 (109-141)	127 (113-142)	128 (105-142)	0.615
Pharmacological treatment				
Statins	105 (40)	63 (54)	26 (61)	0.006
ACEi / ARB	48 (19)	53 (46)	24 (56)	<0.001
Antiplatelet drugs	15 (6)	16 (14)	15 (35)	<0.001

Data are shown as n (percentage in each column), mean ($\pm$ SD), or median (Q1-Q3).

p values for group comparisons are reported

n=82 with <40 years of age were excluded because the 10-year cardiovascular risk with the ACC/AHA-2013 pooled cohort equation cannot be applied.

ACEi: angiotensin-converting enzyme inhibitor; ALT: alanine aminotransferase; ARB: angiotensin receptor blocker; BMI: body mass index; CVD: cardiovascular disease; CSII: continuous subcutaneous insulin infusion; DBP: diastolic blood pressure; eGFR: estimated glomerular filtration rate; SBP: systolic blood pressure; T1D: type 1 diabetes

* Defined as <55 years in men and <65 years in women.

Supplementary Table 7. Performance of all three strategies (Steno-Risk, ESC/EASD-2019, and ACC/AHA-2013) for preclinical atherosclerosis detection.

	AUC	p-value	Value	Sensibility	Specificity	Youden's J statistic
Plaque presence						
Steno-Risk (3 groups)	0.691	<0.001	-	0.889	0.347	0.236
Steno-Risk (continuous)	0.735	<0.001	15.1093	0.705	0.673	0.379
ACC/AHA-2013 (3 groups)	0.673	<0.001	-	0.557	0.771	0.328
ACC/AHA-2013 (continuous)	0.721	<0.001	5.2695	0.698	0.678	0.376
Presence of ≥ 2 plaques						
Steno-Risk (3 groups)	0.736	<0.001	-	0.602	0.778	0.380
Steno-Risk (continuous)	0.772	<0.001	17.5293	0.737	0.721	0.458
ACC/AHA-2013 (3 groups)	0.688	<0.001	-	0.635	0.717	0.352
ACC/AHA-2013 (continuous)	0.755	<0.001	5.3535	0.800	0.635	0.435
Plaque presence						
Steno-Risk >10%	0.618	<0.001	-	0.889	0.347	0.236
Steno-Risk >20%	0.646	<0.001	-	0.483	0.810	0.293
ACC/AHA-2013 >7.5%	0.664	0.027	-	0.557	0.771	0.328
ACC/AHA-2013 >20%	0.569	0.015	-	0.177	0.960	0.137
ESC/EASD-2019 very-high	0.540	0.130	-	0.527	0.553	0.080
Presence of ≥ 2 plaques						
Steno-Risk >10%	0.635	<0.001	-	0.958	0.313	0.271
Steno-Risk >20%	0.690	<0.001	-	0.602	0.778	0.380
ACC/AHA-2013 >7.5%	0.676	<0.001	-	0.635	0.717	0.352
ACC/AHA-2013 >20%	0.579	0.012	-	0.217	0.941	0.158
ESC/EASD-2019 very-high	0.541	0.180	-	0.542	0.538	0.080

p values for every area under the curve (AUC) are reported.

n=82 patients younger than 40 years of age were excluded from calculations according to the ACC/AHA-2013 equation (equation not applicable).

Steno-Risk (3 groups) stands for low, moderate, and high risk; ACC/AHA-2013 (3 groups): low-borderline, intermediate and high risk; Steno-Risk (continuous) and ACC/AHA-2013 (continuous) refers to the absolute value calculated with the equations (10 -year cardiovascular risk).

Supplementary Table 8. Characteristics of the study participants with discordance in CVD risk assessment strategies (Steno Type 1 Risk Engine vs ACC/AHA-2013).

	Concordant non-high-risk (n=263)	Higher risk with ST1RE (n=113)	p- value	Lower risk with ST1RE (n=0)	Concordant high-risk (n=43)	p- value
Clinical characteristics						
Male	128 (49)	53 (47)	0.753	-	33 (77)	-
Age (years)	47.66 (±5.33)	56 (±6.22)	<0.001	-	65.87 (±5.97)	-
Premature CVD in first-degree relatives*	37 (14)	14 (12)	0.663	-	5 (12)	-
Current smokers	69 (26)	42 (37)	0.033	-	14 (33)	-
Cumulative smoking (Pack- years)	0 (0-10)	5 (0-23)	0.016	-	10 (0-44)	-
Hypertension	41 (16)	48 (43)	<0.001	-	29 (67)	-
SBP (mmHg)	125 (117-134)	134 (125-143)	<0.001	-	143 (136-160)	-
DBP (mmHg)	81 (75-86)	80 (74-86)	0.561	-	82 (72-87)	-
BMI (kg/m ²)	25.95 (23.58-28.40)	26.55 (24.36-28.96)	0.110	-	26.22 (23.63-28.30)	-
Waist circumference (cm)				-		-
Female (cm)	86.53 (±11.86)	89.02 (±10.32)	0.207	-	83.33 (±10.89)	-
Male (cm)	94.90 (±10.55)	98.19 (±10)	0.075	-	99.40 (±8.09)	-
T1D duration (years)	25.57 (20.33-32.35)	32.27 (26.17-40.73)	<0.001	-	30.66 (21.38-43.64)	-
Diabetic nephropathy	13 (5)	28 (25)	<0.001	-	7 (16)	-
Diabetic retinopathy	75 (29)	62 (55)	<0.001	-	20 (47)	-
CSII therapy	94 (36)	34 (30)	0.289	-	15 (35)	-
Laboratory characteristics						
Fasting plasma glucose (mg/dL)	149 (110-203)	153 (124-209)	0.139	-	145 (112-198)	-
Hemoglobin A1c (%; (mmol/mol))	7.4 (7-7.9), (57.4)	7.9 (7.4-8.5), (62.8)	<0.001	-	7.7 (7-8.2), (60.7)	-
Serum creatinine (mg/dL)	0.81 (0.71-0.91)	0.84 (0.76-0.99)	0.006	-	0.88 (0.77-1)	-
eGFR (CKD-EPI; ml/min/1.73m ²)	99 (88-105)	85 (75-95)	<0.001	-	86 (74-93)	-
ALT (UI/L)	19 (15-27)	20 (15-26)	0.836	-	21 (18-26)	-
Total cholesterol (mg/dL)	185 (169-205)	186 (167-225)	0.206	-	185 (165-203)	-
HDL-cholesterol (mg/dL)	60 (51-71)	62 (52-73)	0.235	-	59 (47-68)	-
LDL-cholesterol (mg/dL)	109 (96-125)	106 (94-136)	0.998	-	103 (89-123)	-
Triglycerides (mg/dL)	70 (55-91)	82 (67-114)	<0.001	-	85 (73-103)	-

Non-HDL cholesterol (mg/dL)	125 (110-141)	127 (112-151)	0.304	-	128 (105-142)	-
Pharmacological treatment						
Statins	103 (39)	65 (58)	0.001	-	26 (61)	-
ACEi / ARB	45 (17)	56 (50)	<0.001	-	24 (56)	-
Antiplatelet drugs	11 (4)	20 (18)	<0.001	-	15 (35)	-
Preclinical atherosclerosis						
Presence of plaque	92 (35)	66 (58)	<0.001	-	34 (80)	-
Presence of ≥ 2 plaques	44 (17)	46 (41)	<0.001	-	25 (58)	-

Data are shown as n (percentage in each column), mean ($\pm$ SD), or median (Q1-Q3).

p values for group comparisons are reported.

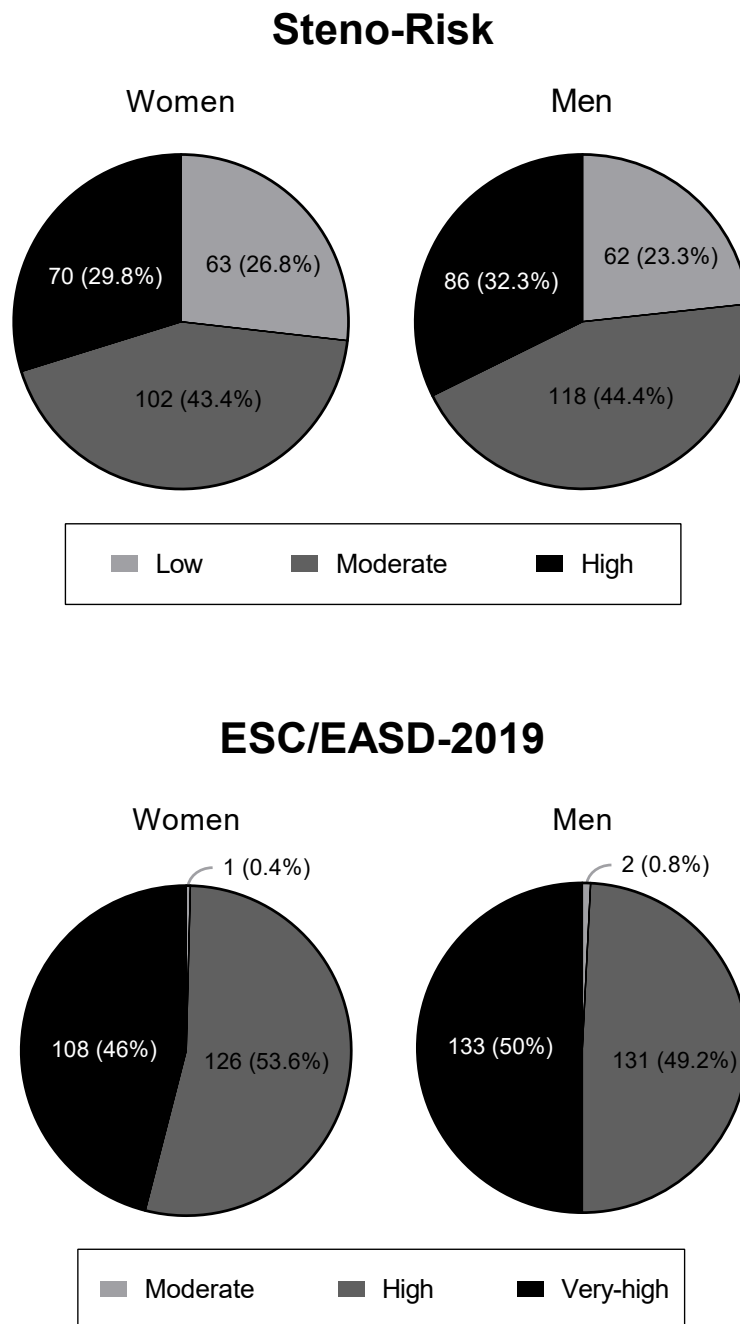
n=82 with <40 years of age were excluded because the 10-year cardiovascular risk with the ACC/AHA-2013 pooled cohort equation cannot be applied.

Concordant non-high-risk: Steno-Risk low/moderate & ACC/AHA-2013 low/borderline/intermediate; Higher risk with ST1RE: Steno-Risk high & ACC/AHA-2013 low/borderline/intermediate; Concordant high-risk: Steno-Risk high & ACC/AHA-2013 high; Lower risk with ST1RE: Steno-Risk low/moderate & ACC/AHA-2013 high.

ACEi: angiotensin-converting enzyme inhibitor; ALT: alanine aminotransferase; ARB: angiotensin receptor blocker; BMI: body mass index; CVD: cardiovascular disease; CSII: continuous subcutaneous insulin infusion; DBP: diastolic blood pressure; eGFR: estimated glomerular filtration rate; SBP: systolic blood pressure; ST1RE: Steno Type 1 Risk Engine; T1D: type 1 diabetes

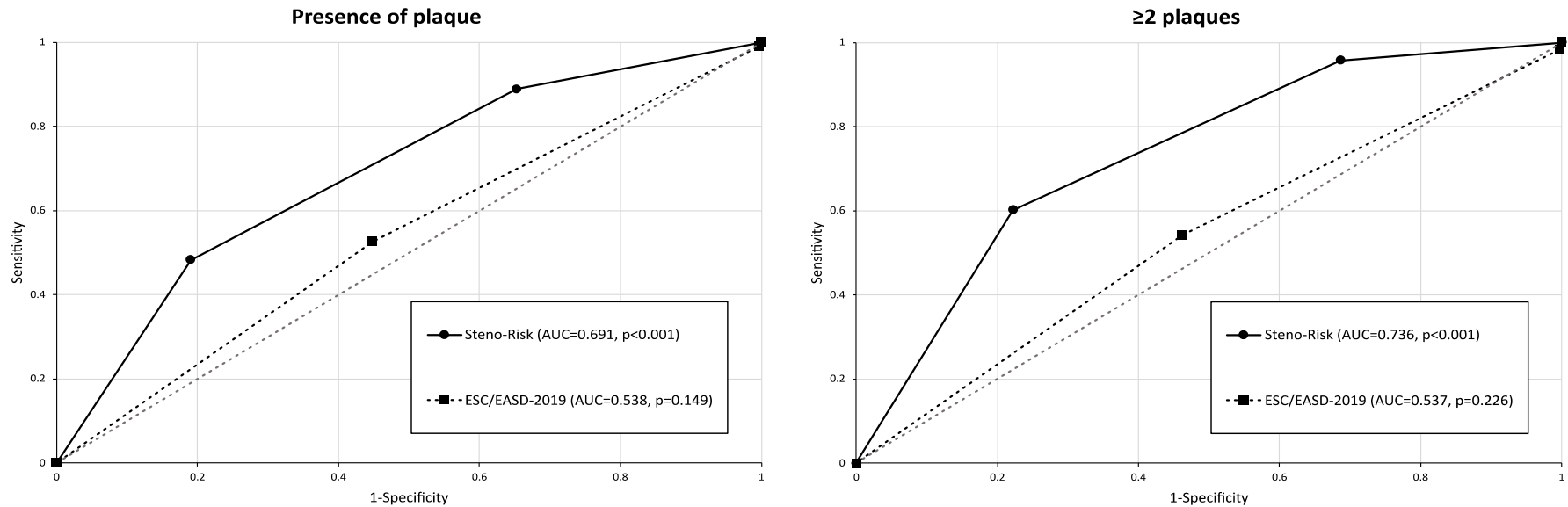
* Defined as <55 years in men and <65 years in women.

Supplementary Figure 1. Distribution of the study subjects according to Steno-Risk, ESC/EASD-2019 strategies, and sex.



Data are shown as n (percentage).

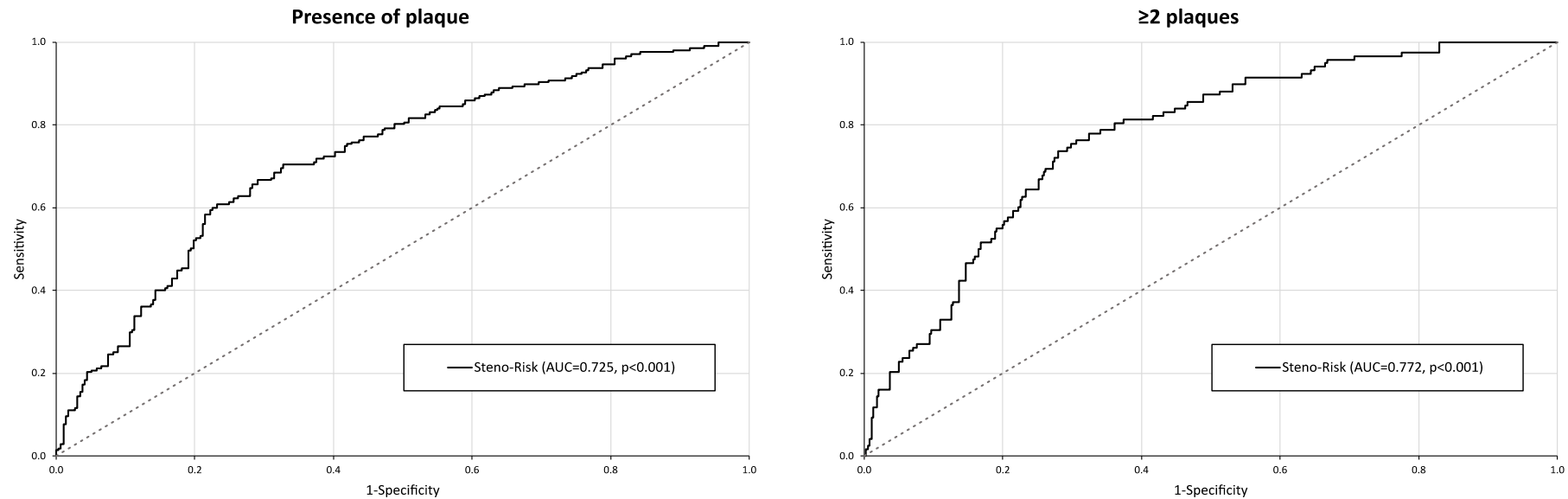
Supplementary Figure 2. Performance of Steno-Risk and ESC/EASD-2019 classifications for the presence and number of plaques in the whole cohort (n=501).



Receiver operating characteristic (ROC) curves with p values for every area under the curve (AUC) are reported and a diagonal reference line is shown.

Left: plaque presence detection for Steno-Risk (low, moderate, high risk) and ESC/EASD-2019 (moderate, high, very-high risk). Right: detection of two or more carotid plaques for Steno-Risk (low, moderate, high risk) and ESC/EASD-2019 (moderate, high, very-high risk).

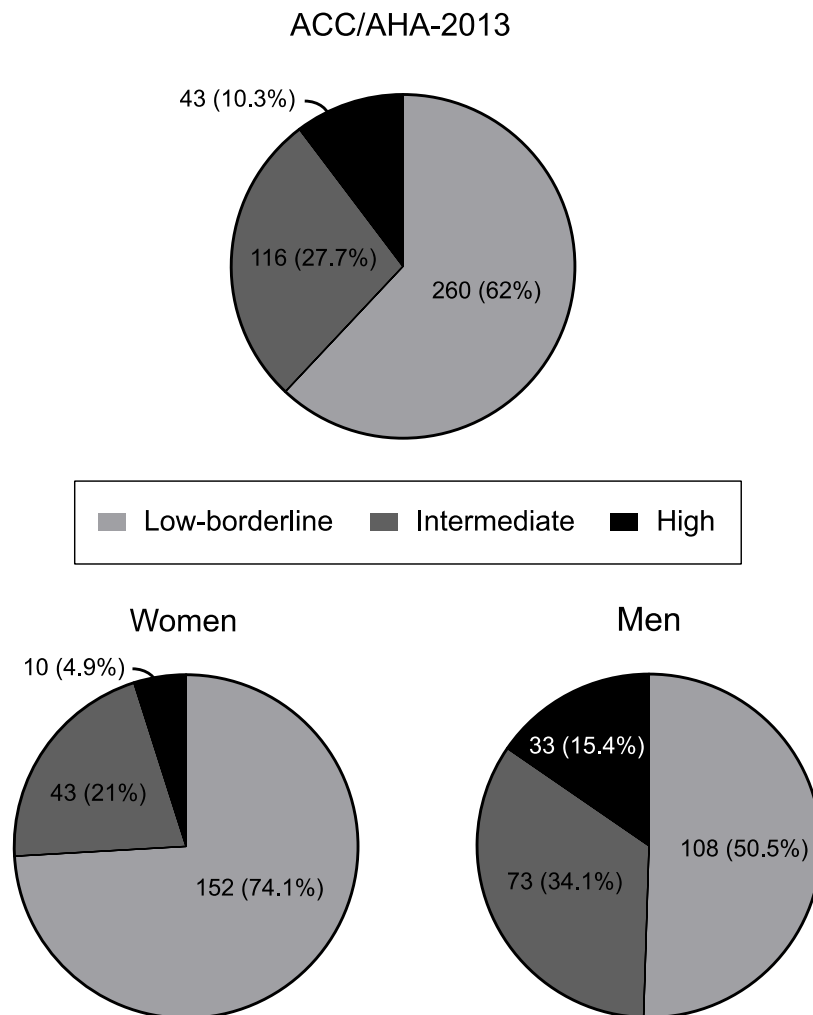
Supplementary Figure 3. Performance of Steno-Risk 10-year CVD absolute risk values for the presence and number of plaques in the whole cohort (n=501).



Receiver operating characteristic (ROC) curves with p values for every area under the curve (AUC) are reported and a diagonal reference line is shown.

Left: plaque presence detection for Steno-Risk (continuous variable; absolute 10-year CVD risk value). Right: detection of two or more carotid plaques for Steno-Risk (continuous variable; absolute 10-year CVD risk value).

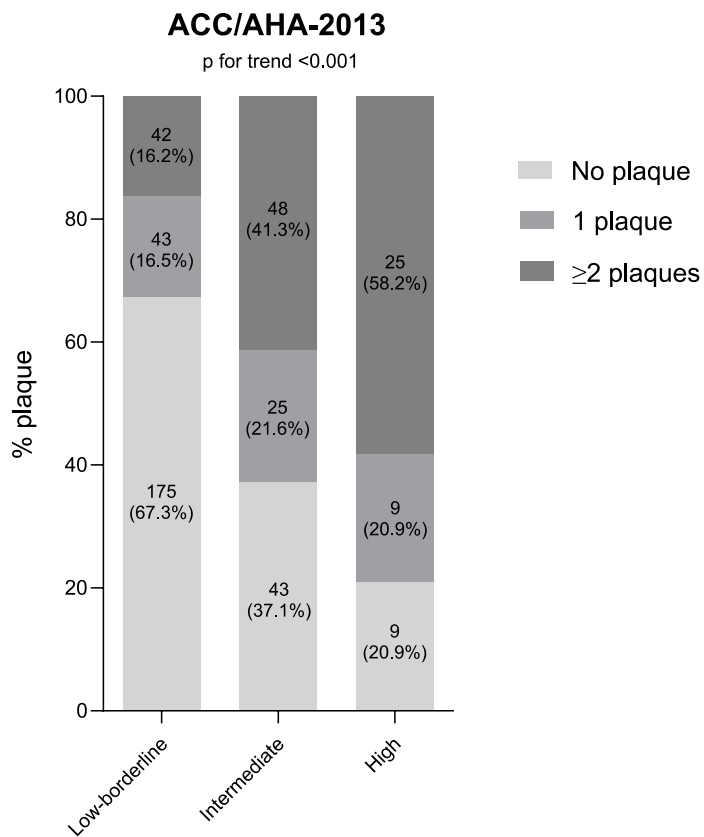
Supplementary Figure 4. Distribution of the study subjects according to the ACC/AHA-2013 pooled cohort equation categories.



Data are shown as n (percentage).

Only n=419 participants between 40-79 years were included.

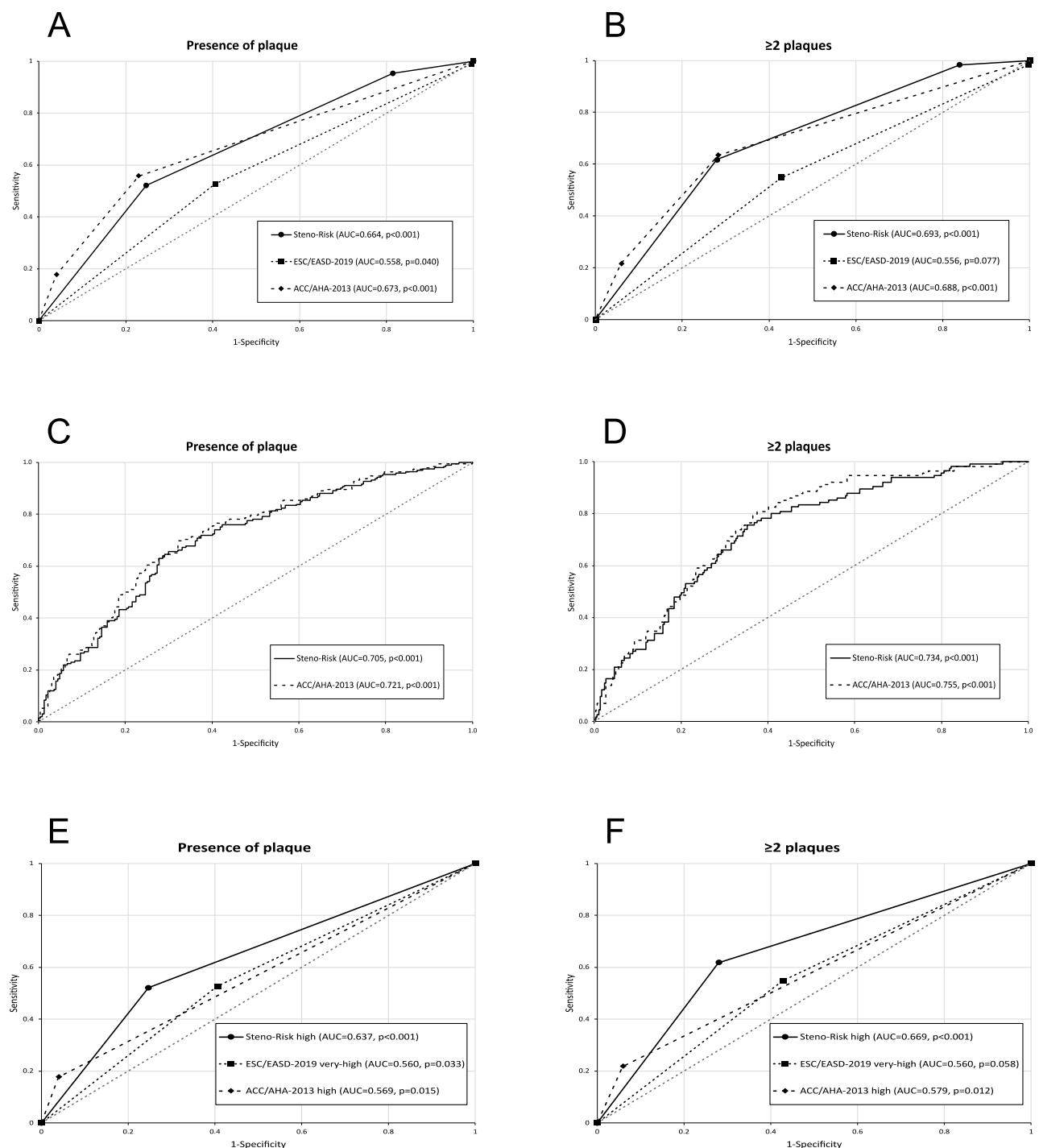
Supplementary Figure 5. Relationships between cardiovascular risk categories and preclinical atherosclerosis for the ACC/AHA-2013 pooled cohort equation.



Data is shown as n (percentage).

Only n=419 participants between 40-79 years were included.

Supplementary Figure 6. Analyses of the performance of all three risk estimation strategies for the detection of preclinical atherosclerosis.



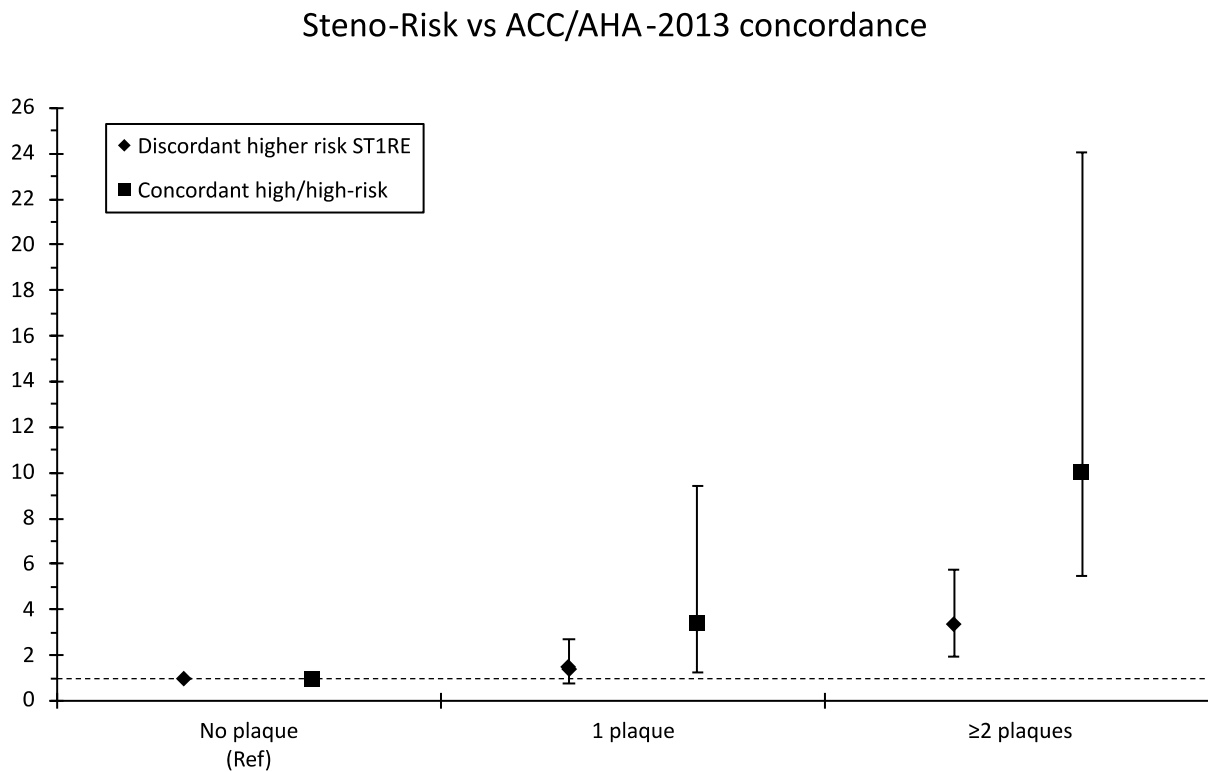
Receiver operating characteristic (ROC) curves with *p* values for every area under the curve (AUC) are reported and a diagonal reference line is shown.

n=82 with <40 years of age were excluded from all ROC curves because the 10-year cardiovascular risk with the ACC/AHA-2013 pooled cohort equation cannot be applied

A: plaque presence detection for Steno-Risk (low, moderate, high risk), ESC/EASD-2019 (moderate, high, very-high risk), and ACC/AHA-2013 (low-borderline, intermediate and high risk). B: detection of two or more carotid plaques for Steno-Risk (low, moderate, high risk), ESC/EASD-2019 (moderate, high, very-high risk),

and ACC/AHA-2013 (low-borderline, intermediate and high risk). C: plaque presence for Steno-Risk and ACC/AHA-2013 using the absolute risk value (continuous variable). D: detection of two or more carotid plaques for Steno-Risk and ACC/AHA-2013 using the absolute risk value (continuous variable). E: plaque presence detection for Steno-Risk high risk (n=156), ESC/EASD-2019 very-high risk (n=194), and ACC/AHA-2013 high risk (n=43). F: detection of two or more carotid plaques for Steno-Risk high risk (n=156), ESC/EASD-2019 very-high risk (n=194), and ACC/AHA-2013 high risk (n=43).

Supplementary Figure 7. Associations of the discordance in estimated risk between Steno-Risk and ACC/AHA-2013 pooled cohort equation with preclinical atherosclerosis by polynomial regression analysis.



Data is shown as odds ratio [95% confidence interval]. No plaque group comparisons are used as reference. Presence of 1 plaque: discordant higher risk ST1RE (OR 1.421 [0.752-2.685], $p=0.279$); concordant high/high-risk (OR 3.4 [1.225-9.437], $p=0.019$). Presence of 2 or more plaques: discordant higher risk ST1RE (OR 3.333 [1.930-5.756], $p<0.001$); concordant high/high-risk (OR 10.055 [4.207-24.030], $p<0.001$).

$n=82$ with <40 years of age were excluded because the 10-year cardiovascular risk with the ACC/AHA-2013 pooled cohort equation cannot be applied.

There was $n=0$ with discordance towards lower risk with Steno-Risk and higher with ACC/AHA-2013.

Concordant high/high risk refers to high risk in Steno-Risk and high risk in ACC/AHA-2013. Discordant higher risk ST1RE refers to high risk in Steno-Risk and low, borderline or intermediate risk in ACC/AHA-2013 equation.

Polynomial regression models adjusted per triglycerides, statins and antiplatelet drugs. ST1RE: Steno Type 1 Risk Engine (Steno-Risk).

ARTÍCULO ORIGINAL 3: LA ADHERENCIA A UNA DIETA MEDITERRÁNEA RESTRINGIDA EN CALORÍAS ESTÁ ASOCIADA CON LA PRESENCIA Y LA CARGA DE ATEROSCLEROSIS CAROTÍDEA EN PERSONAS CON DIABETES TIPO 1

Objetivos: Las personas DTI tienen un mayor riesgo de ECV. La dieta mediterránea está asociada con la reducción de este riesgo, sin embargo, la evidencia en DTI es escasa. Nuestro objetivo fue analizar las relaciones entre la adherencia a la dieta mediterránea restringida en calorías (erMEDd) y la aterosclerosis carotídea.

Métodos: Incluimos sujetos con DTI sin ECV, con al menos una de las siguientes condiciones: edad ≥ 40 años, ERD, o ≥ 10 años de duración de la enfermedad con otro FRCV. La presencia de placas (grosor íntima-media $\geq 1,5$ mm) se determinó mediante ecografía. Se utilizó el cuestionario de 17 ítems de PREDIMED-Plus (PP-17) para evaluar la adherencia a la erMEDd.

Resultados: Se incluyeron 401 individuos (48% hombres, edad $48,3 \pm 11$ años, duración de la diabetes $26,8 \pm 11,4$ años). Aquellos con placas (42%) mostraron una menor adherencia a la erMEDd (PP-17: $8,9 \pm 2,3$ de un máximo de 17 vs. $9,8 \pm 2,5$, $p < 0,001$). Una mayor adherencia a la erMEDd se correlacionó con un mejor perfil metabólico en general. Después de ajustar por múltiples factores de confusión, la adherencia a la erMEDd se asoció independientemente con la aterosclerosis carotídea (OR 0,86 [0,77-0,95] para la presencia de placas y OR 0,85 [0,75-0,97] para ≥ 2 placas). El consumo de frutas y nueces y la preferencia por la carne blanca sobre la roja fue mayor en individuos sin aterosclerosis ($p < 0,05$). El consumo de frutas y nueces se asoció con una menor prevalencia de placas en los modelos ajustados (OR 0,38 [0,19-0,73] y 0,51 [0,29-0,93]).

Conclusiones: Una mayor adherencia a la erMEDd está asociada con menos aterosclerosis carotídea en sujetos con DTI con alto riesgo de ECV. Se deben fomentar estrategias para mejorar e implementar patrones dietéticos saludables en esta población.

RESEARCH ARTICLE

Adherence to an energy-restricted Mediterranean diet is associated with the presence and burden of carotid atherosclerosis in people with type 1 diabetes

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Abstract

Aims: People with type 1 diabetes (T1D) have an increased risk of cardiovascular disease (CVD). The Mediterranean diet is associated with reduced CVD; however, the evidence in T1D is scarce. We aimed to analyse the relationships between adherence to the energy-restricted Mediterranean diet (erMEDd) and carotid atherosclerosis.

Materials and Methods: We included subjects with T1D without CVD, with ≥ 1 of the following: age ≥ 40 years, diabetic kidney disease, or ≥ 10 years of disease duration with another risk factor. Plaque presence (intima-media thickness ≥ 1.5 mm) was determined by ultrasonography. The PREDIMED-Plus 17-item questionnaire (PP-17) was used to assess adherence to the erMEDd.

Results: Four hundred one individuals were included (48% males, age 48.3 ± 11 years, diabetes duration 26.8 ± 11.4 years). Those harbouring plaques (42%) showed lower adherence to the erMEDd (PP-17: 8.9 ± 2.3 of a maximum of 17 vs. 9.8 ± 2.5 , $p < 0.001$). Greater adherence to the erMEDd was correlated with an overall better metabolic profile. After adjusting for multiple confounders, adherence to the erMEDd was independently associated with carotid atherosclerosis (OR 0.86 [0.77–0.95] for plaque presence and OR 0.85 [0.75–0.97] for ≥ 2 plaques). The consumption of fruit and nuts and preference of white over red meat was higher in individuals without atherosclerosis ($p < 0.05$). Fruit and nut consumption was associated with lower plaque prevalence in the fully adjusted models (OR 0.38 [0.19–0.73] and 0.51 [0.29–0.93]).

Conclusions: Greater adherence to the erMEDd is associated with less carotid atherosclerosis in subjects with T1D at high risk of CVD. Strategies to improve and implement healthy dietary patterns in this population should be encouraged.

KEYWORDS

atherosclerosis, cardiovascular disease, carotid plaque, Mediterranean diet, PREDIMED, type 1 diabetes

1 | INTRODUCTION

Cardiovascular disease (CVD) is currently the leading cause of death in people with type 1 diabetes (T1D).^{1–3} In the past, the prevailing cardiovascular risk factor was poor glycaemic control, with the proven benefit of intensive diabetes treatment.⁴ However, we now know that even those with optimal glycaemic control have a 3-fold excess risk compared to non-diabetic matched controls.⁵ Although classical risk factors play an important role in cardiovascular risk in T1D,⁶ the high residual risk seen in this population remains unexplained. This suggests that other extra-glycaemic factors are involved.^{7–9} One of these factors could be the diet composition. To date, there are no randomized clinical trials (RCT) in the T1D population that support a specific eating pattern.¹⁰ However, patterns, such as the Mediterranean diet do have ample evidence of their benefits in other populations. In primary and secondary prevention RCTs, increased adherence to the Mediterranean dietary pattern has been shown to significantly reduce major cardiovascular events.^{11–13} In people with T1D, greater adherence to the Mediterranean diet has been associated with improved glycaemic control^{14,15} and various metabolic parameters.¹⁶ However, little information is available on the relationship of the Mediterranean diet with atherosclerosis and CVD.^{17,18} In this regard, our aim was to analyse the associations between adherence to the energy-restricted Mediterranean diet (erMEDd)^{19,20} assessed through the 17-item energy-restricted Mediterranean Adherence Screener (PP-17) and atherosclerosis measured by carotid ultrasound in people with T1D at high cardiovascular risk.

2 | MATERIALS AND METHODS

2.1 | Participants

This cross-sectional study was conducted to examine T1D individuals without any previous CVD (such as coronary artery disease, ischaemic stroke, peripheral artery disease, or heart failure). The participants were recruited from a specialised Diabetes Unit at the Hospital Clinic in Barcelona, Spain. To be included in the study, all participants had to meet the following criteria: ≥ 40 years of age, have diabetic kidney disease (any stage), and/or a duration of T1D of ≥ 10 years with one or more additional risk factors for CVD. Details can be found in the Supporting Information S2.

2.2 | Clinical and laboratory parameters

All participants provided demographic and clinical data, including a history of microvascular complications, duration of T1D, family history of premature CVD in first-degree relatives, regular physical activity (more than 30 min per day or 3.5 h per week), and medical treatment. High-intensity statin therapy was defined as taking a statin that reduces low-density lipoprotein cholesterol (LDL-c) $\geq 50\%$. As a standardized parameter for statin treatment, we calculated a

statin score,²¹ estimation of lifetime exposure to these drugs, which is calculated as the product of the average received statin dose adjusted to simvastatin (in milligrams per day) for the duration of treatment (in years).

Diabetic retinopathy was assessed by examination of the fundus oculi, which was verified by an ophthalmologist. The albumin-to-creatinine ratio was used to evaluate diabetic kidney disease, with < 30 mg/g considered normal and ≥ 30 mg/g as pathological (verified in at least two out of three consecutive assessments). Treatment with angiotensin-converting enzyme inhibitors or angiotensin II receptor blockers with no prior history of hypertension or CVD was also considered as a diabetic kidney disease.

Weight, height and waist circumference were also recorded. Weight was measured using a calibrated electronic scale with the subject wearing light clothing and barefoot. The body mass index (BMI) was obtained by dividing weight in kilograms by height in square meters, and waist circumference was measured halfway between the last rib and the iliac crest.

The collected samples included blood withdrawn after overnight fasting and first-morning urine. Standardized assays were used to analyse lipid profiles (including total cholesterol, triglycerides, and high-density lipoprotein cholesterol [HDL-c]), glucose, creatinine, and the albumin-to-creatinine ratio. Low-density lipoprotein cholesterol [LDL-c] was calculated using the Friedewald formula. Non-HDL cholesterol was determined as the subtraction of HDL-c from total cholesterol. The estimated glomerular filtration rate (eGFR) was obtained using the Chronic Kidney Disease Epidemiology Collaboration equation.²² Glycated haemoglobin (HbA1c) values (Tosoh G8 Automated HPLC Analyser -Tosoh Bioscience Inc., South San Francisco, CA, USA—DCCT aligned, normal range 4%–6%, [20–42 mmol/mol]) were also recorded. Insulin sensitivity was assessed with the estimated glucose disposal rate (eGDR) formula, a well-validated score in T1D that is expressed in mg/kg/min^{23,24} and calculated as follows: $24.31 - 12.22 \times \text{waist-to-hip ratio} - 3.29 \times \text{hypertension}$ (0 = no, 1 = yes) $- 0.57 \times \text{HbA1c (in \%)}$. Lastly, the fatty liver index (FLI) was calculated as described previously.²⁵

2.3 | Carotid B-mode ultrasound imaging

Carotid atherosclerosis was assessed using a standardized pre-defined protocol as described previously.^{26,27} For more information, see the Supporting Information S2.

2.4 | Evaluation of adherence to the energy-restricted Mediterranean diet

Adherence to the erMEDd was assessed with the validated 17-item questionnaire (PP-17, [Table S1]) used in the intervention group of the ongoing PREDIMED-Plus clinical trial.^{19,20} The questionnaire has a maximum score of 17 points (maximum adherence) and, based on the literature,²⁸ is classified according to approximate tertiles: low

(≤ 7), medium (8–10) and high (11–17). The questionnaire was self-administered on the same day the carotid ultrasound was performed. Compared to the energy-unrestricted 14-item questionnaire used in the PREDIMED study,¹² in addition to assessing the degree of adherence to the Mediterranean diet, the PP-17 emphasises the restriction of certain foods (e.g., refined grains or processed red meat) to reduce the total energy intake.

2.5 | Statistical analysis

Descriptive analysis of the data was performed. The Shapiro-Wilk test and visual analyses of P-P plots were used to assess the normal distribution of continuous variables. For comparisons, appropriate tests were used according to the type of variable (quantitative or qualitative, parametric or nonparametric). Correlations between continuous variables (laboratory and clinical parameters with PP-17 score) were assessed with the pairwise Spearman test (r_s).

To search for independent relationships between carotid atherosclerosis (plaque presence or 2 or more plaques; dependent variable) and adherence to the erMEDd (PP-17 score and individual item data; independent variable), we constructed logistic binary regression models. The first model was sex- and age-adjusted. Model 2 was adjusted for general cardiovascular risk factors including age, sex, statin score, smoking habit, presence of hypertension, BMI, serum non-HDL cholesterol, HDL-c, triglycerides and regular physical activity. Finally, predictors that achieved significance in either of the previous models were further analysed in a third model, which was adjusted for the variables included in model 2 and other T1D-specific risk factors (mean HbA_{1c}, diabetes duration, presence of diabetic kidney disease or retinopathy).

The analyses were performed using SPSS® Statistics v.26 (IBM Corporation) statistical software. The significance level was set at p -value < 0.05 .

3 | RESULTS

3.1 | Subject characteristics

A total of 401 subjects were included (48% males, mean age 48.3 ± 11 years, mean duration of diabetes 26.8 ± 11.4 years, mean HbA_{1c} $7.6 \pm 0.9\%$ [60 mmol/mol]). Globally, 11% had a family history of premature CVD, 44% had never smoked, 22% had hypertension, 30% practised regular physical activity, 15% had obesity, 8% had diabetic kidney disease and 36% had retinopathy. Thirty-four percent were on statin treatment. Overall, $n = 173$ (42%) had carotid plaques ($n = 103$ had ≥ 2 plaques) and were more frequently males, older, active, or former smokers, and had a greater prevalence of hypertension, obesity and cardioprotective drug treatment. They also had significantly worse lipid, renal and glycaemic control serum parameters ($p < 0.05$ for all; Table 1).

Baseline characteristics according to sex and age were also assessed. In brief, men more frequently practised regular physical activity, had a greater BMI (despite similar obesity prevalence), were less frequently on continuous subcutaneous insulin infusion (CSII) therapy, were more often receiving statins and had higher triglyceride and lower HDL-c serum levels ($p < 0.05$ for all, Table S2). Conversely, patients >50 years old had a higher prevalence of hypertension, greater waist circumference, a longer diabetes duration, higher HDL-c and triglycerides, a lower eGFR, and were more frequently on cardioprotective drug therapy ($p < 0.05$ for all; Table S3).

3.2 | Adherence to the energy-restricted Mediterranean diet and its relationship with other clinical and laboratory variables

Overall, the PP-17 score was 9.4 ± 2.4 (of a maximum of 17 points). Analysis of the individual item data (available for $n = 321$) showed that the highest percentage of positive responses ($>70\%$) were those related to the use of olive oil for cooking as opposed to other fats such as butter, margarine or cream (items 1 and 5), lower consumption of sugary soft drinks, pastries and sugar added to beverages (items 6, 9, 13) and higher consumption of white meat such as chicken, turkey, or rabbit instead of red meat (item 11). On the other hand, the items with the lowest number of positive responses ($<30\%$) were those related to fruit consumption (item 3) and wine consumption (item 17; Figure 1).

The PP-17 score was positively correlated with eGDR ($r_s = 0.218$, $p < 0.001$) and HDL-c ($r_s = 0.165$, $p = 0.001$) and negatively correlated with BMI ($r_s = -0.132$, $p = 0.007$), waist circumference ($r_s = -0.207$, $p < 0.001$), FLI ($r_s = -0.168$, $p = 0.010$), fasting glucose ($r_s = -0.128$, $p = 0.010$) and HbA_{1c} ($r_s = -0.1$, $p = 0.047$). Several parameters showed a trend consistent with expectations (negatively with systolic blood pressure, triglycerides, LDL-c and eGFR) although the magnitude of the correlation was very weak ($r_s < 0.1$).

3.3 | Associations between carotid atherosclerosis and adherence to the energy-restricted Mediterranean diet

The PP-17 score was significantly lower among those harbouring carotid plaques (8.9 ± 2.3 vs. 9.8 ± 2.5 , $p < 0.001$). The percentage of positive responses among those without carotid plaques was higher in 12 of the 17 items (Figure 1). This difference was significant in the items related to fruit consumption (item 3; 31% vs. 21%, $p = 0.047$), nut consumption (item 10; 51% vs. 38%, $p = 0.022$) and preference of white over red meat (item 11; 80% vs. 67%, $p = 0.005$).

Regression analyses adjusted for age and sex (model 1) and for non-specific T1D cardiovascular risk factors (model 2) showed a consistent association of adherence to the erMEDd with the presence and extent of carotid atherosclerosis (Table 2). After adding

TABLE 1 Characteristics of the study participants according to the presence of carotid plaque.

	Whole (n = 401)	No plaque presence (n = 237)	Plaque presence (n = 173)	p-value
Clinical characteristics				
Sex (male)	197 (48)	101 (43)	96 (56)	0.010
Age (years)	48.3 (11)	43.9 (9.6)	54.3 (9.9)	<0.001
Premature CVD in first-degree relatives ^a	46 (11)	27 (11)	19 (11)	0.897
Never smokers	182 (44)	120 (51)	62 (36)	0.003
Regular physical activity ^b	124 (30)	78 (33)	46 (27)	0.169
Hypertension	92 (22)	34 (14)	58 (34)	<0.001
SBP (mmHg)	128 (15)	124 (14)	133 (15)	<0.001
DBP (mmHg)	82 (8)	82 (8)	83 (8)	0.568
BMI (kg/m ²)	26 (4.3)	25.6 (4.1)	26.5 (4.5)	0.037
Obesity (BMI ≥ 30 kg/m ²)	61 (15)	26 (11)	35 (20)	0.009
Waist circumference (cm)	91 (13)	88 (12)	94 (14)	<0.001
Female (cm)	86 (12)	84 (11)	88 (14)	0.017
Male (cm)	96 (11)	94 (10)	98 (10)	0.016
T1D duration (years)	26.8 (11.4)	25.1 (10.6)	29.3 (12.1)	<0.001
Diabetic kidney disease	31 (8)	13 (6)	18 (10)	0.063
Diabetic retinopathy	149 (36)	79 (33)	70 (41)	0.138
CSII Therapy	108 (26)	65 (27)	43 (25)	0.559
Conventional lipid profile				
Total cholesterol (mg/dl)	187 (30)	185 (29)	189 (30)	0.236
HDL-cholesterol (mg/dl)	62 (16)	62 (16)	61 (15)	0.222
LDL-cholesterol (mg/dl)	108 (25)	107 (24)	110 (26)	0.223
Triglycerides (mg/dl)	76 (61–101)	70 (58–93)	81 (66–111)	<0.001
Non-HDL cholesterol (mg/dl)	125 (27)	123 (25)	128 (29)	0.043
Other laboratory characteristics				
Fasting plasma glucose (mg/dl)	156 (64)	151 (66)	164 (61)	0.033
Haemoglobin A1c (% (mmol/mol))	7.6 (0.9), (60)	7.4 (0.9), (57)	7.7 (0.9), (61)	0.003
Serum creatinine (mg/dl)	0.88 (0.16)	0.86 (0.15)	0.90 (0.18)	0.007
eGFR (CKD-EPI; ml/min/1.73 m ²)	91 (15)	94 (13)	86 (15)	<0.001
ALT (UI/L)	21 (10)	21 (11)	22 (10)	0.921
Albumin-to-creatinine ratio	4 (2–8)	3 (2–6)	4 (2–11)	0.001
Pharmacological treatment				
Statins	140 (34)	60 (25)	80 (46)	<0.001
ACEi/ARB	91 (22)	35 (15)	56 (32)	<0.001
Antiplatelet drugs	27 (7)	7 (3)	20 (12)	0.001

Note: Data are shown as n (percentage within each column), mean (SD) or median (Q1–Q3). *p* values for group comparisons are reported.

Abbreviations: ACEi, angiotensin-converting enzyme inhibitor; ALT, alanine aminotransferase; ARB, angiotensin receptor blocker; CSII, continuous subcutaneous insulin infusion; CVD, cardiovascular disease; DBP, diastolic blood pressure; eGFR, estimated glomerular filtration rate; HDL, high density lipoprotein; LDL, low density lipoprotein; SBP, systolic blood pressure; T1D, type 1 diabetes.

^aDefined as <55 years in men and <65 years in women.

^bDefined as >3.5 h per week.

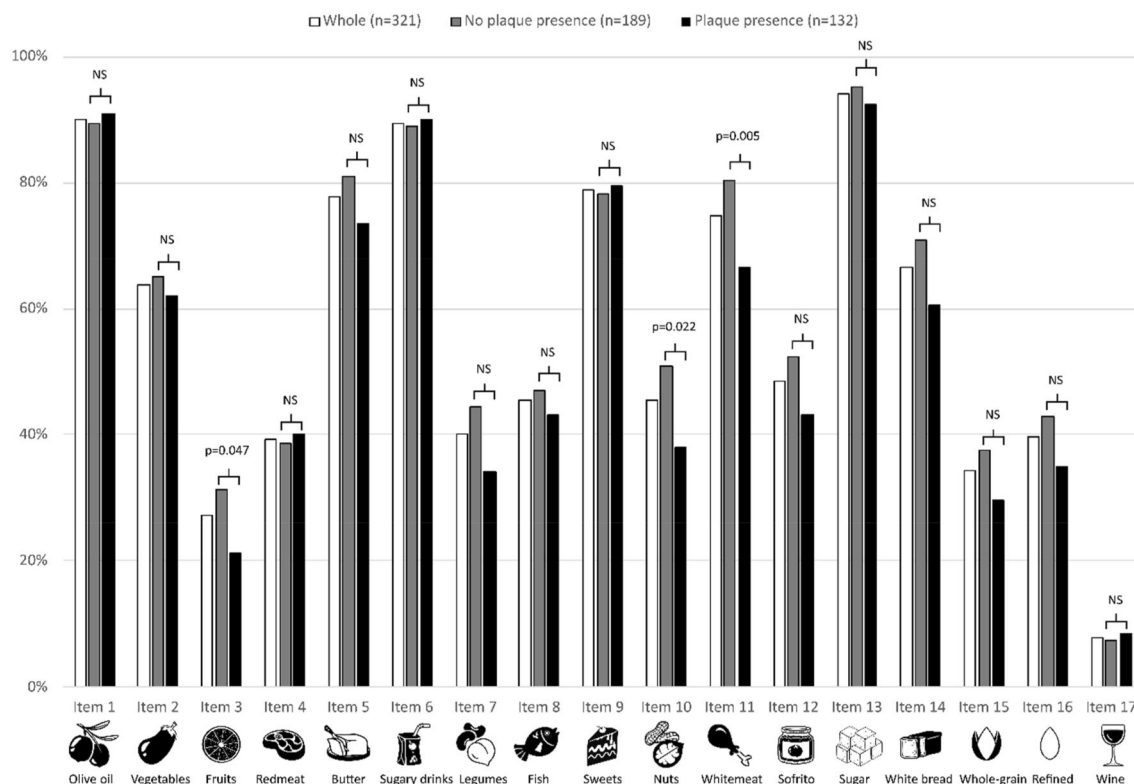


FIGURE 1 The percentage of positive answers to individual items according to carotid plaque presence. Individual item data were available for $n = 321$ ($n = 189$ without plaque presence and $n = 132$ with plaque presence). p -values between groups (no plaque vs. plaque presence) are reported. non-significant.

TABLE 2 Associations between adherence to the erMEDd and carotid atherosclerosis: logistic regression analysis.

	Model 1 ^a		Model 2 ^b	
	OR (95% CI)	p-value	Or (95% CI)	p-value
Presence of carotid plaque				
Grouped score	0.86 (0.78–0.96)	0.004	0.86 (0.78–0.96)	0.006
Item 1	1.42 (0.63–3.23)	0.400	1.78 (0.74–4.26)	0.196
Item 2	1.06 (0.61–1.85)	0.825	1.12 (0.63–2.00)	0.708
Item 3	0.45 (0.24–0.83)	0.010	0.41 (0.22–0.78)	0.006
Item 4	0.94 (0.54–1.62)	0.820	0.85 (0.48–1.50)	0.574
Item 5	0.70 (0.38–1.30)	0.259	0.68 (0.37–1.27)	0.227
Item 6	1.05 (0.45–2.42)	0.916	0.98 (0.42–2.29)	0.958
Item 7	0.63 (0.36–1.07)	0.088	0.61 (0.35–1.06)	0.081
Item 8	0.93 (0.55–1.56)	0.786	0.88 (0.51–1.52)	0.647
Item 9	1.14 (0.60–2.19)	0.686	1.07 (0.55–2.09)	0.836
Item 10	0.55 (0.32–0.94)	0.028	0.57 (0.33–0.99)	0.050
Item 11	0.57 (0.31–1.04)	0.067	0.53 (0.28–0.99)	0.049
Item 12	0.66 (0.39–1.11)	0.116	0.66 (0.38–1.14)	0.133
Item 13	0.71 (0.24–2.05)	0.520	0.56 (0.19–1.69)	0.303
Item 14	1.09 (0.62–1.89)	0.770	1.12 (0.63–1.98)	0.697
Item 15	0.80 (0.45–1.41)	0.432	0.90 (0.49–1.62)	0.714

(Continues)

TABLE 2 (Continued)

	Model 1 ^a		Model 2 ^b	
	OR (95% CI)	p-value	Or (95% CI)	p-value
Item 16	0.89 (0.52–1.55)	0.684	0.96 (0.55–1.69)	0.890
Item 17	0.71 (0.28–1.82)	0.482	0.80 (0.31–2.12)	0.657
Presence of 2 or more carotid plaques				
Grouped score	0.85 (0.75–0.96)	0.007	0.85 (0.75–0.97)	0.017
Item 1	0.96 (0.38–2.44)	0.926	1.23 (0.44–3.42)	0.697
Item 2	0.70 (0.37–1.35)	0.289	0.73 (0.36–1.48)	0.382
Item 3	0.46 (0.22–0.98)	0.044	0.42 (0.19–0.94)	0.037
Item 4	0.73 (0.38–1.42)	0.356	0.68 (0.33–1.40)	0.295
Item 5	0.62 (0.31–1.27)	0.193	0.54 (0.25–1.14)	0.106
Item 6	0.68 (0.26–1.82)	0.445	0.58 (0.21–1.62)	0.295
Item 7	0.47 (0.24–0.93)	0.029	0.41 (0.20–0.85)	0.017
Item 8	0.85 (0.45–1.58)	0.602	0.72 (0.36–1.42)	0.340
Item 9	0.82 (0.38–1.79)	0.629	0.65 (0.28–1.48)	0.299
Item 10	0.62 (0.33–1.18)	0.145	0.79 (0.40–1.59)	0.515
Item 11	0.42 (0.21–0.85)	0.015	0.36 (0.17–0.78)	0.009
Item 12	0.62 (0.33–1.16)	0.132	0.51 (0.25–1.02)	0.056
Item 13	1.23 (0.37–4.16)	0.735	0.86 (0.23–3.15)	0.815
Item 14	0.87 (0.46–1.65)	0.673	0.83 (0.42–1.65)	0.590
Item 15	1.03 (0.52–2.03)	0.935	1.26 (0.60–2.66)	0.547
Item 16	1.17 (0.60–2.26)	0.644	1.35 (0.67–2.71)	0.400
Item 17	0.94 (0.33–2.73)	0.916	1.05 (0.33–3.37)	0.929

Note: A logistic regression model (OR and 95% confidence interval) is presented. Individual item data were available for $n = 321$ ($n = 189$ without plaque presence and $n = 132$ with plaque presence). Item description [criteria to score 1 point]: 1. Do you use only extra-virgin olive oil for cooking, salad dressings, and spreads? [Yes]; 2. How many servings of vegetables/garden produce do you consume per day? [≥ 2 (≥ 1 portion raw or in a salad)]; 3. How many fruit units (including natural fruit juices) do you consume per day? [≥ 3]; 4. How many servings of red meat, hamburgers, or meat products do you consume per week? [≤ 1]; 5. How many servings of butter, margarine, or cream do you consume per week? [< 1]; 6. How many sugary beverages or sugar-sweetened fruit juices do you drink per week? [< 1]; 7. How many servings of legumes do you consume per week? [≥ 3]; 8. How many servings of fish or shellfish do you consume per week? [≥ 3]; 9. How many times per week do you consume commercial sweets or pastries (not homemade), such as cakes, cookies, sponge cake, or custard? [< 3]; 10. How many servings of nuts (including peanuts) do you consume per week? [≥ 3]; 11. Do you preferentially consume chicken, turkey, or rabbit instead of beef, pork, hamburgers, or sausages? [Yes]; 12. How many times per week do you consume vegetables, pasta, rice, or other dishes seasoned with sofrito (sauce made with tomato and onion, leek, or garlic simmered in olive oil)? [≥ 2]; 13. Do you add sugar to beverages (coffee, tea)? [No]; 14. How many servings of white bread do you consume per day? [≤ 1]; 15. How many times per week do you consume whole-grain cereals and pasta? [≥ 5]; 16. How many times per week do you consume refined grain pasta or white rice? [< 3]; 17. How many glasses of wine do you drink per day? [two to three for men; one to two for women].

^aModel 1: adjusted for sex and age.

^bModel 2: adjusted for sex and age, the presence of hypertension, smoking status (never vs. current or smoker), regular physical activity (> 3.5 h per week), body mass index, non-HDL cholesterol, HDL-cholesterol, triglycerides and statin score.

various T1D-specific cardiovascular risk factors to the model (model 3), greater adherence to the erMEDd remained associated with a lower probability of both atherosclerosis and a lower atherosclerotic burden (OR 0.86 [0.77–0.95], $p = 0.005$ for plaque presence and OR 0.85 [0.75–0.97], $p = 0.019$ for ≥ 2 plaques; Figure 2). In subjects for whom individual questionnaire data were available ($n = 321$), positive responses to several of the questionnaire items were also inversely associated with the presence and number of plaques (Table 2 and Figure 2). The consumption of more than three pieces of fruit per day

including natural juice (item 3) showed a significant association in all the models analysed (fully adjusted model: OR 0.38 [0.19–0.73], $p = 0.004$ for plaque presence and OR 0.42 [0.19–0.94], $p = 0.035$ for 2 plaques). Furthermore, consumption of more than 3 servings (30 g) of nuts per week (item 10) was associated with the presence of atherosclerosis (fully adjusted model: OR 0.51 [0.29–0.93], $p = 0.027$). Finally, the intake of more than 3 servings of legumes (dish or 150 g) per week (item 7) and the preference for white meat (chicken, turkey, rabbit) over red meat (beef, pork, hamburger or

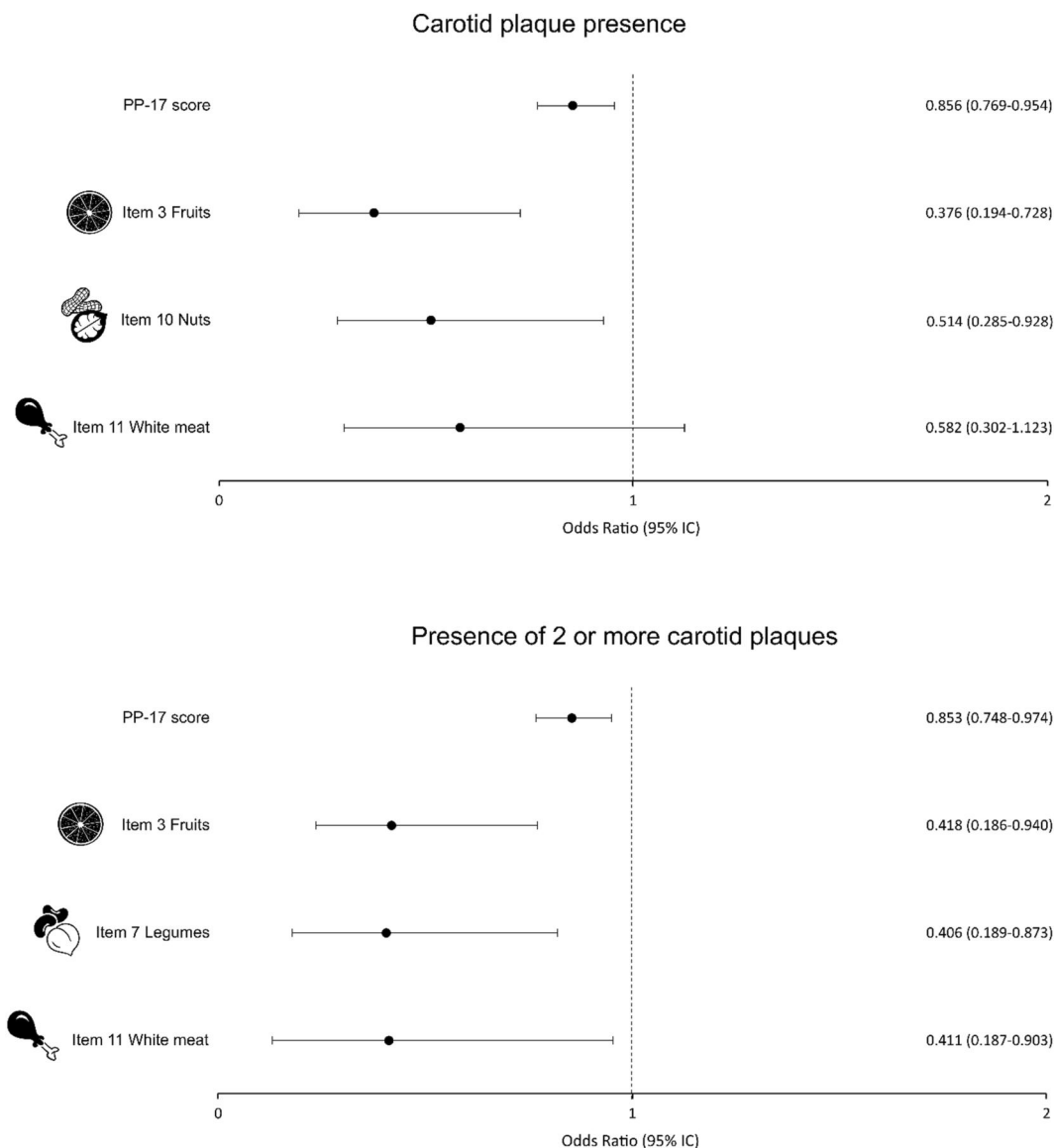


FIGURE 2 Association of adherence to the erMEDd with carotid plaque presence and number: fully adjusted logistic regression model (model 3). A logistic regression model (OR and 95% confidence interval) is presented. Individual item data were available for $n = 321$ ($n = 189$ without plaque presence and $n = 132$ with plaque presence). Model three is adjusted for all model 2 variables (adjusted for sex and age, presence of hypertension, smoking status (never vs. current or former smoker), regular physical activity (>3.5 h per week), body mass index, non-HDL cholesterol, HDL-cholesterol, triglycerides and statin score) and mean HbA_{1c} in the last 5 years, the presence of diabetic kidney disease and/or retinopathy and diabetes duration.

sausage) (item 11) were consistently associated with atherosclerotic burden (≥ 2 plaques) (fully-adjusted model: OR 0.41 [0.19–0.87], $p = 0.021$ and OR 0.41 [0.19–0.90], $p = 0.027$, respectively).

4 | DISCUSSION

The main results of the present study show that adherence to the erMEDd correlates globally with a better metabolic profile and is inversely associated with the presence and number of carotid plaques in people with T1D at high cardiovascular risk. Individual food groups that were associated with atherosclerosis were those related

to the consumption of fruits, nuts, and legumes and a preference for white meat over red meat. To the best of our knowledge, this is one of the first studies to examine the association between adherence to the Mediterranean diet and the occurrence of atherosclerosis and the first analysing a more restrictive version of the Mediterranean diet (erMEDd).

Although the cut-off points for interpreting adherence using the 17-item questionnaire are not standardised based on previous publications by the PREDIMED-Plus research group,²⁸ adherence to erMEDd in our study can be considered as a medium. Despite previously published studies using different questionnaires and, therefore, being not fully comparable, they seem to agree that the degree

of adherence to the Mediterranean diet in the adult population with T1D is in the medium range. Vidal-Peracho et al. reported a medium adherence rate using the 14-item PREDIMED questionnaire. In addition, they found lower adherence in the diabetic group compared with the control group. It should be noted that the analyses were performed comparing the group with diabetes (the vast majority with type 2 diabetes) with the control group; therefore, the results cannot be extrapolated to our study population. Furthermore, the control group was significantly older than the group with diabetes, which implies a selection bias, and thus the results should be interpreted with caution.²⁹ On the other hand, a multicentre study conducted in our same region (Catalonia, Spain) in people with T1D found greater adherence to the Mediterranean diet compared to healthy people matched for age and sex using the alternate Mediterranean Diet Score questionnaire. Also, having T1D, being physically active, being older, female, and living in a rural/semi-rural environment (only for the group with T1D) were all associated with greater adherence to the Mediterranean diet in the adjusted regression analyses. In populations with T1D from a non-Mediterranean region,^{30,31} the results regarding the eating pattern are discordant, which suggests that it depends to a great extent on other factors such as the culture of each region.

Our analyses showed that greater adherence to the eMEDd in the whole sample was correlated with an overall favourable metabolic profile (higher eGDR and HDL-c and lower BMI, waist circumference, FLI, fasting glucose, and HbA1c). Similar results have been found in cross-sectional analyses in the PREDIMED-Plus study,²⁸ in which higher adherence to eMEDd was associated with lower triglyceride levels, BMI and waist circumference. Notably, the sample consisted of people aged 55–75 years who had overweight or obesity and <30% had associated type 2 diabetes. Furthermore, several meta-analyses of RCTs support the use of the Mediterranean diet as one of the best strategies for good glycaemic and metabolic control in people with type 2 diabetes.^{32,33} Although the evidence in people with T1D is scarce, previously published data are in line with our findings. Despite a recent meta-analysis³⁴ conducted on people with T1D with overweight or obesity showing benefits in multiple metabolic variables, it failed to find significant differences in weight control or metabolic syndrome components between the Mediterranean diet and other dietary patterns such as the low-fat diet, intermittent fasting or continuous calorie restriction. The results should be interpreted with caution as four studies were included and only two were RCTs. Two cross-sectional studies^{14,15} also found an association between adherence to the Mediterranean diet and better glycaemic control in children and adolescents with T1D. Another study conducted in youth with T1D¹⁶ showed the benefit of a number of dietary habits typical of the Mediterranean diet (consumption of dairy products at breakfast, extra virgin olive oil or fish) with lower HbA1c and blood pressure.

Focusing on cardiovascular events and atherosclerosis in the non-T1D population, experimental and observational evidence indicates a consistent benefit of the Mediterranean diet on cardiovascular events, with a less clear benefit on mortality.^{11–13,35–37} The

PREDIMED study¹² was a large RCT that showed about a 30% lower incidence of major cardiovascular events over 4.8 years of follow-up in subjects following a Mediterranean diet with extra virgin olive oil or nuts in comparison to a low-fat diet. It should be noted that the study was conducted in subjects at primary prevention and high cardiovascular risk and that approximately one half of the participants had type 2 diabetes. In the subgroup analysis, participants with diabetes had a beneficial effect on CVD similar to that observed in the total cohort. In addition, in a sub-cohort of the PREDIMED study, IMT regressed in the group following a Mediterranean diet supplemented with nuts, while the control group showed progression and there were no significant differences in the arm following a Mediterranean diet supplemented with extra-virgin olive oil.³⁸

In addition, the effect of an extra-virgin olive oil enriched Mediterranean diet was also evaluated in the CORDIOPREV RCT¹³ in people with established coronary heart disease (54% with type 2 diabetes), showing similar cardiovascular benefits (near 30%) compared to a low-fat diet over a 7-year follow-up period. Also, the CORDIOPREV trial³⁹ consistently showed a reduction in IMT in the group assigned to the Mediterranean diet, whereas the group following a low-fat diet showed no change. Recently, a large ($n = 8116$) cross-sectional study⁴⁰ carried out in our region showed that greater adherence to the Mediterranean diet was associated with less carotid and/or femoral atherosclerosis in subjects without CVD or diabetes and was considered to be at low/moderate cardiovascular risk.

There are currently no RCTs or large observational studies investigating the effect of the Mediterranean diet on CVD in people with T1D. However, there is preliminary evidence of its association with atherosclerosis. An analysis of the Coronary Artery Calcification in Type 1 Diabetes (CACTI) study cohort¹⁷ showed a clear association between increased adherence to the Mediterranean diet and the Dietary Approaches to Stop Hypertension (DASH) diet and reduced pericardial adiposity (as a surrogate marker of CVD), although it failed to demonstrate reduced progression of coronary artery calcification (CAC score) after 6 years of follow-up. Another study⁴¹ in T1D individuals aged 10–30 years from the SEARCH for Diabetes in Youth Study cohort found no association between the Mediterranean diet and arterial stiffness. It is important to mention that both studies were conducted in the United States and that adherence to the Mediterranean diet, as assessed by different questionnaires, was generally rated as low or moderate; therefore, these conclusions may not apply to our population belonging to a Mediterranean region and with a presumably higher degree of adherence to the diet. In contrast, a longitudinal study conducted in Iran¹⁸ including a large sample of subjects with T1D ($n = 1849$) found an independent and consistent association between adherence to the Mediterranean diet and a lower incidence of microvascular complications and CVD. It should be noted that the adherence was analysed dichotomously and using a very conservative cut-off point ($>7/14$ considered 'adherent'). Less than 10% of the sample were considered 'non-adherent' and those with adherence considered medium and high were analysed together, so the results may have been subject to bias. In summary,

the little evidence published to date shows either a neutral or a positive effect of the Mediterranean diet on atherosclerosis and CVD; however, there is also no evidence or reason why the benefits seen in the non-T1D population should not be seen in people with T1D.

Finally, the food groups with a beneficial effect on atherosclerosis were fruit, nuts, legumes, and white meat instead of red meat. Fruit contains a myriad of nutrients that can act synergistically on various mechanisms that can reduce cardiovascular risk, cancer risk, and even all-cause mortality.⁴² On the other hand, a recent umbrella review suggested that nut and seed consumption is associated with lower all-cause mortality, CVD, cancer deaths and diabetes mortality.⁴³ Nuts are an excellent source of polyunsaturated fatty acids in addition to several other beneficial nutrients (i.e. fibre, phytosterols, and polyphenols).⁴⁴ Likewise, high consumption of legumes has been associated with a lower incidence of CVD and coronary disease.⁴⁵ Lastly, the association between red meat consumption and health remains controversial and the underlying mechanisms are unclear. A recent meta-analysis⁴⁶ showed an association between meat consumption and the risk of CVD and diabetes. The magnitude of the effect was greater with the consumption of processed compared to unprocessed meat and was observed mainly in Western countries (in contrast with Eastern countries).

Our study has limitations. First, adherence to the Mediterranean diet was assessed using questionnaires and no biomarkers of dietary adherence were evaluated, which could imply a recall bias or under/over-reporting. Second, the cross-sectional design does not allow inferring causality, and thus, the results must be confirmed in prospective studies. Third, given that the study only included high-risk individuals, extrapolation to other patients with a different cardiovascular profile should be made with caution. However, although age and diabetes duration could differ, the other key characteristics of cardiovascular risk, such as the mean BMI, the prevalence of smoking habit, hypertension, or microvascular complications, were very similar to those of other recent data sets representative of our region.⁴⁷ Fourth, although the PP-17 questionnaire assesses adherence to an energy-restricted Mediterranean diet, no other methods for quantifying caloric intake were used, which should be taken into account when interpreting the results. Nonetheless, the present study has several strengths. On the one hand, it includes a large sample and the data have been carefully collected. On the other hand, carotid plaques were determined by ultrasonography as a proxy of CVD risk.⁴⁸

5 | CONCLUSIONS

Greater global adherence to the *erMEDd* and a greater consumption of fruit, nuts, legumes and white meat instead of red meat are independently associated with the presence and extent of carotid atherosclerosis in subjects with T1D and high cardiovascular risk. Our results should serve to highlight the importance of adopting the

necessary measures to increase and maintain adherence to the Mediterranean diet in this population.

AUTHOR CONTRIBUTIONS

All authors have discussed the results and commented on the final version of the manuscript. Tonet Serés-Noriega, Clara Viñals, Verónica Perea, Alex Mesa, Laura Boswell, Karla Mariaca, Jesús Blanco, Irene Vinagre, Adriana Pané, Camila Milad, Clara Solà, Enric Esmatjes, Ignacio Conge, Marga Giménez and Antonio J. Amor acquired and processed all clinical data; Antonio J. Amor performed the US measurements; Tonet Serés-Noriega and Antonio J. Amor contributed to data analysis and interpretation, wrote, reviewed and edited the manuscript. Antonio J. Amor and Verónica Perea contributed to the study concept and design. Marga Giménez, Verónica Perea, Clara Viñals, Jesús Blanco, Irene Vinagre, Ignacio Conge and Antonio J. Amor supervised the study and participated in data analysis and interpretation. Tonet Serés-Noriega and Antonio J. Amor designed the figures and had final responsibility for the decision to submit for publication. Antonio J. Amor is the guarantor of this work and, as such, had full access to the data in the study and takes responsibility for the integrity and the accuracy of the data analysis.

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CONFLICT OF INTEREST STATEMENT

The authors declare that they have no conflicts of interest.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available on request from the corresponding author. The data are not publicly available due to privacy or ethical restrictions.

ETHICS STATEMENT

All patients provided informed consent, and the study received approval from the Ethical Committee of the hospital (approval number HCB/2020/0195).

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PEER REVIEW

The peer review history for this article is available at <https://www.webofscience.com/api/gateway/wos/peer-review/10.1002/dmrr.3783>.

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SUPPORTING INFORMATION

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Supplementary material corresponding to:

Adherence to an energy-restricted Mediterranean diet is associated with the presence and burden of carotid atherosclerosis in people with type 1 diabetes

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Supplementary Tables number: 3

Supplementary Material and Methods

Participants

Additional risk factors considered in the study included the presence of diabetic retinopathy, active smoking, hypertension (defined as systolic blood pressure of ≥ 140 mmHg and/or diastolic blood pressure of ≥ 90 mmHg, or being under treatment with antihypertensive drugs), a family history of premature CVD in first-degree relatives (defined as any CVD occurring < 65 years in women and < 55 in men), triglyceride levels > 150 mg/dL, low levels of high-density lipoprotein cholesterol (HDL-cholesterol [HDL-c]; < 40 mg/dL in males and < 45 mg/dL in females), the presence of hypoglycemia unawareness (defined as a score > 3 in the Clarke Test, using the validated Spanish version[1], severe hypoglycemia events (defined as an episode of confirmed hypoglycemia requiring external assistance for recovery), in the last 2 years, and a history of preeclampsia/eclampsia in women. Subjects with pancreatic disease or clinical suspicion of latent autoimmune diabetes in adults (LADA) were excluded from the study.

All patients provided informed consent, and the study received approval from the Ethical Committee of the hospital (approval number HCB/2020/0195).

Carotid B-mode ultrasound imaging

Carotid plaques were defined as focal echo-structures encroaching into the arterial lumen with an intima-media thickness (IMT) ≥ 1.5 mm, or by at least 50% of the surrounding IMT value, as measured from the media-adventitia interface to the intima–lumen surface. An expert endocrinologist (A.J.A) performed all the procedures, and IMT measurements were assessed using semiautomatic software.

References

- [1] Jansa M, Quirós C, Giménez M, Vidal M, Galindo M, Conget I. Psychometric analysis of the Spanish and Catalan versions of a questionnaire for hypoglycemia awareness. *Medicina Clínica (English Edition)* 2015;144:440–4. <https://doi.org/10.1016/j.medcle.2015.12.012>.

Supplementary Table S1. Adherence to energy-restricted Mediterranean diet: 17-item questionnaire (adapted from PREDIMED-Plus trial):

Item	Criteria for 1 point
1. Do you use only extra-virgin olive oil for cooking, salad dressings, and spreads?	Yes
2. How many servings of vegetables/garden produce do you consume per day? (serving: 200g, consider side dishes as half a serving)	≥ 2 (≥ 1 portion raw or in a salad)
3. How many fruit units (including natural fruit juices) do you consume per day?	≥ 3
4. How many servings of red meat, hamburgers, or meat products do you consume per week? (serving: 100-150g)	≤ 1
5. How many servings of butter, margarine, or cream do you consume per week? (serving: 12g)	< 1
6. How many sugary beverages or sugar-sweetened fruit juices do you drink per week?	< 1
7. How many servings of legumes do you consume per week? (serving: 150g)	≥ 3
8. How many servings of fish or shellfish do you consume per week? (serving: 100-150g of fish or 4-5 units or 200g of shellfish)	≥ 3
9. How many times per week do you consume commercial sweets or pastries (not homemade), such as cakes, cookies, sponge cake, or custard?	< 3
10. How many servings of nuts (including peanuts) do you consume per week? (serving: 30g)	≥ 3
11. Do you preferentially consume chicken, turkey, or rabbit instead of beef, pork, hamburgers, or sausages?	Yes
12. How many times per week do you consume vegetables, pasta, rice, or other dishes seasoned with sofrito (sauce made with tomato and onion, leek, or garlic simmered in olive oil)?	≥ 2
13. Do you add sugar to beverages (coffee, tea)?	No
14. How many servings of white bread do you consume per day? (serving: 75g)	≤ 1
15. How many times per week do you consume whole-grain cereals and pasta?	≥ 5
16. How many times per week do you consume refined grain pasta or white rice?	< 3
17. How many glasses of wine do you drink per day?	2-3 for men; 1-2 for women

Supplementary Table S2. Characteristics of the study participants according to sex.

	Male (n=197)	Female (n=213)	p-value
Clinical characteristics			
Age (years)	48.1 (10.6)	48.5 (11.3)	0.674
Premature CVD in first-degree relatives*	20 (10)	26 (12)	0.510
Never smokers	84 (43)	98 (46)	0.493
Regular physical activity [†]	79 (40)	45 (21)	<0.001
Hypertension	48 (24)	44 (21)	0.369
SBP (mmHg)	131 (13)	124 (16)	<0.001
DBP (mmHg)	83 (7)	81 (9)	0.033
BMI (kg/m ²)	26.4 (3.7)	25.6 (4.7)	0.046
Obesity (BMI≥30 kg/m ²)	32 (16)	29 (14)	0.467
Waist circumference (cm)	96 (11)	86 (12)	<0.001
T1D duration (years)	26.7 (11.5)	26.9 (11.4)	0.856
Diabetic kidney disease	17 (9)	14 (7)	0.431
Diabetic retinopathy	67 (34)	82 (39)	0.345
CSII therapy	36 (18)	59 (28)	<0.001
Conventional lipid profile			
Total cholesterol (mg/dL)	183 (29)	190 (30)	0.023
HDL-cholesterol (mg/dL)	56 (13)	67 (16)	<0.001
LDL-cholesterol (mg/dL)	110 (26)	107 (24)	0.212
Triglycerides (mg/dL)	78 (63-103)	73 (58-95)	0.046
Non-HDL cholesterol (mg/dL)	127 (28)	123 (26)	0.144
Other laboratory characteristics			
Fasting plasma glucose (mg/dL)	159 (60)	154 (68)	0.406
Haemoglobin A1c (%, (mmol/mol))	7.6 (0.9), (60)	7.5 (0.9), (58)	0.425
Serum creatinine (mg/dL)	0.96 (0.13)	0.80 (0.15)	<0.001
eGFR (CKD-EPI; ml/min/1.73m ²)	93 (13)	89 (16)	0.003
ALT (UI/L)	24 (9)	19 (11)	<0.001
Albumin-to-creatinine ratio	3 (2-7)	4 (3-9)	0.003
Pharmacological treatment			
Statins	81 (41)	59 (28)	<0.004
ACEi / ARB	49 (25)	42 (20)	0.210
Antiplatelet drugs	16 (8)	11 (5)	0.228

Data are shown as n (percentage within each column), mean (SD) or median (Q1-Q3).

p values for group comparisons are reported

ACEi: angiotensin-converting enzyme inhibitor; ALT: alanine aminotransferase; ARB: angiotensin receptor blocker; CVD: cardiovascular disease; CSII: continuous subcutaneous insulin infusion; DBP: diastolic blood pressure; eGFR: estimated glomerular filtration rate; HDL: high density lipoprotein; LDL: low density lipoprotein; SBP: systolic blood pressure; T1D: type 1 diabetes

* Defined as <55 years in men and <65 years in women.

[†] Defined as >3.5 hours per week.

Supplementary Table S3. Characteristics of the study participants according to age.

	<50 years old (n=245)	≥50 years old (n=165)	p-value
Clinical characteristics			
Premature CVD in first-degree relatives*	28 (11)	18 (11)	0.870
Never smokers	118 (48)	64 (39)	0.061
Regular physical activity [†]	162 (66)	124 (75)	0.051
Hypertension	24 (10)	68 (41)	<0.001
SBP (mmHg)	123 (13)	134 (15)	<0.001
DBP (mmHg)	82 (8)	82 (9)	0.823
BMI (kg/m ²)	25.8 (4.2)	26.2 (4.5)	0.403
Obesity (BMI≥30 kg/m ²)	33 (14)	28 (17)	0.316
Waist circumference (cm)	89 (12)	93 (13)	0.002
Female (cm)	84 (12)	88 (12)	0.004
Male (cm)	95 (10)	98 (12)	0.049
T1D duration (years)	24.5 (9.5)	30.3 (13.1)	<0.001
Diabetic kidney disease	14 (6)	17 (10)	0.085
Diabetic retinopathy	91 (37)	58 (35)	0.681
CSII therapy	68 (28)	40 (24)	0.428
Conventional lipid profile			
Total cholesterol (mg/dL)	185 (28)	189 (32)	0.290
HDL-cholesterol (mg/dL)	59 (15)	65 (16)	<0.001
LDL-cholesterol (mg/dL)	110 (25)	105 (25)	0.050
Triglycerides (mg/dL)	73 (59-96)	78 (65-111)	0.038
Non-HDL cholesterol (mg/dL)	126 (27)	123 (27)	0.286
Other laboratory characteristics			
Fasting plasma glucose (mg/dL)	156 (65)	157 (63)	0.854
Haemoglobin A1c (%, (mmol/mol))	7.5 (1), (58)	7.6 (0.9), (60)	0.122
Serum creatinine (mg/dL)	0.88 (0.15)	0.88 (0.18)	0.735
eGFR (CKD-EPI; ml/min/1.73m ²)	96 (13)	83 (13)	<0.001
ALT (UI/L)	20 (10)	23 (12)	0.007
Albumin-to-creatinine ratio	3 (2-6)	5 (3-11)	<0.001
Pharmacological treatment			
Statins	56 (23)	84 (51)	<0.001
ACEi / ARB	28 (11)	63 (38)	<0.001
Antiplatelet drugs	4 (2)	23 (14)	<0.001

Data are shown as n (percentage within each column), mean (SD) or median (Q1-Q3).

p values for group comparisons are reported

ACEi: angiotensin-converting enzyme inhibitor; ALT: alanine aminotransferase; ARB: angiotensin receptor blocker; CVD: cardiovascular disease; CSII: continuous subcutaneous insulin infusion; DBP: diastolic blood pressure; eGFR: estimated glomerular filtration rate; HDL: high density lipoprotein; LDL: low density lipoprotein; SBP: systolic blood pressure; T1D: type 1 diabetes

* Defined as <55 years in men and <65 years in women.

[†] Defined as >3.5 hours per week.

ARTÍCULO ORIGINAL 4: IMPACTO DE LA PREECLAMPSIA Y LA PARIDAD EN LAS DISCREPANCIAS ACORDE AL SEXO EN LA ATEROSCLEROSIS CAROTÍDEA SUBCLÍNICA EN LA DIABETES TIPO I

Objetivos: El riesgo excesivo de eventos cardiovasculares fatales y no fatales es aproximadamente el doble en mujeres que en hombres con DTI. Nuestro objetivo fue evaluar el impacto de la preeclampsia y la paridad en las discrepancias por sexo en la aterosclerosis preclínica y en el rendimiento diagnóstico de una escala de riesgo cardiovascular.

Métodos: Estudio transversal. Los criterios de inclusión fueron: DTI sin ECV y con edad ≥ 40 años, ERD, y/o ≥ 10 años de duración de la diabetes con otro FRCV. La aterosclerosis carotídea se determinó mediante ecografía. El RCV se estimó mediante la ecuación Steno-Risk ($<10\%$, bajo; $10-20\%$, moderado; $\geq 20\%$, alto).

Resultados: Se incluyeron 728 sujetos (48,5% mujeres, edad mediana 47 años, duración mediana de la diabetes de 25,9 años, 39,4% con placas carotídeas). Las mujeres nulíparas y las mujeres con hijos sin preeclampsia previa tuvieron un menor riesgo de placa carotídea que los hombres (OR 0,48 [0,28-0,82]; OR 0,51 [0,33-0,79], respectivamente), sin diferencias en el grupo con preeclampsia. La prevalencia de placa carotídea aumentó a medida que aumentó el RCV estimado por Steno-Risk en todos los subgrupos, excepto en el grupo con preeclampsia. El AUC de Steno-Risk para identificar ≥ 2 placas carotídeas fue menor en el grupo con preeclampsia (hombres: 0,7886; mujeres nulíparas: 0,9026; mujeres sin preeclampsia: 0,8230; grupo con preeclampsia: 0,7841; p entre grupos = 0,042). Ni la adición de la paridad ni la preeclampsia al Steno-Risk llevaron a un aumento estadísticamente significativo en el AUC.

Conclusiones: El riesgo de placa carotídea en mujeres en comparación con los hombres disminuyó a medida que disminuyó la exposición a factores obstétricos. Sin embargo, la adición de estos factores no mejoró la predicción de la ecuación Steno-Risk.

Impact of Preeclampsia and Parity on Sex-based Discrepancies in Subclinical Carotid Atherosclerosis in Type 1 Diabetes

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Abstract

Context: The excess risk of fatal and nonfatal cardiovascular events is roughly twice as high in women than in men with type 1 diabetes.

Objective: To evaluate the impact of preeclampsia and parity on sex-based discrepancies in preclinical atherosclerosis and on the diagnostic performance of a cardiovascular risk scale.

Design: Cross-sectional study.

Setting: Single tertiary hospital.

Patients: A total of 728 people with type 1 diabetes (48.5% women) without cardiovascular disease and age ≥ 40 years, nephropathy, and/or ≥ 10 years of diabetes duration with another risk factor.

Intervention: Standardized carotid ultrasonography.

Main Outcome Measures: Carotid plaque determined by ultrasonography and cardiovascular risk estimated according to the Steno T1 Risk Engine (Steno-Risk).

Results: Nulliparous women and parous women without previous preeclampsia had a lower risk for carotid plaque than men (adjusted odds ratio: .48, 95% confidence interval [.28-.82]; adjusted odds ratio: .51 [.33-.79], respectively), without differences in the preeclampsia group. The prevalence of carotid plaque increased as the estimated cardiovascular risk increased in all subgroups except for preeclampsia group. The area under the curve of the Steno-Risk for identifying ≥ 2 carotid plaques was lower in the preeclampsia group (men: .7886; nulliparous women: .9026; women without preeclampsia: .8230; preeclampsia group: .7841; P between groups = .042). Neither the addition of parity nor preeclampsia in the Steno-Risk led to a statistically significant increase in the area under the curve.

Conclusion: The risk for carotid plaque in women compared with men decreased as exposure to obstetric factors diminished. However, the addition of these factors did not improve the prediction of the Steno-Risk.

Key Words: carotid atherosclerosis, preeclampsia, parity, type 1 diabetes, cardiovascular risk scale

Abbreviations: aOR, adjusted odds ratio; AUC, area under the curve; CVD, cardiovascular disease; CVRF, cardiovascular risk factor; eGFR, estimated glomerular filtration rate; HbA1c, glycated hemoglobin; HDL, high-density lipoprotein; IMT, intima-media thickness; LDL, low-density lipoprotein; OR, odds ratio; Steno-Risk, Steno T1 Risk Engine.

Despite improvements in the treatment and management of type 1 diabetes, this population still experiences higher rates of cardiovascular disease (CVD) than the general population (1, 2), with sex discrepancies. Although a higher number of cardiovascular events have been reported in men than in women with type 1 diabetes, the excess risk of fatal and nonfatal

cardiovascular events is roughly twice as high in women than in men (compared with their counterparts without diabetes) (3, 4).

Unlike men, women are exposed to specific cardiovascular risk factors (CVRF), such as those related to the reproductive area. Indeed, international guidelines on the prevention of

CVD recommend considering preeclampsia as an additional individual risk-enhancing clinical factor in the general population (5, 6). Several studies have reported the increased risk of CVD in women who developed preeclampsia during pregnancy (7-9). In fact, our group showed that a history of preeclampsia was associated with more advanced preclinical carotid atherosclerosis not only in the general population but also in women with type 1 diabetes (10). In addition to adverse pregnancy outcomes, parity has also been described as a CVRF, showing a J-shaped association between parity and incident CVD (11, 12). However, data in the type 1 diabetes population are scarce and are mainly focused on the relationship between parity and microvascular complications and CVRF (13, 14).

Given the importance of CVD in people with type 1 diabetes, tools to predict cardiovascular events are essential to tailoring cardiovascular risk management according to the risk of each individual. Specific type 1 diabetes scales that take into account specific CVRF of this entity (such as diabetes duration or diabetes-related complications) have been developed and show better performance than the classical scales. In this setting, the Steno T1 Risk Engine (Steno-Risk) has shown to have an independent association with the identification of several markers of subclinical atherosclerosis and CVD events in several type 1 diabetes populations (15-17).

As mentioned previously, there is growing evidence of the role of sex-specific reproductive factors in the risk of CVD in women with type 1 diabetes. However, in contrast to the general population, there is no evidence about whether these factors could improve the discrimination of specific type 1 diabetes scales, such as Steno-Risk (18). Considering this background, this study aimed to evaluate the risk of preclinical atherosclerosis according to sex-specific reproductive factors, such as preeclampsia and parity. Additionally, the diagnostic performance of adding these factors to the Steno-Risk equation for the identification of preclinical atherosclerosis plaque was studied.

Materials and Methods

Study Design and Participants

This was a cross-sectional investigation of a specific prospective protocol for CVD risk assessment in subjects with type 1 diabetes (which included a structured evaluation of CVRF and a standardized carotid ultrasonography to assess subclinical atherosclerosis) (15, 19). Briefly, this protocol included patients diagnosed with type 1 diabetes and no previous personal history of CVD (coronary artery disease, ischemic stroke, peripheral vascular disease, or heart failure), but with conditions associated with high risk according to the standards proposed by the main CVD prevention guidelines (20-22): (A) age ≥ 40 years, (B) individuals with any stage of diabetic nephropathy regardless of age, and (C) individuals of any age, with at least 10 years of disease duration and 1 additional CVRF (active smoking habit, diabetic retinopathy, hypertension, triglycerides >150 mg/dL, low high-density lipoprotein [HDL] cholesterol [<40 mg/dL in men, <45 mg/dL in women], family history of premature CVD in first-degree relatives [<55 years of age in men and <65 years in women], severe hypoglycemia [defined as an episode of confirmed hypoglycemia requiring external assistance for recovery], or hypoglycemia unawareness [defined as a score >3 in the Clarke test], with the validated Spanish version (23), and history

of preeclampsia/eclampsia in at least 1 previous pregnancy in women).

The study protocol was conducted according to the Declaration of Helsinki. All patients provided informed consent, and the study was approved by the Ethical Committee of the Hospital Clínic, Barcelona, Spain (approval number HCB/2017/0977).

Clinical and Laboratory Measures

Both demographic and clinical data including duration of type 1 diabetes, family history of premature CVD in first-degree relatives, history of microvascular diabetes complications, and medical treatment (multiple-dose insulin, continuous subcutaneous insulin infusion, lipid-lowering agents, and antihypertensive and antiplatelet drugs) were obtained from medical records.

Diabetic nephropathy was assessed according to the albumin-to-creatinine ratio, with <30 mg/g considered as normal and ≥ 30 mg/g as diabetic nephropathy (confirmed in at least 2 of 3 consecutive determinations). The use of angiotensin-converting enzyme inhibitors or angiotensin II receptor blockers, without a history of hypertension or CVD, was also considered as diabetic nephropathy. Fundus oculi was used for the diagnosis of diabetic retinopathy, which was always confirmed by an ophthalmologist.

Anthropometric measurements (weight, height, and waist circumference) were also obtained. Patients were weighed wearing light clothing and being barefoot, with the use of a calibrated electronic scale. The body mass index was calculated as weight in kilograms divided by the square height in meters. The midpoint between the lowest rib and the iliac crest was used to measure waist circumference.

Laboratory parameters were measured in fasting blood and first-morning urine spot samples. Lipid profile (including total cholesterol, triglycerides, and HDL cholesterol), glucose, creatinine, and albumin-to-creatinine ratio were assessed with standardized assays. Low-density lipoprotein (LDL) cholesterol was determined with the Friedewald formula. Non-HDL cholesterol was calculated as the deduction of HDL cholesterol from total cholesterol. The estimated glomerular filtration rate (eGFR) was assessed with the Chronic Kidney Disease Epidemiology Collaboration equation. Glycated hemoglobin (HbA1c) values (Tosoh G8 Automated HPLC Analyzer; Tosoh Bioscience, South San Francisco, CA) (Diabetes Control and Complications Trial aligned, normal range 4-6% [20-42 mmol/mol]) were also recorded.

Sex-specific Reproductive Factors

All women with type 1 diabetes were asked about their history of previous pregnancies. The diagnosis of preeclampsia included internationally recommended criteria (24) and was generally defined as de novo hypertension (≥ 140 mm Hg systolic and/or ≥ 90 mm Hg diastolic) after 20 weeks of gestation and proteinuria (300 mg/24 hours or $\geq 1+$ in the dipstick test). All the gestational information (even in women without pregnancy complications) was confirmed by verifying medical records.

Cardiovascular Risk Estimation

Cardiovascular risk was assessed with the Steno-Risk equation (17). Briefly, Steno-Risk estimates the 10-year risk of fatal or nonfatal CVD (ischemic heart disease, ischemic stroke, heart failure, and peripheral artery disease) based on

10 variables (age, sex, diabetes duration, HbA1c, systolic blood pressure, LDL cholesterol, albuminuria, eGFR, smoking habit, and regular exercise [≥ 3.5 hours/week]). Individuals were classified as having low ($<10\%$), moderate (10% - 19.9%), or high ($\geq 20\%$) risk, accordingly.

Carotid B-mode Ultrasound Imaging

Carotid plaques were evaluated with high-resolution B-mode ultrasound (ACUSON X700 [Siemens Healthineers] or Aplio a450 [Canon]) with the same electric linear array 5- to 10-MHz transducer. Predefined and standardized imaging protocols to measure carotid intima-media thickness (IMT) were used, and the presence of plaque was determined as previously described (15, 25). Carotid images were visualized with B-mode and color Doppler in longitudinal and transverse planes to appraise circumferential asymmetry. Carotid plaques were defined according to focal echo structures intruding into the arterial lumen by at least 50% of the surrounding IMT value or when IMT was at least 1.5 mm as measured from the media-adventitia interface to the intima-lumen surface (26). Two experienced endocrinologists performed all the procedures, and IMT and plaque ascertainment and measurements were made by the same researcher (A.J.A.) using semiautomatic software.

Statistical Analysis

Data are presented as median [25th and 75th percentiles] or number (percentage) unless otherwise indicated. The Mann-Whitney *U* test, Kruskal-Wallis, Pearson χ^2 test, and ANOVA were performed, as appropriate, for comparisons between groups. The Bonferroni test was used as a post hoc analysis to make pairwise comparisons, correcting for multiple analyses.

Logistic regression modeling was used to estimate the association between sex and carotid plaque burden (presence of at least 1 carotid plaque or presence of ≥ 2 carotid plaque). Sex was included in the model as a binary variable (men/women) or categorical variable (men, nulliparous women, and parous women with/without history of preeclampsia), with men being the reference category. Two adjusted models were performed. Model 1 included the same variables of Steno-Risk: age, diabetes duration, HbA1c, systolic blood pressure, LDL cholesterol, albuminuria, eGFR, smoking habit, and regular exercise. Model 2 included model 1 plus statin use. Interaction analysis was performed between sex and age ($\leq$ or >50 years).

Furthermore, receiver operating characteristic curves were used to assess the optimal model for identifying individuals with carotid plaque. Area under the curve (AUC) comparisons were performed between the Steno-Risk equations according to sex category. Additionally, among women with type 1 diabetes, the diagnostic performance of adding preeclampsia and parity (number of live births) to the Steno-Risk equation for the identification of preclinical atherosclerosis plaque was evaluated. All analyses were performed using STATA version 14.0 (Stata Corp., College Station, TX, USA). A 2-sided $P < .05$ was considered statistically significant.

Results

Subject Characteristics

A total of 728 subjects were included in the study, with a median age of 47.0 [40.9-55.1] years (60.4% aged ≤ 50 years), a median duration of diabetes of 25.9 [20.1-33.7] years, a

median HbA1c of 7.4% [6.9%-8%], and 38.6% were smokers (Supplemental Table 1 (27)). Among women ($n = 353$, 48.5% of the sample), 125 (35.4%) were nulliparous, 175 (49.6%) were parous without a history of preeclampsia, and 51 (15%) had a history of preeclampsia. Parous women reported having 1 (48.2%), 2 (47.4%), or 3+ (4.5%) live births, without significant differences in the history of preeclampsia between groups (Supplemental Table 2 (27)). Nulliparous women presented a better cardiovascular profile, with lower rates of hypertension, lower systolic and diastolic blood pressure, higher HDL cholesterol, and lower use of statins compared with men, without significant differences in rate of premature CVD in first-degree relatives, smoking habit, HbA1c, diabetes duration, or diabetic nephropathy (Table 1). Conversely, the rates of retinopathy were higher in nulliparous women compared with men (46.5% vs 35.7%, $P < .05$). Despite women with previous preeclampsia having higher HDL cholesterol concentrations than men, these levels were lower compared with their counterparts without preeclampsia or without children (Table 1). The remaining characteristics are shown in Table 1.

Prevalence of Preclinical Carotid Atherosclerosis

In the whole cohort, 39.4% of subjects had at least 1 carotid plaque (22.8% with 2 or more plaques) with significant between-sex differences (men: 43.5% vs women: 35.2%; $P = .023$). Indeed, the crude and adjusted logistic regression model showed that women had a lower risk for carotid plaque than men (crude odds ratio [OR]: .68 [95% CI, .50-.92]; adjusted OR [aOR]: .54 [95% CI, .38-.78]).

When dividing the whole cohort according to parity and previous preeclampsia, nulliparous women had a lower prevalence of carotid plaque (26%) than their counterparts (vs 38.8% in parous women without preeclampsia, $P = .025$; vs 41.2% in parous women with preeclampsia, $P = .046$) and men (vs 43.5%, $P < .001$) (Table 2). However, when adjusting for well-known CVRF (age, diabetes duration, HbA1c, systolic blood pressure, LDL cholesterol, albuminuria, eGFR, smoking habit, and regular exercise), both parity and preeclampsia were associated with carotid plaque. Not having children or no history of preeclampsia was associated with a lower risk of carotid plaque compared with men (aOR: .48 [.28-.82]; aOR: .51 [.33-.79], respectively). Conversely, the risk of carotid plaque in parous women with a history of preeclampsia was not significantly different than men (aOR: .87 [.44-1.74]). The inclusion of statin use did not modify the results (Table 2).

An interaction analysis between age and sex was statistically significant (P for interaction = .037); thus, the logistic models were performed stratifying for age ($\leq$ or >50 years). The association observed between the presence of carotid plaque and sex-specific reproductive factors was maintained among subjects aged younger than 50 years (nulliparous women: aOR: .41 [95% CI, .18-.89]; parous women without preeclampsia: aOR .43 [95% CI, .21-.86]). However, the associations were blunted among those aged >50 years (Table 2).

Last, the same analysis was performed for the presence of 2 or more carotid plaques, showing similar results (Table 2).

Relationships Between Estimated Risk and Preclinical Carotid Atherosclerosis

The use of the Steno-Risk equation classified low, moderate, and high cardiovascular risk in 25.1%, 42.4%, and 32.5%

Table 1. Characteristics of the study participants according to sex-specific reproductive factors

	Men (n = 375)	Nulliparous women (n = 127)	Parous women without preeclampsia (n = 175)	Parous women with previous preeclampsia (n = 51)	P between groups
Clinical characteristics					
Age, y	46.9 (40.7-55.0)	43.2 (35.3-52.2) ^a	49.9 (43.3-58.0) ^{a,b}	46.3 (41.3-54.5)	<.001
Premature CVD in first-degree relatives ^c	41 (10.9)	13 (10.2)	23 (13.1)	7 (13.7)	.795
Current smokers	96 (25.6)	40 (31.5)	51 (29.1)	14 (27.5)	.588
Hypertension	100 (26.7)	17 (13.4) ^a	39 (22.3)	16 (31.4)	.011
SBP (mm Hg)	129 (121-138)	120 (113-130) ^a	127 (112-136) ^a	127 (113-136)	<.001
DBP (mm Hg)	83 (77-87)	81 (74-85) ^a	81 (76-87)	80 (74-86)	.001
BMI (kg/m ²)	26.2 (24.2-28.7)	24.9 (22.3-28.1)	25.0 (22.5-28.1)	24.7 (22.3-28.7)	.001
Waist circumference, cm	96 (89-103)	85 (77-91.5) ^a	84 (77-95) ^a	86 (77-94) ^a	<.001
Diabetes duration, y	25.7 (19.8-33.4)	25.5 (18.9-34.8)	26.4 (20.6-34.3)	30.2 (24.1-34.4)	.081
Diabetic nephropathy	37 (9.9)	12 (9.6)	12 (6.9)	7 (13.7)	.465
Diabetic retinopathy	134 (35.7)	59 (46.5) ^a	52 (29.7)	23 (45.1)	.014
CSII therapy	83 (22.1)	38 (29.9)	68 (39.0) ^a	26 (51.0) ^a	<.001
Laboratory characteristics					
Fasting plasma glucose, mg/dL	147 (111-196)	145 (111-189)	148 (104-199)	146 (104-200)	.964
HbA1c, %, mmol/mol	7.4 (6.9-8.0)	7.3 (6.9-7.9)	7.5 (6.9-8.1)	7.7 (6.9-8.1)	.460
	57.4 (51.9-63.6)	56.6 (51.5-62.3)	58.5 (52.3-65.0)	60.1 (51.9-65.0)	
Serum creatinine, mg/dL	.93 (.84-1.02)	.77 (.69-.86) ^a	.76 (.69-.83) ^a	.77 (.66-.87) ^a	<.001
eGFR, CKD-EPI, mL/min/1.73 m ²	95.1 (85.9-104.7)	95.0 (79.5-106.7)	92.1 (79.6-102.1) ^a	92.6 (80.6-104.5)	.027
ALT, IU/L	22 (18-30)	17 (13-21) ^a	17 (13-21) ^a	17 (14-20) ^a	<.001
Total cholesterol, mg/dL	183 (165-201)	194 (172-213) ^a	190 (175-212) ^a	185 (161-210)	<.001
HDL cholesterol, mg/dL	54 (46-62)	66 (57-78) ^a	66 (55-78) ^a	60 (49-70) ^{a,b,d}	<.001
LDL cholesterol, mg/dL	109 (94-126)	109 (93-127)	111 (92-124)	107 (90-124)	.874
Triglycerides, mg/dL	80 (64-107)	71 (58-95)	70 (57-91) ^a	70 (54-103)	<.001
Non-HDL cholesterol, mg/dL	127.5 (110-144)	126 (107-140)	127 (107-139)	123 (110-142)	.410
Pharmacological treatment					
Statins	160 (42.7)	35 (27.6) ^a	61 (34.9)	16 (31.4)	.012
ACEi/ARB	104 (27.7)	21 (16.5)	38 (21.7)	19 (37.6) ^b	.010
Antiplatelet drugs	34 (9.1)	8 (6.3)	10 (5.7)	6 (11.8)	.346

Data are n (% in each column) or median (interquartile range). P values for between-group comparisons are reported.

Abbreviations: ACEi, angiotensin-converting enzyme inhibitor; ALT, alanine aminotransferase; ARB, angiotensin receptor blocker; BMI, body mass index; CSII, continuous subcutaneous insulin infusion; CKD-EPI, Chronic Kidney Disease Epidemiology Collaboration; CVD, cardiovascular disease; DBP, diastolic blood pressure; eGFR, estimated glomerular filtration rate; HbA1c, glycated hemoglobin; HDL, high-density lipoprotein; LDL, low-density lipoprotein; SBP, systolic blood pressure.

^aP < .05 vs men.

^bP < .05 vs nulliparous women.

^cDefined as aged <55 years in men and <65 years in women.

^dP < .05 vs parous women without preeclampsia.

of men, respectively, and 35.3%, 36.9%, and 27.8% of women, respectively ($P = .010$). When taking into account pregnancy factors, the most prevalent Steno-Risk category was moderate in all groups except for nulliparous women, with 51.2% of the latter being classified as low risk ($P < .001$) (Fig. 1).

In the whole cohort, the prevalence of carotid plaque increased as the estimated cardiovascular risk increased (low risk: 11.7%; moderate risk: 38.4%; high risk: 68.2%; P for trend <.001). As shown in Fig. 1, similar results were found when the sample was stratified for sex and pregnancy factors, except for parous women with preeclampsia. In this group, no significant association was observed between Steno-Risk categories and the presence of carotid plaque (low risk: 25%; moderate risk: 45%; high risk: 53.3%; $P = .251$). We also investigated whether Steno-Risk predicted the presence of

carotid plaque by sex. Steno-Risk showed good performance in identifying 1 or more carotid plaques regardless of sex and sex-specific reproductive factors (men: AUC .7651; nulliparous women: AUC .7988; women without preeclampsia: AUC .8353; women with preeclampsia: AUC .6206; $P = .051$) (Fig. 2). However, the AUC was significantly lower in the preeclampsia group compared with the other 3 groups for the prediction of the presence of 2 or more carotid plaques (men: AUC .7886; nulliparous women: AUC .9026; women without preeclampsia: AUC .8230; women with preeclampsia: AUC .7841; $P = .042$) (Fig. 2).

Finally, we evaluated the diagnostic performance of the Steno-Risk equation alone and with the addition of pregnancy factors to the model for the prediction of carotid plaque among women with type 1 diabetes. Neither the inclusion of parity (number of live births) nor preeclampsia in the

Table 2. Logistic regression models for presence of carotid plaque according to sex and age

	Plaque presence	Crude model		Adjusted model 1		Adjusted model 2	
	n/N (%)	OR (95% CI)	P	OR (95% CI)	P	OR (95% CI)	P
Whole sample							
≥1 carotid plaque							
Men	163/375 (43.5)	ref.		ref.		ref.	
Nulliparous women	33/127 (26.0)	.46 (.29-.71)	.001	.48 (.28-.82)	.007	.49 (.29-.84)	.010
Parous women without PE	67/175 (38.3)	.81 (.56-1.16)	.252	.51 (.33-.79)	.003	.52 (.34-.82)	.004
Parous women with PE	21/51 (41.2)	.91 (.50-1.65)	.757	.87 (.44-1.74)	.703	.91 (.45-1.81)	.781
≥2 carotid plaques							
Men	96/375 (25.6)	ref.		ref.		ref.	
Nulliparous women	16/127 (12.6)	.42 (.24-.74)	.003	.38 (.19-.76)	.006	.40 (.20-.79)	.009
Parous women without PE	42/175 (24)	.91 (.60-1.39)	.687	.56 (.34-.92)	.022	.57 (.35-.95)	.031
Parous women with PE	11/51 (21.6)	.80 (.39-1.62)	.534	.76 (.33-1.75)	.525	.80 (.35-1.85)	.607
Subjects aged ≤50 years							
≥1 carotid plaque							
Men	65/229 (28.4)	ref.		ref.		ref.	
Nulliparous women	10/88 (11.4)	.32 (.16-.66)	.002	.41 (.18-.89)	.024	.44 (.20-.97)	.042
Parous women without PE	14/88 (15.9)	.48 (.25-.90)	.023	.43 (.21-.86)	.018	.46 (.23-.95)	.034
Parous women with PE	13/35 (37.1)	1.49 (.71-3.14)	.292	1.65 (.72-3.76)	.237	1.77 (.77-4.10)	.181
≥2 carotid plaques							
Men	29/229 (12.7)	ref.		ref.		ref.	
Nulliparous women	2/88 (2.3)	.16 (.04-.69)	.014	.16 (.03-.075)	.020	.16 (.03-.79)	.025
Parous women without PE	5/88 (5.7)	.42 (.16-1.11)	.080	.34 (.11-.98)	.045	.35 (.12-1.02)	.055
Parous women with PE	4/35 (11.4)	.89 (.29-2.70)	.837	.92 (.28-3.06)	.893	.93 (.28-3.10)	.907
Subjects aged >50 years							
≥1 carotid plaque							
Men	98/146 (67.1)	ref.		ref.		ref.	
Nulliparous women	23/39 (59.0)	.70 (.34-1.45)	.343	.55 (.24-1.26)	.161	.55 (.24-1.26)	.158
Parous women without PE	53/87 (60.9)	.76 (.44-1.32)	.338	.58 (.31-1.08)	.085	.58 (.31-1.07)	.084
Parous women with PE	8/16 (50.0)	.49 (.17-1.38)	.178	.32 (.10-1.02)	.054	.31 (.10-1.01)	.052
≥2 carotid plaques							
Men	67/146 (45.9)	ref.		ref.		ref.	
Nulliparous women	14/39 (35.9)	.66 (.32-1.37)	.266	.57 (.25-1.32)	.188	.58 (.25-1.34)	.200
Parous women without PE	37/87 (42.5)	.87 (.51-1.49)	.618	.76 (.42-1.38)	.366	.76 (.42-1.39)	.375
Parous women with PE	7/16 (43.8)	.92 (.32-2.59)	.870	.79 (.25-2.54)	.694	.83 (.25-2.67)	.753

Data are n/N(%) and OR and 95% CI. Adjusted model 1 included: age, diabetes duration, glycated hemoglobin, systolic blood pressure, low-density lipoprotein cholesterol, albuminuria, estimated glomerular filtration rate, smoking habit, and regular exercise. Adjusted model 2 included model 1 plus statins use.

Abbreviation: PE, preeclampsia.

Steno-Risk model led to a statistically significant increase in the AUC (Steno-Risk equation alone: AUC .7921 vs plus parity: AUC .7920, $P = .969$; Steno-Risk equation alone: AUC .7808 vs plus preeclampsia: .7887, $P = .208$) (Supplemental Table 2 (27)). There were also no significant differences in the diagnostic performance when stratifying for age or when evaluating the identification of 2 or more carotid plaques (Supplemental Table 3, Supplemental Table 4 (27)).

Discussion

In the present study, the inclusion of specific reproductive factors modified the association between preclinical carotid atherosclerosis and sex. Although the lowest risk of carotid

plaque was observed in nulliparous women, there was no significant difference in carotid plaque burden between men and women with a history of preeclampsia. In fact, the diagnostic performance of the Steno-Risk scale for the identification of carotid plaque was lower in women with previous preeclampsia. However, the inclusion of either parity or preeclampsia in the Steno-Risk model did not improve the prediction of cardiovascular risk. To the best of our knowledge, no previous study has evaluated the role of parity and preeclampsia in the risk of carotid atherosclerosis in a type 1 diabetes population.

CVD in women has been widely underrecognized and underestimated (28). Indeed, women were less likely to be prescribed cardiovascular medication such as statins than men in

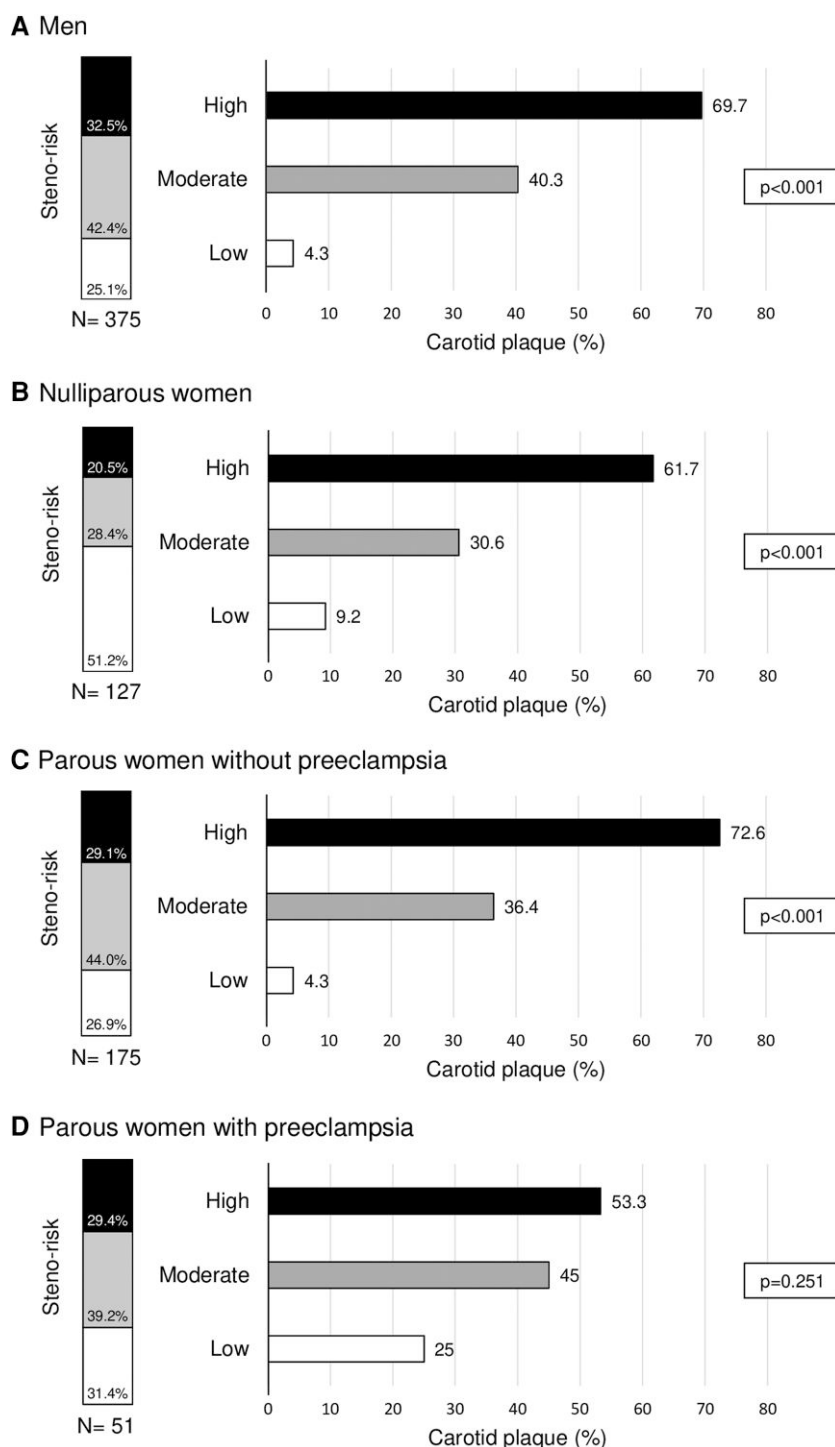


Figure 1. Cardiovascular risk categories (as determined by the Steno-Risk equation) were stratified based on sex-specific reproductive factors and the prevalence of carotid plaque within each of these cardiovascular risk categories. Steno-Risk, Steno T1 Risk Engine.

primary prevention, both in the general population (29) and, as our results reinforced, in women with type 1 diabetes. In part, this could be explained by the fact that women were more often classified as having low/moderate cardiovascular risk according to international guidelines on CVD prevention (30). Thus, additional individual risk-enhancing clinical factors, such as sex-specific variables, should be taken into account (5, 22). In this context, the deleterious impact of preeclampsia on cardiovascular health years after pregnancy

has been described (8, 9). Our group has previously shown a higher prevalence of carotid atherosclerosis burden when a history of preeclampsia was present among the type 1 diabetes population (10). Data from the present study, including almost 350 women with type 1 diabetes, confirmed the aforementioned results. The prevalence of carotid plaques observed was twice as high in the preeclampsia group compared with that of nulliparous women. Interestingly, the lowest odds for carotid plaque in women with type 1 diabetes

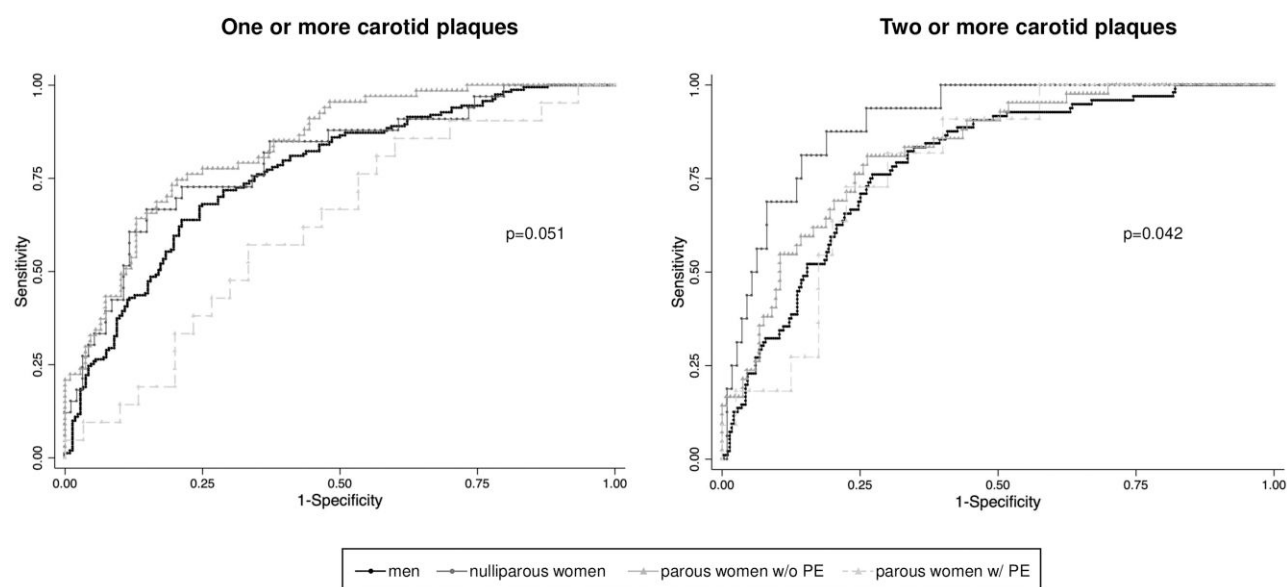


Figure 2. Area under the curve of the Steno-Risk scale for identification of carotid plaque according to sex categories. Steno-Risk, Steno T1 Risk Engine.

compared with men disappeared when preeclampsia was taken into account. Although the higher absolute risk of CVD in men with type 1 diabetes compared with women has been largely described (31–33), no previous study has included obstetrics factors in the evaluation of sex disparities in CVD among the type 1 diabetes population. Overall, our results highlight that women with type 1 diabetes who developed preeclampsia during pregnancy should receive patient counseling about their future cardiovascular health and start preventive and therapeutic measures to reduce cardiovascular risk.

In the present study, nulliparous women with type 1 diabetes showed a better cardiovascular profile (ie, lower rates of hypertension and higher HDL cholesterol) but had higher rates of retinopathy compared with men. In addition to hypertension, poor glycemic control is the main modifiable risk factor for diabetic retinopathy (34). In our cross-sectional study, there was no significant difference in HbA1c levels. However, these data could not fully capture the glycemic control throughout diabetes duration. Indeed, parous women were more likely to use advanced diabetes technology such as continuous subcutaneous insulin infusion therapy (Table 1 of our study (35)). On the other hand, although it is known that pregnancy is associated with progression of diabetic retinopathy (36), follow-up studies comparing women with or without children found that the prevalence and progression of diabetic retinopathy occur less frequently in parous women compared with nulliparous women (37, 38). Taken together, it is possible that the intensive self-management behaviors adopted in pregnancy and the increased uptake of diabetes technologies could help women who have conceived to improve their glucose control compared with women who have never conceived. In the same line as CVRF, nulliparous women showed an approximately 50% decreased risk of carotid plaque presence compared with men. In the general population, multiparity has been associated with a higher risk of CVD (11, 39). Indeed, a recent Mendelian randomization study highlighted that the correlation between the number of live births and CVD is likely to be a direct causal link, beyond sociodemographic and clinical factors (12).

Nonetheless, few studies have focused on women with type 1 diabetes (13, 40, 41). A cohort from the T1D Exchange Network and Clinic Registry, including 497 women with type 1 diabetes over a 5-year follow-up period, did not show differences between nulliparous ($n = 469$) and parous women ($n = 28$) in relation to either in microvascular complications or cardiovascular events (13). Compared with our study, those women were younger (aged < 30 years) with a shorter diabetes duration (median < 18 years), and assessment of subclinical carotid atherosclerosis was not carried out. On the other hand, our findings show that the “protective” effect of nulliparity varied across age groups. The lower risk of carotid plaque in nulliparous women compared with men was no longer observed among those aged > 50 years. Previous large nationwide cohort studies have described a reduction in the sex difference in the absolute number of cardiovascular events after age 50 years (3, 33). In this context, our findings suggest that factors such as age, the onset of menopause (42), and other established risk factors such as glycemic control (33) may exert a greater influence on the risk of subclinical atherosclerosis at that age. This influence could potentially diminish the protective effect of parity.

As mentioned previously, the risk of carotid plaque compared with men decreased as exposure to obstetric factors diminished (parity and preeclampsia). However, adding this information to a validated calculator for cardiovascular risk, such as Steno-Risk, did not improve discrimination for the identification of subclinical atherosclerosis among women with type 1 diabetes. These results were in accordance with the previous large studies in the general population (18, 43). A hypothesis was that the incremental information provided by obstetric factors may have been partly captured by any subsequent increases in classical risk factors. However, despite its limitations, a recent Scientific Statement from the American Heart Association emphasized the importance of recognizing adverse pregnancy outcomes when CVD risk is evaluated in women (44). In this regard, we also observed that with the Steno-Risk scale the discrimination of women with previous preeclampsia was weaker than for the other 3 groups (men,

nulliparous women, and parous women without preeclampsia). Indeed, the rate of carotid plaque was approximately 3 times higher in the low-risk group among women with previous preeclampsia in comparison to the other 3 groups. Overall, these results highlight that the cardiovascular risk classification obtained by the Steno-Risk scale among women with previous preeclampsia should be considered with caution, and other tools (such as carotid ultrasonography) should be applied for more accurate risk reclassification, especially in the low-risk category.

Our study has several strengths. This is the first study that assesses the presence of subclinical atherosclerosis by taking into account both parity and a history of preeclampsia in a cohort of subjects with type 1 diabetes. Second, we selected a large sample using standardized procedures to reduce variability, and thereby provide more accurate results. Last, the revision of all the medical records regarding gestational information avoided recall bias, a flaw frequently observed in previous retrospective studies (45). However, our study also has limitations. First, the study was carried out in a single tertiary hospital and only prespecified high-risk patients without CVD were included. Thus, extrapolation to other patients with a different setting or cardiovascular profile should be made with caution. Second, data of some reproductive factors were missing, such as age at menarche, age at first birth, or menopausal status. Despite this limitation, the inclusion of an easily collected (having children yes/no) variable into routine clinical practice could overcome the well-known recall bias of adverse obstetric outcomes in the cardiovascular risk reclassification (46). Third, there were no data about mild hypertensive disorders of pregnancy such as gestational hypertension. This could lead to the inclusion of women with higher cardiovascular risk in the control group (parous without preeclampsia). Despite this limitation, we found significant differences between groups highlighting the importance of the identification of preeclampsia in the cardiovascular risk reclassification in women with type 1 diabetes. Finally, the cross-sectional design does not allow assessment of the appearance of atherosclerotic disease or cardiovascular events. However, the presence of carotid plaque has been associated with incident CVD in large follow-up studies in the general population (47, 48). Furthermore, the predictive capacity for future cardiovascular events of carotid plaque has also been demonstrated in subjects with a coronary artery calcium score (the gold standard for CVD reclassification) of 0 (5, 22, 49). This is particularly interesting because women are more likely to have coronary artery calcium scores of 0 than men (50).

In conclusion, the present study highlights the importance of specific reproductive factors in the presence of preclinical carotid atherosclerosis, especially in younger women with type 1 diabetes. Furthermore, the presence of a history of preeclampsia identifies a subset of women in whom the diagnostic performance of a specific cardiovascular risk scale, such as Steno-Risk, was poor. Overall, both parity and preeclampsia are easily identifiable factors during routine clinical practice and thereby provide a window of opportunity for tailoring better strategies to prevent CVD in this high-risk population.

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Disclosures

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Data Availability

Some or all datasets generated during and/or analyzed during the current study are not publicly available but are available from the corresponding author on reasonable request.

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Supplementary material

Corresponding to:

Impact of Preeclampsia and Parity on Sex-based Discrepancies in Subclinical Carotid Atherosclerosis in Type 1 Diabetes

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Supplemental Tables: 4

Supplemental table 1. Characteristics of the study participants according to sex

	Total cohort (n=728)	Men (n=375)	Women (n=353)	P
Clinical characteristics				
Age (years)	47.0 (40.9-55.1)	46.9 (40.8-55.0)	47.4 (41.2-55.2)	0.741
Premature CVD in first-degree relatives*	84 (11.5)	52 (10.9)	43 (12.2)	0.340
Current smokers	201 (38.6)	96 (25.6)	105 (29.8)	0.122
Hypertension	172 (23.6)	100 (26.7)	72 (20.4)	0.047
SBP (mmHg)	127 (118-136)	129 (121-138)	124 (113-135)	<0.001
DBP (mmHg)	82 (76-87)	81 (75-86)	83 (77-87)	<0.001
BMI (kg/m ²)	25.8 (23.-28.4)	26.2 (24.2-28.7)	25.0 (22.3-28.4)	<0.001
Waist circumference (cm)	91 (83-100)	96 (89-103)	85 (77-94)	<0.001
T1D duration (years)	25.9 (20.1-33.7)	25.7 (19.8-33.4)	26.6 (20.7-34.4)	0.364
Diabetic nephropathy	68 (9.3)	37 (9.9)	31 (8.8)	0.615
Diabetic retinopathy	268 (36.8)	134 (35.7)	134 (38.0)	0.534
CSII therapy	215 (29.5)	83 (22.1)	132 (37.4)	<0.001
Laboratory characteristics				
Fasting plasma glucose (mg/dL)	147 (109-196)	147 (111-196)	146 (106-196)	0.659
HbA1c [% mmol/mol]	7.4 (6.9-8) 57.4 (51.9-63.9)	7.4 (6.9-8) 57.4 (51.9-63.6)	7.4 (6.9-8) 57.4 (51.9-63.9)	0.632
Serum creatinine (mg/dL)	0.84 (0.75-0.96)	0.93 (0.84-1.02)	0.76 (0.69-0.84)	<0.001
eGFR, CKD-EPI (mL/min/1.73 m ²)	94.3 (83.7-104.3)	95.1 (85.9-104.7)	92.5 (80.2-103.8)	0.010
ALT (IU/L)	19 (15-26)	22 (18-30)	17 (13-21)	<0.001
Total cholesterol (mg/dL)	186 (168-206)	183 (165-201)	190 (172-212)	<0.001
HDL cholesterol (mg/dL)	59 (50-70)	54 (46-62)	65.7 (55-77)	<0.001
LDL cholesterol (mg/dL)	109 (93.5-125.5)	109 (94-126)	110 (93-124)	0.652
Triglycerides (mg/dL)	75 (60-100)	80 (64-107)	70 (57-93)	<0.001
Non-HDL cholesterol (mg/dL)	127 (109-142)	127.5 (110-144)	126 (107-140)	0.117

Pharmacological treatment				
Statins	272 (37.4)	160 (42.7)	112 (31.7)	0.002
ACEi/ARB	182 (25.0)	104 (27.7)	78 (22.1)	0.079
Antiplatelet drugs	58 (8.0)	34 (9.1)	24 (6.8)	0.259

Data are n (% in each column), or median (interquartile range).

P values for men vs women.

CSII, continuous subcutaneous insulin infusion; DBP, diastolic blood pressure; SBP, systolic blood pressure.

* Defined as <55 years of age in men and <65 years in women.

Supplemental table 2. Characteristics of the study participants according to number of live births.

	Number of live births				
	0 (n=127)	1 (n=109)	2 (n=107)	3+ (n=10)	p
Clinical characteristics					
Age (years)	43.2 (35.3-52.2)	47.0 (41.2-53.7) †	51.4 (44.2-58.3) †	58.9 (45.3-65.1) †‡	<0.001
Premature CVD in first-degree relatives*	13 (10.2)	17 (15.6)	11 (10.3)	2 (20)	0.463
Current smokers	40 (31.5)	28 (25.7)	34 (31.8)	3 (30)	0.740
Hypertension	17 (13.4)	22 (20.2) †	32 (29.9)	1 (10)	0.016
SBP (mmHg)	120 (113-130)	125 (109-135)	128 (117-138) †‡	126 (116-134)	0.009
DBP (mmHg)	81 (74-85)	80 (75-86)	80 (74-87)	85 (81-88)	0.408
BMI (kg/m ²)	24.9 (22.3-28.1)	24.6 (22.2-27.1)	25.7 (22.5-29.0)	25.6 (24.7-29.9)	0.252
Waist circumference (cm)	85 (77-91.5)	84 (77-94)	86 (77-95.5)	89.3 (75-97.5)	0.610
Diabetes duration (years)	25.5 (18.9-34.8)	26.5 (21.3-33.7)	27.5 (22.1-34.4)	23.9 (18.2-40.3)	0.577
Diabetic nephropathy	12 (9.6)	10 (9.2)	7 (6.5)	2 (20)	0.397
Diabetic retinopathy	59 (46.5)	41 (37.6)	33 (30.8)	1 (10)	0.023
CSII therapy	38 (29.9)	43 (39.5)	48 (44.9)	3 (30)	0.111
History of preeclampsia	-	24 (22.0)	26 (24.3)	1 (10)	0.680
Carotid plaque	33 (26.0)	44 (40.4)	38 (35.6)	6 (60)	0.032
Laboratory characteristics					
Fasting plasma glucose (mg/dL)	145 (111-189)	157 (113-200)	135 (98-196)	157 (90-203)	0.641
HbA1c [%; mmol/mol]	7.3 (6.9-7.9) 56.6 (51.5-62.3)	7.5 (6.9-8.1) 58.1 (51.9-65.0)	7.5 (6.9-8.0) 58.5 (52.3-64.2)	7.9 (7.5-8.6) 62.7 (58.5-70.5)	0.291
Serum creatinine (mg/dL)	0.77 (0.69-0.86)	0.76 (0.67-0.84)	0.78 (0.68-0.84)	0.76 (0.76-0.77)	0.774

eGFR, CKD-EPI (mL/min/1.73 m ²)	95.0 (79.5-106.7)	93.4 (81.7-103.9)	91.6 (78.6-100.4)	87.1 (84.4-93.4)	0.178
ALT (IU/L)	17 (13-21)	16.1 (12-21)	17 (13.8-21)	20 (16-35)	0.456
Total cholesterol (mg/dL)	194 (172-213)	190 (176-212)	188 (168-209)	187.5 (179-217)	0.425
HDL cholesterol (mg/dL)	66 (57-78)	66 (54-79)	63 (53-74.2)	65 (54-76)	0.580
LDL cholesterol (mg/dL)	109 (93-127)	112 (94-125)	108 (90-121)	109.5 (93-122)	0.673
Triglycerides (mg/dL)	71 (58-95)	72 (55-96)	68 (57-84)	68.5 (57-105)	0.865
Non-HDL cholesterol (mg/dL)	126 (107-140)	127 (111-141)	123 (106-137)	120 (107-138)	0.707
Pharmacological treatment					
Statins	35 (27.6)	25 (22.9)	48 (44.9) †‡	4 (40)	0.003
ACEi/ARB	21 (16.5)	26 (23.9)	29 (27.1)	2 (20)	0.255
Antiplatelet drugs	8 (6.3)	8 (7.3)	8 (7.5)	0	0.824

Data are n (% in each column), or median (interquartile range).

P values for between-group comparisons are reported.

Abbreviations: CSII, continuous subcutaneous insulin infusion; CVD: cardiovascular disease; DBP, diastolic blood pressure; SBP, systolic blood pressure.

* Defined as <55 years of age in men and <65 years in women.

† p<0.05 vs nulliparous; ‡ p<0.05 vs 1 live birth

Supplemental table 3. Area under the curve for the identification of one or more carotid plaques

	One or more carotid plaque			
	n	AUC	95% CI	p
Women				
Steno-risk alone	353	0.7921	0.7433-0.8409	0.938
Steno-risk plus parity		0.7925	0.7434-0.8416	
Steno-risk alone	226	0.7808	0.7202-0.8414	0.208
Steno-risk plus PE		0.7887	0.7300-0.8474	
Women ≤ 50 years				
Steno-risk alone	211	0.6806	0.5908-0.7705	0.914
Steno-risk plus parity		0.6771	0.5802-0.7739	
Steno-risk alone	123	0.6979	0.5914-0.8044	0.441
Steno-risk plus PE		0.7415	0.6494-0.8336	
Women > 50 years				
Steno-risk alone	142	0.7233	0.6382-0.8084	0.306
Steno-risk plus parity		0.7250	0.6401-0.8098	
Steno-risk alone	103	0.7283	0.6296-0.8271	0.619
Steno-risk plus PE		0.7377	0.6405-0.8349	

Abbreviations: AUC, area under the curve; PE, preeclampsia.

Supplemental table 4. Area under the curve for the identification of two or more carotid plaques

	Two or more carotid plaque			
	n	AUC	95% CI	p
Women				
Steno-risk alone	353	0.8478	0.8038-0.8919	0.961
Steno-risk plus parity		0.8482	0.8039-0.8925	
Steno-risk alone	226	0.8168	0.7575-0.8760	0.208
Steno-risk plus PE		0.8164	0.7572-0.8757	
Women ≤ 50 years				
Steno-risk alone	211	0.7841	0.6975-0.8707	0.692
Steno-risk plus parity		0.7582	0.6239-0.8924	
Steno-risk alone	123	0.7466	0.6230-0.8702	0.711
Steno-risk plus PE		0.7261	0.5703-0.8820	
Women > 50 years				
Steno-risk alone	142	0.7192	0.6350-0.8034	0.821
Steno-risk plus parity		0.7159	0.6319-0.7999	
Steno-risk alone	103	0.6922	0.5897-0.7947	0.724
Steno-risk plus PE		0.6938	0.5914-0.7961	

Abbreviations: AUC, area under the curve; PE, preeclampsia.

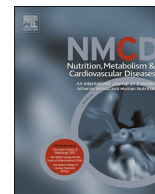
ARTÍCULO ORIGINAL 5: EL PERFIL AVANZADO DE LIPOPROTEÍNAS IDENTIFICA MEJOR LA ATEROSCLEROSIS QUE LOS LÍPIDOS CONVENCIONALES EN LA DIABETES TIPO 1 CON ALTO RIESGO CARDIOVASCULAR

Objetivos: Las personas con DTI presentan alteraciones en las lipoproteínas que podrían contribuir a su mayor riesgo de ECV. Evaluamos la relación entre las alteraciones de lipoproteínas y la aterosclerosis en pacientes con DTI.

Métodos: Estudio transversal en sujetos con DTI sin ECV previa, pero de alto RCV (≥ 40 años, ERD, o ≥ 10 años de evolución de la diabetes con otro FRCV). La presencia de placa (grosor íntima-media $\geq 1,5$ mm) en los diferentes segmentos carotídeos se determinó mediante ecografía. El perfil avanzado de lipoproteínas se analizó mediante resonancia magnética nuclear (^1H -RMN).

Resultados: Incluimos a 189 pacientes (42% mujeres, $47,8 \pm 10,7$ años, duración de la diabetes $27,3 \pm 10,1$ años, HbA1c 7,5% [7-8]). Aquellos con placas carotídeas (35%) eran mayores, con una mayor duración de la diabetes, mayor prevalencia de hipertensión, y mostraron niveles de partículas de LDL (LDL-P) y HDL (HDL-P) más bajos y de menor diámetro, pero partículas de VLDL (VLDL-P) más elevadas. Algunos parámetros relacionados con LDL, HDL y VLDL se asociaron con la aterosclerosis en modelos ajustados por sexo, edad y uso de estatinas ($p < 0,05$), pero después de ajustar por múltiples factores de confusión, incluidos los parámetros lipídicos convencionales, solo HDL-P (OR 0,440 [0,204-0,951]), HDL-P media (OR 0,754 [0,590-0,963]; $p = 0,024$), contenido de colesterol en HDL-P (OR 0,692 [0,495-0,968]), número de LDL-P por ^1H -RMN/colesterol LDL convencional (OR 1,144 [1,026-1,275]), y las relaciones entre el número de partículas no HDL por ^1H -RMN y el colesterol no-HDL convencional (OR 1,178 [1,019-1,361]) permanecieron asociados con la aterosclerosis.

Conclusiones: En adultos con DTI y alto RCV, las variables relacionadas con HDL, LDL y el número total de partículas aterogénicas están independientemente asociadas con la aterosclerosis preclínica. El perfil avanzado de lipoproteínas podría usarse para identificar a aquellos con mayor riesgo de ECV.



Advanced lipoprotein profile identifies atherosclerosis better than conventional lipids in type 1 diabetes at high cardiovascular risk

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Advanced lipoprotein;
Nuclear magnetic resonance;
LDL particles

Abstract *Background and aims:* People with type 1 diabetes (T1D) present lipoprotein disturbances that could contribute to their increased cardiovascular disease (CVD) risk. We evaluated the relationship between lipoprotein alterations and atherosclerosis in patients with T1D.

Methods and results: Cross-sectional study in subjects with T1D, without previous CVD, but high-risk (≥ 40 years, nephropathy, or ≥ 10 years of evolution of diabetes with another risk factor). The presence of plaque (intima-media thickness ≥ 1.5 mm) in the different carotid segments was determined by ultrasound. The advanced lipoprotein profile was analysed by magnetic resonance imaging (^1H NMR). We included 189 patients (42% women, 47.8 ± 10.7 years, duration of diabetes 27.3 ± 10.1 years, HbA1c 7.5% [7–8]). Those with carotid plaques (35%) were older, with longer diabetes duration, had a higher prevalence of hypertension, and showed lower and smaller LDL particles (LDL-P) and HDL particles (HDL-P), but higher VLDL particles (VLDL-P). Some LDL, HDL and VLDL-related parameters were associated with atherosclerosis in sex, age and statin use adjusted models ($p < 0.05$), but after adjusting for multiple confounders, including conventional lipid parameters, only HDL-P (OR 0.440 [0.204–0.951]; $p = 0.037$), medium HDL-P (OR 0.754 [0.590–0.963]; $p = 0.024$), HDL-P cholesterol content (OR 0.692 [0.495–0.968]; $p = 0.032$), ^1H NMR LDL-P number/conventional LDL-cholesterol (OR 1.144 [1.026–1.275]; $p = 0.015$), and ^1H NMR non-HDL particle number/conventional non-HDL-cholesterol ratios (OR 1.178 [1.019–1.361], $p = 0.026$) remained associated with atherosclerosis. *Conclusions:* In adults with T1D at high-risk, variables related to HDL, LDL and total atherogenic particle number are independently associated with preclinical atherosclerosis. Advanced lipoprotein profiling could be used to identify those at the highest risk of CVD.

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1. Introduction

In people with type 1 diabetes (T1D), cardiovascular disease (CVD) is the leading cause of mortality [1]. Despite greater awareness of risk factors, better glycaemic control and patient education, and a global reduction in the incidence of cardiovascular events, people with T1D still present a four to eight-fold higher risk of CVD than the general European population [2,3]. Moreover, even in those with optimal glycaemic control (time-updated HbA1c $\leq$ 6.9% or 51.9 mmol/mol), cardiovascular death is almost triple compared with their non-diabetic counterparts [4]. Although classic factors such as high levels of LDL cholesterol (LDL-C), plasma triglycerides or low HDL cholesterol (HDL-C) have been consistently associated with CVD [5,6], they do not explain all the excess risk observed in this population. In addition, individuals with T1D and optimal glycaemic control usually have apparently normal or even “beneficial” conventional lipid profiles (normal or low LDL-C and plasma triglycerides and normal or even elevated HDL-C). However, they appear to have numerous qualitative and functional alterations in their lipoprotein subclasses [7]. Thus, the current evidence takes us away from the classic glucocentric scenario and makes it imperative to understand, and subsequently manage, other factors involved in their high residual cardiovascular risk [8–10], such as the abovementioned lipoprotein disturbances. To determine these disturbances there are different techniques: ultracentrifugation, gradient gel electrophoresis, high-performance liquid chromatography, or nuclear magnetic resonance spectroscopy (^1H NMR). The latter is more efficient due to the shorter performance time, high throughput and lower variability between laboratories [11].

Against this background, we previously studied the advanced lipoprotein profile by ^1H NMR in a cohort of Mediterranean adults with T1D at increased cardiovascular risk, as well as their differences from the general population [12]. In the present investigation, our objectives are to describe the ^1H NMR-assessed lipoprotein profile in our sample of T1D adults at high risk, analyse their associations with other clinical and laboratory parameters and also their relationship with the presence of carotid atherosclerosis measured by ultrasound.

2. Methods

2.1. Participants

This was a cross-sectional study of T1D patients without prior CVD (coronary artery disease, ischaemic stroke, peripheral artery disease, or heart failure) and high CVD risk according to the European CVD prevention guidelines [13]: a) age $\geq$ 40 years; b) presence of any stage of diabetic kidney disease; c) or $\geq$ 10 years of diabetes duration and at least one additional CVD risk factor. The following CVD risk factors were considered: family history of premature CVD (defined as $<$ 55 years in men and $<$ 65 years in women), active smoking habit, hypertension, low HDL-cholesterol

levels ($<$ 40 mg/dL in males, $<$ 45 mg/dL in females), high triglycerides levels ($>$ 150 mg/dL), being already on statins, a former episode of preeclampsia/eclampsia, the presence of diabetic retinopathy, impaired hypoglycemia awareness or a previous episode of severe hypoglycemia in the last two years. Subjects were recruited from a specialised Diabetes Unit of the Hospital Clinic in Barcelona, Spain. Details about the study subjects have already been published [14]. All patients provided informed consent, and the study was approved by the Ethical Committee of the hospital (number of approval HCB/2017/0977).

2.2. Clinical and laboratory parameters

Details of clinical and laboratory measurements can be found in [Supplementary material](#).

2.3. Carotid B-mode ultrasound imaging

The presence of carotid plaque was determined as described previously [14,15]. For further details see [Supplementary material](#).

2.4. ^1H NMR spectroscopy analysis of lipoproteins (advanced lipoprotein profile)

Two-dimensional diffusion-ordered ^1H NMR spectroscopy (DOSY) to assess plasma lipoprotein analysis was performed as described elsewhere [16]. The specifications can be found in the [Supplementary material](#).

2.5. Statistical analysis

All statistical analyses are summarized in the [Supplementary material](#).

3. Results

3.1. Subject's characteristics

A total of 189 subjects were included (42% women, mean age 47.8 ± 10.7 years, mean duration of diabetes 27.3 ± 10.1 years). Overall, 50% were never smokers, 31% had hypertension, 15% had obesity (BMI $\geq$ 30 kg/m²), 11% had nephropathy, and 38% had retinopathy. There were 44% of patients on statin treatment. In the whole cohort, 35% had at least one carotid plaque, with no between-gender differences ($p = 0.127$). As expected, those with atherosclerosis were older and had an increased prevalence of hypertension, a longer duration of diabetes, and elevated triglyceride levels ($>$ 150 mg/dL). However, they had lower levels of total cholesterol and non-HDL cholesterol, mainly at the expense of better control of LDL-C levels ($p < 0.05$ for all) and a trend for increased use of lipid-lowering drugs ($p = 0.082$). The remaining characteristics are shown in [Table 1](#).

Baseline characteristics according to sex and statin treatment were also assessed. In brief, women presented an overall better lipid profile (increased levels of HDL-

Table 1 Characteristics of the study participants according to the presence of carotid plaque.

	Whole (n = 189)	No plaque presence (n = 123)	Plaque presence (n = 66)	p-value
Clinical characteristics				
Sex (male)	109 (58)	66 (54)	43 (65)	0.127
Age (years)	47.84 (± 10.67)	45.04 (± 10.30)	53.07 (± 9.35)	<0.001
Premature CVD in first-degree relatives ^a	25 (13)	19 (15)	6 (9)	0.219
Never smokers	95 (50)	63 (51)	32 (49)	0.720
Hypertension	58 (31)	29 (24)	29 (44)	0.004
SBP (mmHg)	126 (117–137)	123 (116–132)	133 (119–143)	0.001
DBP (mmHg)	80.37 (± 8.91)	80.19 (± 8.94)	80.70 (± 8.91)	0.709
BMI (kg/m ²)	26.26 (± 3.96)	26.27 (± 4.17)	26.24 (± 3.56)	0.958
Obesity (BMI ≥ 30 kg/m ²)	29 (15)	20 (16)	9 (14)	0.633
Waist circumference (cm)	92.20 (± 12.32)	91.52 (± 12.33)	93.44 (± 12.31)	0.311
Female (cm)	86.11 (± 11.28)	85.98 (± 11.00)	86.43 (± 12.21)	0.872
Male (cm)	96.72 (± 11.08)	96.46 (± 11.38)	97.19 (± 10.73)	0.742
T1D duration (years)	27.35 (± 10.11)	26.08 (± 8.84)	29.71 (± 11.85)	0.031
Diabetic nephropathy	21 (11)	13 (11)	8 (12)	0.746
Diabetic retinopathy	72 (38)	46 (37)	26 (39)	0.788
CSII therapy	75 (40)	51 (42)	24 (36)	0.495
Conventional lipid profile				
Total cholesterol (mg/dL)	184.33 (± 31.27)	189.66 (± 27.98)	174.40 (± 34.71)	0.001
HDL-cholesterol (mg/dL)	56 (48–69)	56 (49–70)	54 (46–67)	0.059
Low HDL-cholesterol ^b	26 (14)	13 (11)	13 (20)	0.082
LDL-cholesterol (mg/dL)	107.88 (± 24.51)	112.18 (± 20.60)	100 (± 28.96)	0.003
LDL-cholesterol <100 mg/dL	67 (35)	30 (24)	37 (56)	<0.001
LDL-cholesterol <70 mg/dL	11 (6)	3 (2)	8 (12)	0.007
Triglycerides (mg/dL)	72 (56–100)	71 (56–87)	79 (56–121)	0.102
Triglycerides >150 mg/dL	15 (8)	6 (5)	9 (14)	0.034
Non-HDL cholesterol (mg/dL)	124.85 (± 27.27)	128.50 (± 23.37)	118.03 (± 32.47)	0.022
Remnant cholesterol (mg/dL)	14 (11–19)	14 (11–17)	16 (11–24)	0.075
Other laboratory characteristics				
Fasting plasma glucose (mg/dL)	145 (106–190)	140 (102–188)	153 (112–193)	0.308
Haemoglobin A1c (% (mmol/mol))	7.52 (7–8), (58.7)	7.5 (7–7.93), (58.5)	7.57 (7.13–8.17), (59.2)	0.549
Serum creatinine (mg/dL)	0.83 (0.72–0.95)	0.81 (0.72–0.93)	0.86 (0.73–0.98)	0.267
eGFR (CKD-EPI; ml/min/1.73 m ²)	99 (87–108)	102 (90–109)	95 (81–104)	0.008
ALT (U/L)	19 (15–26)	19 (15–24)	20 (16–27)	0.331
Albumin-to-creatinine ratio	4 (2–7)	4 (2–7)	5 (3–9)	0.187
Pharmacological treatment				
Statins	84 (44)	49 (40)	35 (53)	0.082
ACEi/ARB	62 (33)	32 (26)	30 (46)	0.007
Antiplatelet drugs	17 (9)	9 (7)	8 (12)	0.271

Data are shown as n (percentage) within each column, mean ($\pm$ SD) or median (Q1–Q3).

p values for group comparisons are reported and shown in bold if statistically significant.

ACEi: angiotensin-converting enzyme inhibitor; ALT: alanine aminotransferase; ARB: angiotensin receptor blocker; CVD: cardiovascular disease; CSII: continuous subcutaneous insulin infusion; DBP: diastolic blood pressure; eGFR: estimated glomerular filtration rate; HDL: high density lipoprotein; LDL: low density lipoprotein; SBP: systolic blood pressure; T1D: type 1 diabetes.

^a Defined as <55 years in men and <65 years in women.

^b Defined as HDL-cholesterol <40 mg/dL in men and <50 mg/dL in women.

cholesterol and lower levels of triglycerides and remnant cholesterol) and were more frequently on CSII therapy than men ($p < 0.05$ for all; [Supplementary Table 1](#)). On the other hand, those patients on statin treatment were older, had a higher prevalence of hypertension, lower LDL-C levels and eGFR, and were also more prone to receive other cardioprotective drugs ($p < 0.05$ for all; [Supplementary Table 2](#)).

3.2. Relationships between ¹H NMR-assessed advanced lipoprotein profile and other clinical and laboratory variables

The relationships between ¹H NMR advanced lipoprotein profile according to several traits are shown in [Fig. 1](#). As expected, the strongest correlations ($r_s > 0.4$) were

between variables related to VLDL and HDL (positively and inversely, respectively) with adiposity variables (waist circumference and BMI). Circulating leukocyte levels, as a marker of inflammation, were also moderately correlated with VLDL-related variables ($r_s = 0.2$ – 0.3); and glycaemic control showed a weak-to-moderate correlation with most VLDL- and LDL-related variables ($r_s = 0.1$ – 0.3). There was no correlation with the presence of retinopathy or nephropathy (data not shown), although there was a weak correlation of urinary albumin-to-creatinine ratio with VLDL-related variables ($r_s = 0.1$ – 0.2). Furthermore, when analysing those without statin treatment ($n = 105$), most of the correlations were maintained, and positive correlations were also observed between adiposity parameters and LDL-related parameters, mainly at the expense of small LDL-P ($r = 0.3$ – 0.4 ; [Supplementary Fig. 1](#)).

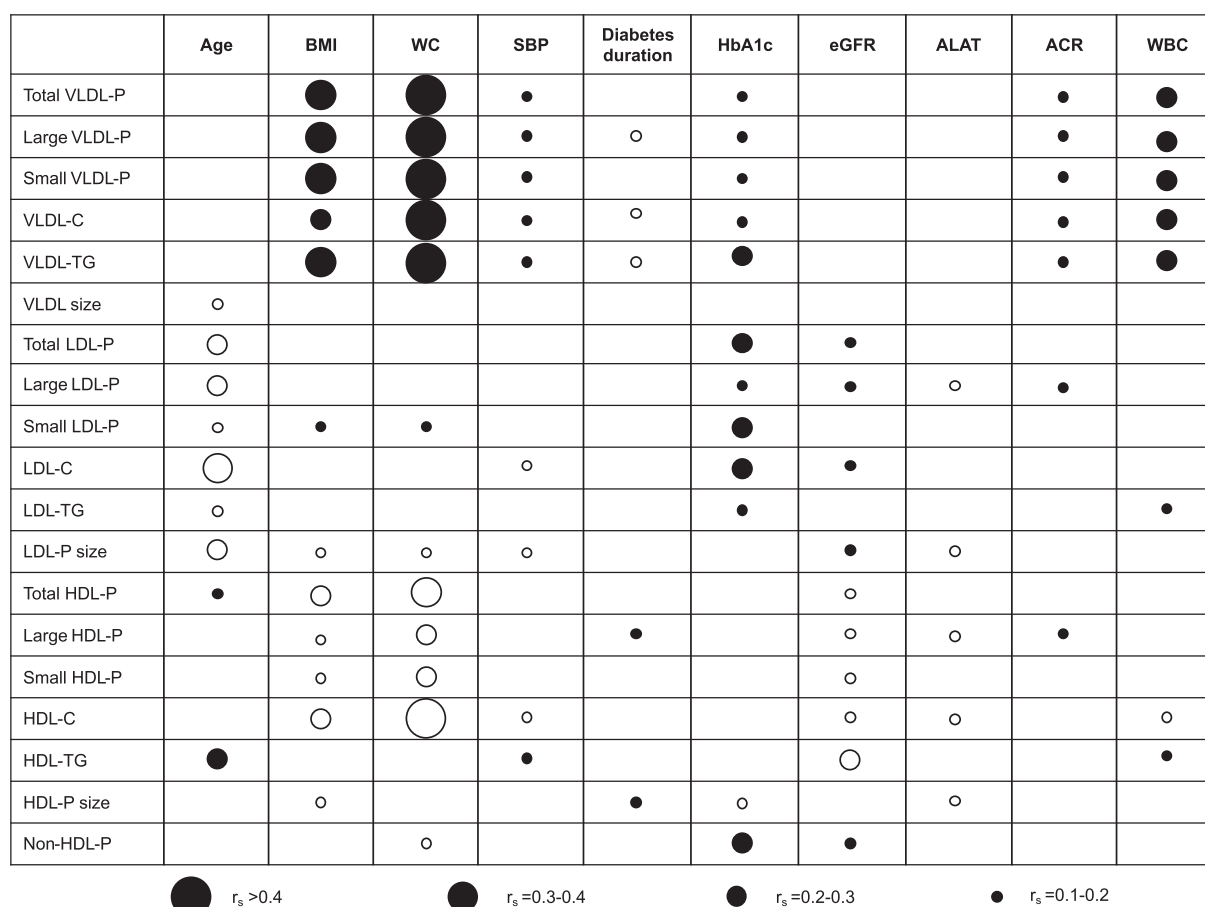


Figure 1 Associations between NMR-assessed advanced lipoprotein profile and clinical and laboratory parameters. Solid and open circles indicate positive and negative relationships, respectively. ACR: albumin-to-creatinine ratio; ALAT: alanine aminotransferase; BMI: body mass index; eGFR: estimated glomerular filtration rate; FLI: fatty liver index; HDL: high-density lipoprotein; HDL-C: cholesterol content in HDL; HDL-P: HDL particles; HDL-TG: triglyceride content in HDL; LDL: low-density lipoprotein; LDL-C: cholesterol content in LDL; LDL-P: LDL particles; LDL-TG: triglyceride content in LDL; NMR: nuclear magnetic resonance; SBP: systolic blood pressure; VLDL: very low-density lipoprotein; VLDL-C: cholesterol content in VLDL; VLDL-P: VLDL particles; VLDL-TG: triglyceride content in VLDL; WBC: white blood cells; WC: waist circumference.

The advanced lipoprotein profile was also evaluated according to sex and statin treatment. Women had an overall better profile, with lower VLDL of all subtypes, higher HDL-P (and cholesterol carried by them) and the ratio between LDL-P and HDL-P ($p < 0.001$ for all comparisons), despite a similar number of LDL and non-HDL particles ($p = 0.412$ and $p = 0.983$, respectively; [Supplementary Table 3](#)). Regarding lipid-lowering drugs, statin users also presented a better profile, with lower LDL-P/HDL-P ratio ($p < 0.01$), total particles/HDL-P ratio ($p < 0.01$) and lower non-HDL particles than non-statin users ($p = 0.019$) principally as a result of lower medium and large LDL-P, despite a non-significant difference in total LDL-P number ($p = 0.206$; [Supplementary Table 4](#)).

3.3. Relationships between conventional and ^1H NMR-assessed advanced lipoprotein profile with preclinical atherosclerosis

Overall, those with carotid plaques had a worse lipoprotein profile than those without atherosclerosis (higher number of VLDL-P, smaller LDL-P and a lower number of HDL-P

[and lower cholesterol content in these] ($p < 0.05$ for all)), despite lower levels of total atherogenic lipoproteins (non-HDL particles), mainly due to the reduced presence of medium and large LDL-P ($p < 0.022$ for all) ([Table 2](#)). In age, sex and statin treatment-adjusted models ([Supplementary Table 5](#)), conventionally assessed remnant cholesterol, small-LDL-P/total LDL-P ratio, VLDL-P number (and its cholesterol content) and some atherogenic ratios (LDL-P/LDL-C (conventional), LDL-P number/LDL-C (^1H NMR), Non-HDL-P/Non-HDL-C (conventional) and, both conventional, triglycerides/remnant-C ratio) showed direct associations with preclinical atherosclerosis ($p < 0.05$ for all). Conversely, most HDL-related variables (conventional HDL-C, HDL-P, medium HDL-P, and ^1H NMR-assessed HDL-C) were negatively associated with the presence of carotid plaque ($p < 0.05$ for all).

Those parameters that achieved statistical significance in the previous model were further analysed in the fully-adjusted model ([Supplementary Fig. 2](#)). In conventional analysis, both remnant and total cholesterol remained largely unchanged (positively and negatively related, respectively). For the advanced lipoprotein profile, while

Table 2 ^1H NMR-assessed advanced lipoprotein profile according to the presence of carotid plaque.

	Whole (n = 189)	No plaque presence (n = 123)	Plaque presence (n = 66)	p-value
VLDL-P number (nmol/L)				
Total	31.39 (25.77–41.96)	30.75 (25.49–37.75)	34.08 (27.99–49.47)	0.012
Large	0.945 (0.712–1.202)	0.923 (0.711–1.06)	1.023 (0.727–1.457)	0.032
Medium	3.167 (2.123–4.660)	3.016 (2.146–4.213)	3.672 (1.958–5.687)	0.205
Small	27.27 (23.32–34.83)	26.72 (22.55–31.37)	30.22 (24.38–42.10)	0.008
Ratio large/total	0.0279 (0.0255–0.0299)	0.0282 (0.0257–0.0299)	0.0269 (0.0249–0.0298)	0.084
VLDL-P composition (mg/dL)				
VLDL-C	9.463 (7.038–13.758)	9.163 (6.754–12.39)	10.58 (7.77–18.25)	0.041
VLDL-TG	43.31 (35.79–58.50)	42.17 (35.19–52)	46.63 (36.62–67.90)	0.010
Ratio VLDL-C/VLDL-TG	0.2125 (± 0.0487)	0.2117 (± 0.0476)	0.2140 (± 0.0510)	0.752
VLDL-P size (nm)	42.03 (41.77–42.32)	42.05 (41.79–42.35)	42 (41.58–42.30)	0.166
LDL-P number (nmol/L)				
Total	1261.23 (± 206.39)	1287.47 (± 193.82)	1212.33 (± 221.28)	0.022
Large	172.26 (155.08–189.96)	174.96 (161.36–189.65)	163.67 (147.44–191.44)	0.010
Medium	350.90 (277.79–421.28)	358.53 (313.24–440.87)	286.42 (242.94–401.54)	0.001
Small	715.03 (658.62–772.01)	715.89 (665.33–772.24)	705.05 (634.88–770.44)	0.368
Ratio small/total	0.5797 (± 0.0637)	0.5688 (± 0.0547)	0.5999 (± 0.0740)	0.003
LDL-P composition (mg/dL)				
LDL-C	124.86 (± 21.60)	128.10 (± 20.24)	118.82 (± 22.87)	0.007
LDL-TG	13.35 (11.88–15.77)	13.84 (12.18–15.87)	12.93 (10.38–15.65)	0.135
Ratio LDL-C/LDL-TG	9.196 (8.027–10.164)	9.224 (8.032–10.184)	9.093 (7.982–10.082)	0.641
LDL size (nm)	20.98 (± 0.31)	21.03 (± 0.28)	20.90 (± 0.36)	0.013
HDL-P number ($\mu\text{mol/L}$)				
Total	30.64 (27.83–34.68)	31.32 (28.2–36.36)	29.37 (27.08–32.85)	0.036
Large	0.2656 (0.2483–0.2905)	0.2677 (0.2505–0.2970)	0.2640 (0.2410–0.2840)	0.353
Medium	10.42 (9.31–11.74)	10.61 (9.43–12.03)	9.85 (8.84–11.28)	0.010
Small	20.58 (± 4.10)	20.91 (± 4.23)	19.98 (± 3.82)	0.137
Ratio small/total	0.6511 (± 0.0384)	0.6496 (± 0.0356)	0.6538 (± 0.0433)	0.493
HDL-P composition (mg/dL)				
HDL-C	60.75 (53.61–70.24)	63.20 (55.67–73.67)	57.12 (52–65.60)	0.003
HDL-TG	17.06 (± 4.40)	16.72 (± 4.64)	17.68 (± 3.87)	0.155
Ratio HDL-C/HDL-TG	3.892 (± 1.103)	4.081 (± 1.092)	3.539 (± 1.043)	0.001
HDL size (nm)	8.273 (± 0.067)	8.275 (± 0.062)	8.268 (± 0.076)	0.570
Other atherogenic variables				
Non-HDL-P (nmol/L)	1238.2 (1132.9–1374.1)	1290.2 (1155.9–1374)	1186.9 (1039.1–1380)	0.013
Ratio LDL-P/HDL-P	40.24 (33.83–46.74)	40.79 (33.85–46.90)	38.83 (32.67–46.61)	0.498
Ratio total-P/HDL-P	41.69 (34.58–48.31)	42.22 (34.93–48.75)	41.04 (33.74–48.26)	0.647
Ratio LDL-P/LDL-C (^1H NMR)	10.09 (9.88–10.28)	10.04 (9.84–10.23)	10.16 (9.99–10.43)	0.004

Data are shown as mean ($\pm$ SD) or median (Q1–Q3).

p values for group comparisons are reported and shown in bold if statistically significant.

HDL: high-density lipoprotein, HDL-C: cholesterol content in HDL, HDL-P: HDL particles, HDL-TG: triglyceride content in HDL, LDL: low-density lipoprotein, LDL-C: cholesterol content in LDL, LDL-P: LDL particles, LDL-TG: triglyceride content in LDL, ^1H NMR: nuclear magnetic resonance spectroscopy, Non-HDL-P: Non-HDL particles, total-P: total particles, VLDL: very low-density lipoprotein, VLDL-C: cholesterol content in VLDL, VLDL-P: VLDL particles, VLDL-TG: triglyceride content in VLDL.

all the ^1H NMR-assessed HDL parameters continued inversely associated with atherosclerosis ($p < 0.05$ for all), VLDL-P-related parameters lost their statistical significance ($p > 0.076$ for all). However, when only those with suboptimal glycaemic control were assessed ($\text{HbA1c} \geq 8\%$ or 63.9 mmol/mol , $n = 48$), most VLDL-related parameters persisted positively associated with atherosclerosis (data not shown). Interestingly, most of the advanced lipoprotein ratios remained strongly associated: the small LDL-P/Total LDL-P ratio (per 0.1 increase, OR 1.932 [1.098–3.4]; $p = 0.022$), the ^1H NMR LDL-P number/conventional LDL-C (per 0.5-unit increase, OR 1.150 [1.038–1.275], $p = 0.036$) and the Non-HDL-P/conventional non-HDL-C (per 0.5-unit increase, OR 1.170 [1.018–1.346, $p = 0.028$; [Supplementary Fig. 2](#)). Finally, the conventional triglycerides/HDL-C ratio lost significance (per 0.5-unit increase, OR 1.115 [0.986–1.261], $p = 0.084$). Of note,

this ratio showed weak or negligible correlation with LDL-P size parameters such as LDL-P size, small LDL-P/total LDL-P ratio, and LDL-P/LDL-C ratio, both assessed by ^1H NMR and conventional (data not shown). No major changes were observed when the fully-adjusted models were applied only in those without lipid-lowering treatment ($n = 105$; $p < 0.05$ for most of the analysis), except for the small LDL-P/Total LDL-P ratio and the Non-HDL-P/conventional non-HDL-C ($p = 0.127$ and $p = 0.132$, respectively; [Supplementary Fig. 3](#)).

Those ^1H NMR-derived variables that remained significantly associated with the presence of plaque in fully-adjusted models were further adjusted by conventional lipid variables (except for variables strongly correlated; see the methods section; [Fig. 2](#)). For HDL-related variables, no major changes were found after including conventional LDL-C and triglycerides ($p < 0.05$ for HDL-P, medium HDL-

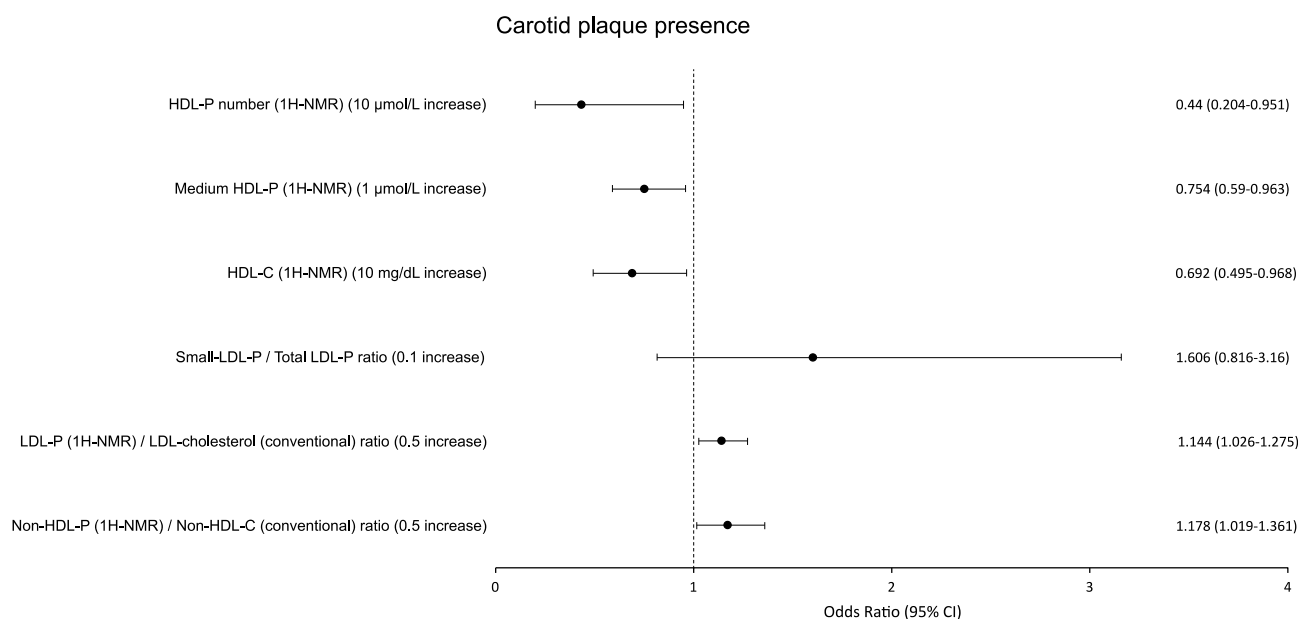


Figure 2 Relationships between ^1H NMR-assessed advanced lipoprotein profile with the presence of carotid plaque in regression analyses. Adjusted per sex, age, body mass index, diabetes duration, systolic blood pressure, presence of diabetic retinopathy, smoking status, glycated haemoglobin (HbA1c), urine-to-creatinine ratio, statin score and antihypertensive treatment (angiotensin-converting enzyme inhibitor or angiotensin receptor blocker). HDL-related variables were further adjusted per conventional LDL-C and triglycerides. LDL-P/LDL-C (conventional) ratio was further adjusted per conventional HDL-C and triglycerides. Small LDL-P/total LDL-P ratio was further adjusted per conventional LDL-C, HDL-C and triglycerides. Non-HDL-P/Non-HDL-C (conventional) ratio was further adjusted per conventional HDL-C and triglycerides. Odds Ratio with confidence intervals (95%). HDL: high-density lipoprotein, HDL-C: cholesterol content in HDL, HDL-P: HDL particles, LDL-C: cholesterol content in LDL, LDL-P: LDL particles, ^1H NMR: nuclear magnetic resonance spectroscopy, Non-HDL-C: cholesterol content in non-HDL, Non-HDL-P: non-HDL particles.

P and HDL-P cholesterol content). For LDL-related variables, the ^1H NMR LDL-P number/conventional LDL-C ratio remained largely unchanged (per 0.5-unit increase; OR 1.144 [1.026–1.275]; $p = 0.015$) after the inclusion of conventional HDL-C and triglycerides, whereas the association with small LDL-P/Total LDL-P ratio was blunted ($p = 0.170$), after the inclusion of conventional LDL-C, HDL-C and triglycerides. Finally, the Non-HDL-P/Non-HDL-C (conventional) maintained the association (per 0.5-unit increase, OR 1.178 [1.019–1.361], $p = 0.026$), after further adjustment per conventional HDL-C and triglycerides.

4. Discussion

In our cohort of adults with T1D at high cardiovascular risk, those harbouring plaques had an overall worse lipoprotein profile (i.e., higher VLDL-P, lower HDL-P and smaller LDL-P). As expected, most VLDL-related variables were correlated with adiposity parameters (i.e., BMI and waist circumference), glycaemic control, and inflammation (i.e., leukocyte count). Finally, in the regression analyses, most HDL-related and several LDL-related variables were significantly associated, negatively and positively respectively, with the presence of atherosclerosis. Importantly, the ^1H NMR LDL-P number/conventional LDL-C and the ^1H NMR Non-HDL-P/conventional non-HDL-C ratios remained significantly associated independently of other multiple confounders, including conventional lipid parameters. As far as we are aware, no prior study has

associated this new ^1H NMR-derived ratios with preclinical atherosclerosis in this population, especially in the setting of a high background statin use.

4.1. Advanced lipoprotein profile according to sex and statin treatment

In our sample, women showed an overall better advanced lipoprotein profile (Supplementary Table 3) with lower VLDL-related parameters and higher HDL-related parameters despite a similar concentration of some atherogenic lipoproteins (LDL-P and non-HDL-particle number). Our results are in consonance with our previously reported analyses [12] in a large and multicentre study of Mediterranean adults with T1D ($n = 508$), where it was also pointed out that the women's known protective profile, with lower total atherogenic lipoproteins versus men in the general population, was blunted in the T1D cohort. Some older studies also observed similar between-sex differences but with higher LDL-P subfractions among men [17,18]. Notably, in those cohorts, the use of lipid-lowering therapy was very low ($<10\%$) in comparison to nearly half of our sample (Table 1), thus the results are not directly comparable. Some other studies using different techniques to assess the advanced lipoprotein profile as biochemical analyses [19], ultracentrifugation or liquid chromatography [20] showed conflicting results. However, it is important to note that the use of different methodologies may not correlate well with each other [21]. Further, in a meta-analysis [22], women with T1D showed

a 2-to-3-fold increased excess risk of coronary heart disease compared to men, and a recently published study of the DCCT/EDIC cohorts showed that despite a more favourable cardiovascular risk profile, women with T1D did not have lower rates of CVD than men [23]. These results highlight the complexity of CVD physiopathology in T1D, particularly among women, where other mechanisms, apart from lipid factors, are probably also involved [9].

Statin treatment reduces not only the cholesterol content of most apolipoprotein B-lipoproteins but also, more modestly, their triglyceride content [24]. In addition, reductions are observed in the concentrations of VLDL-P (mainly the smaller ones) and in large, medium and, to a lesser extent, small LDL-P, which has also been verified in Mendelian randomization studies [25]. The effects seen in the general population were similar to those in our study, except for a discrete increase rather than a decrease in the concentration of small VLDL-P (Supplementary Table 4). The presence of insulin resistance or worse glycaemic control could impact the amount and distribution of VLDL-P in T1D subjects [7]. In this regard, among our participants, those under statin therapy showed a trend (although not significant) towards a higher BMI and waist circumference, which could explain our results (Supplementary Table 2).

4.2. ¹H NMR -assessed lipoproteins correlations with adiposity and other clinical and laboratory parameters

In our cohort, the strongest univariate associations were observed between VLDL-related and HDL-related parameters with adiposity markers, such as BMI and waist circumference (Fig. 1). Even more, LDL-P, mainly at the expense of small LDL-P, was correlated with adiposity in those without statin treatment (Supplementary Fig. 1). These results align with the known associations of insulin resistance with atherogenic dyslipidaemia [26] characterised by increased serum triglycerides, decreased HDL-C and smaller LDL-P. Additionally, our analyses are also in concordance with previous studies performed in a larger T1D sample [12] and by using a hyperinsulinaemic-euglycaemic clamp [20]. Moreover, using ¹H NMR spectroscopy, similar lipoprotein alterations have been observed in other conditions associated with insulin resistance, such as non-alcoholic fatty liver disease [27] or newly diagnosed type 2 diabetes [15].

Hyperglycaemia is associated with detrimental disturbances in lipoprotein fractions [7]. Data from the DCCT/EDIC cohort showed associations between a worse glycaemic control and ¹H NMR-assessed total LDL-P concentration, small VLDL-P and HDL-P [17]. Furthermore, those randomized to conventional instead of intensive diabetes treatment showed detrimental lipoprotein disturbances with, principally, increased levels of LDL-P (and the smaller subfraction) and small HDL-P, with a similar conventional lipid profile except for higher triglycerides levels [28]. As expected, we found weak and direct associations between all the VLDL-related and LDL-related parameters

with HbA1c, and also an inverse association with HDL-P size (Fig. 1), which are also in consonance with our previous reports [12].

4.3. Relationships between the advanced lipoprotein profile and atherosclerosis

One of the main findings of our study is the independent and positive association of atherosclerosis with the ¹H NMR LDL-P number/conventional LDL-C and the ¹H NMR Non-HDL-P/conventional non-HDL-C ratios and an inverse correlation with various HDL-related parameters (Fig. 2 and Supplementary Fig. 2). Although conventional LDL-C levels have been strongly associated with CVD in T1D [5,6], the performance of this conventional parameter in predicting future CVD events could be hampered especially given the high use of lipid-lowering drugs in high-risk populations [29]. Thus, ¹H NMR-assessed advanced lipoprotein profile could help to uncover some traits associated with CVD risk. The ¹H NMR LDL-P number/conventional LDL-C ratio may serve as a surrogate of the atherogenic small and dense LDL-P, and we believe that a greater discrepancy between LDL-P and conventional LDL-C, may be an important factor involved in the high residual risk of this population. We previously observed, in a bigger sample, that individuals with T1D may have greater discrepancies compared to their non-diabetic counterparts [12]. In fact, in the general population, the total LDL-P number could outperform conventional LDL-C levels in CVD prediction [30]. Further, some other studies suggested that a higher discordance between LDL-P and conventional LDL-C could identify those more prone to suffer from future CVD [31]. Additionally, the direct association of the ¹H NMR Non-HDL-P/conventional non-HDL-C ratio with atherosclerosis reinforces the importance of this discordance not only in LDL particles but also in the overall number of atherogenic lipoprotein particles. Pending confirmatory studies, our data suggest that the ¹H NMR LDL-P number/conventional LDL-C and the ¹H NMR Non-HDL-P/conventional non-HDL-C ratios could be promising biomarkers in the T1D population, especially considering the strong and independent association with preclinical carotid atherosclerosis, even after adjusting for conventional lipid profile parameters. Specifically, it might be used in those individuals with conventional LDL-C close to the target, in whom a high particle content could be especially detrimental in the atherogenic process.

Concerning the T1D population, data from the Finnish Diabetic Nephropathy (FinnDiane) Study [32] pointed to a greater predictive utility of total atherogenic particles (assessed as apolipoprotein B plasma concentration) instead of LDL-C. Nonetheless, some analyses performed 20–30 years ago, found no association between advanced lipoprotein profile and atherosclerosis [18], and others showed conflicting results between various lipoprotein parameters measured by ¹H NMR-spectroscopy with carotid artery IMT [33] or coronary artery disease [34]. It should be noted that at the time of these studies, diabetes management was different and glycaemic control, among

other parameters, was largely suboptimal. In addition, the setting of our study, and a probable greater adherence to the Mediterranean diet pattern, could be accompanied by beneficial changes in the lipoprotein profile [35], so the results may be substantially different. In this sense, a more recent study supports the possible protective role of medium HDL-P in atherosclerosis seen in our sample [36]. Furthermore, another study carried out in a Mediterranean setting, highlights the direct relationship of most VLDL-related parameters with an early marker of atherosclerosis (arterial stiffness) [37]. It should be mentioned that, although we did not find associations of VLDL-related parameters with the presence of carotid plaque in the fully-adjusted regression models for the whole sample (Fig. 2 and Supplementary Fig. 2), these associations were maintained when analysing only those with a worse glycaemic control ($\text{HbA1c} \geq 8\%$ or 63.9 mmol/mol), which was expected given the effects of suboptimal glycaemic control on lipoproteins [7].

4.4. Limitations and strengths

Our study has many strengths. First, we analysed the associations of the advanced lipoprotein profile using a precise and validated technique (^1H NMR -spectroscopy) with the presence of carotid plaque by ultrasound, as a proven proxy for CVD [38]. Second, the data collection was thorough in terms of clinical variables and treatments that could interfere with the results (i.e., lipid-lowering therapy), so there is less probability of bias in this regard. However, limitations should also be acknowledged. First, the type of study does not allow us to assess the causality or incidence of CVD thus these promising results need to be confirmed in commensurate prospective studies. Second, the inclusion criteria limit the extrapolation of our results to adults with T1D and high cardiovascular risk, so it should be interpreted in said context. Finally, the use of statins in almost half of the participants could have influenced the results. However, we used a statin score as a proxy of the exposure of this drug in the statistical models to overcome this limitation about atherosclerosis. Furthermore, the sensitivity analysis performed in subjects without lipid-lowering drugs yielded similar results.

5. Conclusions

In summary, our study shows that in our cohort of adults with T1D at high cardiovascular risk, the presence of preclinical carotid atherosclerosis implies detrimental overall lipoprotein subclasses disturbances, some of them (i.e., VLDL-related parameters) associated with adiposity, HbA1c and inflammation markers. Finally, some specific alterations were negatively (i.e., HDL-related parameters) or positively associated (^1H NMR LDL-P number/conventional LDL-C and ^1H NMR Non-HDL-P/conventional non-HDL-C ratios) with carotid plaques, and remained associated independently of other multiple confounders, including conventional lipid parameters. The higher residual cardiovascular risk present in individuals with T1D

could be partly explained by these lipoprotein alterations and their inclusion in the clinical practice should be evaluated in future prospective and well-designed studies. The identification of new risk markers may make it possible to identify those patients who may benefit from more aggressive and, above all, earlier treatment.

Author contributions

All authors have discussed the results and commented on the final version of the manuscript. TS-N, EO, MG, VP, LB, KM, CF, AM, CV, JB, IV, AP, LB, EE, IC and AJA acquired and processed all clinical data; AJA performed the US measurements; TS-N and AJA contributed to data analysis and interpretation, wrote, reviewed and edited the manuscript. AJA and VP contributed to the study concept and design. MG, VP, CV, JB, IV, IC and AJA supervised the study and participated in data analysis and interpretation. TS-N and AJA designed the figures and had final responsibility for the decision to submit for publication. AJA is the guarantor of this work and, as such, had full access to all the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis.

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Declaration of competing interest

The authors declare they have no conflict of interest.

Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.numecd.2023.03.025>.

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Supplementary material and methods

Corresponding to:

Advanced lipoprotein profile identifies atherosclerosis better than conventional lipids in type 1 diabetes at high cardiovascular risk

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Clinical and laboratory parameters

As a standardized parameter for statin treatment, we calculated a statin score[1], an estimation of lifetime exposure to these drugs and obtained as the product of the duration of treatment in years by the average dose received of statin drugs adjusted to simvastatin in milligrams per day.

The examination of the fundus oculi was used to diagnose diabetic retinopathy, which was always verified by a specialized ophthalmologist. Albumin-to-creatinine ratio was used to evaluate diabetic nephropathy, with <30 mg/g considered normal and ≥ 30 mg/g as diabetic nephropathy (verified on at least two out of three consecutive assessments). With no history of hypertension or CVD, the use of angiotensin-converting enzyme inhibitors or angiotensin II receptor blockers was also considered diabetic nephropathy.

Weight, height, waist circumference, and hip circumference were also recorded. The patient's weight was measured using a calibrated electronic scale while wearing light clothing and without footwear. The body mass index (BMI) was obtained by dividing weight in kilograms by height in square meters., the waist circumference was measured at halfway between the last rib and iliac crest. Finally, hip circumference was measured around the widest portion of the buttocks, with the tape parallel to the floor.

Blood and first-morning urine samples were collected and analysed. No participant reported stress the day before blood was collected (strenuous exercise, minor illness, etc.). Standardized assays were used to analyse lipid profiles (including total cholesterol, triglycerides, and HDL-cholesterol), glucose, creatinine, and the albumin-to-creatinine ratio. The Friedewald formula was used to calculate conventional LDL-C. Non-HDL cholesterol was determined by subtracting HDL-C from the total cholesterol, and remnant cholesterol as total cholesterol minus HDL and LDL-C. The estimated glomerular filtration rate (eGFR) was obtained by the Chronic Kidney Disease Epidemiology Collaboration equation. Glycated haemoglobin (HbA1c) values (Tosoh G8 Automated HPLC Analyzer -Tosoh Bioscience Inc., South San Francisco, CA, USA - DCCT aligned, normal range 4-6%, (20-42 mmol/mol)) were also recorded. HbA1c values are shown as the mean of up to 2 annual determinations over the last 5 years.

Carotid B-mode ultrasound imaging

Carotid ultrasound images were generated using high-resolution B-mode ultrasound (Acuson X300 or Acuson X700 [Siemens]) with an electric linear array 5-10 MHz transducer. The presence of carotid plaque and the carotid intima-media thickness (IMT) were determined using predefined and standardized imaging protocols. B-mode and colour Doppler were used to visualize carotid plaques, both in longitudinal and transverse planes, to consider circumferential asymmetry. Carotid plaques were defined as focal echo-structures intruding into the arterial lumen with an $IMT \geq 1.5$ mm, or by at least 50% of the surrounding IMT value, as measured from the media-adventitia interface to the intima-lumen surface[2]. A trained endocrinologist (A.J.A) performed all the procedures, and IMT measurements were assessed using semiautomatic software. The mean and mean-maximum IMT of all carotid territories (common carotid, bulb, and internal carotid) were recorded, in addition to the maximum height of the plaque. In the presence of carotid plaques, the maximum IMT was equal to the highest carotid plaque height. Peak systolic and end-diastolic velocities were used to evaluate carotid stenosis, and also the planimetric area in transversal view was measured when significant carotid plaques ($IMT > 2.5$ mm) were spotted.

^1H -NMR spectroscopy analysis of lipoproteins (advanced lipoprotein profile)

This technique evaluates the lipid concentrations (i.e., cholesterol and triglycerides), particle number and size of three different lipoprotein classes (VLDL, LDL, and HDL), along with the particle number of nine subclasses (large, medium and small VLDL, LDL, and HDL). Shortly, the methyl signal of the plasma 2D ^1H -NMR spectra was deconvoluted by using 9 Lorentzian functions to ascertain the lipid concentration of the large, medium and small lipoprotein subclasses mentioned previously, and the diffusion coefficient associated with each analytical function, which is associated with the particle size. Moreover, we combined the lipid concentration and diffusion coefficient-derived particle volume to quantify the number of particles needed to transport the determined lipid concentration of each lipoprotein subclass. Further, weighted average VLDL, LDL, and HDL particle sizes were assessed from the subclass concentrations by summing the known diameter of each subclass and multiplying by its relative percentage of subclass particle number. The mean particle diameter for the subclasses and the estimated ranges were as follows: large VLDL particles (VLDL-P), 75.2 nm (> 60 nm); medium VLDL-P, 52.1 nm (45–60 nm); small VLDL-P, 37.1 nm (35–45 nm); large

LDL particles (LDL-P), 22.8 nm (22.5–27 nm); medium LDL-P, 20.5 nm (20–22.5 nm); small LDL-P 18.9 nm (18–20 nm); large HDL particles (HDL-P), 10.1 nm (9–13 nm); medium HDL-P, 8.7 nm (8.2–9 nm); small HDL-P, 7.8 nm (< 8.2 nm). In the end, the cholesterol and triglyceride content of every lipoprotein class was also analysed with the ¹H-NMR-based Liposcale® test[3]. The plasma samples were separated by centrifugation and frozen at –80°C after collection, there was no defrosting procedure before the analysis.

Statistical analysis

We carried out a descriptive analysis of the data. The Kolmogorov-Smirnov test was used to verify the normal distribution of each continuous variable. Results for qualitative variables are expressed as frequency and percentage, for quantitative normal variables as mean ± standard deviation, and non-normal distribution variables as median (first quartile-third quartile). The comparison of binomial qualitative variables was made by means of the Chi-square test, quantitative variables with the Student's T test, and quantitative variables with non-normal distribution by means of the Mann-Whitney U test. All the analysed correlations between variables were assessed with the pairwise R of Spearman test.

To search for independent relationships between preclinical carotid atherosclerosis (carotid plaque presence; dependent variable) and conventional lipid and ¹H-NMR-assessed advanced lipoprotein parameters (independent variable), we constructed logistic binary regression models. In the first model, age, sex and statin treatment were included. Those parameters that remained statistically significant were analysed in the fully adjusted model (including age, sex, statin score, smoking habit, diabetes duration, HbA1c, BMI, systolic blood pressure, use of antihypertensive treatment (angiotensin-converting enzyme inhibitor or angiotensin receptor blocker), presence of diabetic retinopathy and albumin-to-creatinine ratio). Finally, the ¹H-NMR-assessed parameters still achieving statistical significance in the fully adjusted model were further evaluated in a model including the main conventional lipid parameters (LDL-C, HDL-C and triglycerides) but excluding those showing strong correlations ($r_s > 0.6$), to avoid multicollinearity biases.

All the analyses were performed using IBM® SPSS® Statistics v.26 (IBM Corporation, Armonk, New York) statistical software. The significance level was set as a p-value <0.05.

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Supplementary tables and figures

Corresponding to:

Advanced lipoprotein profile identifies atherosclerosis better than conventional lipids in type 1 diabetes at high cardiovascular risk

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Total Supplemental Tables: 5

Total Supplemental Figures: 3

Supplementary Table 1. Characteristics of the study participants according to sex.

	Women (n=80)	Men (n=109)	p-value
Clinical characteristics			
Age (years)	47.62 (±11.10)	48.01 (±10.39)	0.807
Premature CVD in first-degree relatives*	9 (11)	16 (15)	0.492
Never smokers	39 (49)	56 (51)	0.721
Hypertension	23 (29)	35 (32)	0.621
SBP (mmHg)	125 (113-135)	126 (118-140)	0.087
DBP (mmHg)	77.99 (±8.65)	82.11 (±8.72)	0.002
BMI (kg/m ²)	25.65 (±4-23)	26.70 (±3.70)	0.069
Obesity (BMI≥30 kg/m ²)	12 (15)	17 (16)	0.911
Waist circumference (cm)	86.11 (±11.28)	96.75 (±11.08)	<0.001
T1D duration (years)	27.99 (±9.27)	26.88 (±10.70)	0.458
Diabetic nephropathy	8 (10)	13 (12)	0.677
Diabetic retinopathy	29 (36)	43 (39)	0.654
CSII therapy	41 (51)	34 (31)	0.005
Conventional lipid profile			
Total cholesterol (mg/dL)	193.95 (±29.22)	177.27 (±30.96)	<0.001
HDL-cholesterol (mg/dL)	68 (55-79)	52 (44-59)	<0.001
Low HDL-cholesterol [†]	7 (9)	19 (17)	0.087
LDL-cholesterol (mg/dL)	110.19 (±21)	106.19 (±26.75)	0.253
LDL-cholesterol <100 mg/dL	26 (33)	41 (38)	0.477
LDL-cholesterol <70 mg/dL	1 (1)	10 (9)	0.022
Triglycerides (mg/dL)	67 (51-86)	80 (61-112)	0.001
Triglycerides >150 mg/dL	2 (3)	13 (12)	0.018
Non-HDL cholesterol (mg/dL)	125.38 (±25.48)	124.46 (±28.63)	0.820
Remnant cholesterol (mg/dL)	13 (10-17)	16 (12-22)	<0.001
Other laboratory characteristics			
Fasting plasma glucose (mg/dL)	142 (115-191)	147 (106-191)	0.994
Haemoglobin A1c (%; (mmol/mol))	7.63 (7-7.94), (59.9)	7.48 (7.12-8.04), (58.3)	0.789
Serum creatinine (mg/dL)	0.72 (0.66-0.82)	0.89 (0.80-0.99)	<0.001
eGFR (CKD-EPI; ml/min/1.73m ²)	97 (84-107)	100 (89-109)	0.319
ALT (UI/L)	17 (14-21)	22 (17-30)	<0.001
Albumin-to-creatinine ratio	5 (3-8)	3 (2-6)	0.030
Pharmacological treatment			
Statins	35 (44)	49 (45)	0.869
ACEi / ARB	26 (33)	36 (33)	0.939
Antiplatelet drugs	9 (11)	8 (7)	0.353

Data are shown as n (percentage) within each column, mean (±SD) or median (Q1-Q3).

p values for group comparisons are reported and shown in bold if statistically significant.

ACEi: angiotensin-converting enzyme inhibitor; ALT: alanine aminotransferase; ARB: angiotensin receptor blocker; BMI: body mass index; CVD: cardiovascular disease; CSII: continuous subcutaneous insulin infusion; DBP: diastolic blood pressure; eGFR: estimated glomerular filtration rate; HDL: high-density lipoprotein; LDL: low-density lipoprotein; SBP: systolic blood pressure; T1D: type 1 diabetes

* Defined as <55 years in men and <65 years in women.

[†] Defined as HDL-cholesterol <40 mg/dL in men and <50 mg/dL in women.

Supplementary Table 2. Characteristics of the study participants according to statin treatment.

	Statin non-users (n=105)	Statin users (n=84)	p-value
Clinical characteristics			
Sex (male)	60 (57)	49 (58)	0.869
Age (years)	45.83 (±10.68)	50.36 (±10.17)	0.004
Premature CVD in first-degree relatives*	12 (11)	13 (16)	0.414
Never smokers	54 (51)	41 (49)	0.720
Hypertension	23 (22)	35 (42)	0.003
SBP (mmHg)	124 (115-135)	127 (119-140)	0.058
DBP (mmHg)	80.43 (±8.19)	80.29 (±9.78)	0.913
BMI (kg/m ²)	25.80 (±3.70)	26.83 (±4.21)	0.075
Obesity (BMI≥30 kg/m ²)	14 (13)	15 (18)	0.391
Waist circumference (cm)	90.70 (±12.23)	94.08 (±12.26)	0.062
Female (cm)	84.69 (±12.36)	87.94 (±9.59)	0.203
Male (cm)	95.29 (±10.01)	98.55 (±12.13)	0.130
T1D duration (years)	26.76 (±9.53)	28.09 (±10.80)	0.370
Diabetic nephropathy	10 (10)	11 (13)	0.438
Diabetic retinopathy	37 (35)	35 (42)	0.366
CSII therapy	37 (35)	38 (45)	0.163
Conventional lipid profile			
Total cholesterol (mg/dL)	188.10 (±27.56)	179.62 (±34.97)	0.071
HDL-cholesterol (mg/dL)	55 (47-68)	57 (49-70)	0.313
Low HDL-cholesterol [†]	17 (16)	9 (11)	0.277
LDL-cholesterol (mg/dL)	113.44 (±20.79)	100.91 (±27.04)	0.001
LDL-cholesterol <100 mg/dL	23 (22)	44 (53)	<0.001
LDL-cholesterol <70 mg/dL	3 (3)	8 (10)	0.051
Triglycerides (mg/dL)	70 (55-103)	76 (58-95)	0.263
Triglycerides >150 mg/dL	6 (6)	9 (11)	0.206
Non-HDL cholesterol (mg/dL)	129.49 (±22.31)	119.05 (±31.63)	0.012
Remnant cholesterol (mg/dL)	14 (11-20)	15 (11-18)	0.298
Other laboratory characteristics			
Fasting plasma glucose (mg/dL)	143 (103-190)	148 (118-193)	0.366
Haemoglobin A1c (%, (mmol/mol))	7.5 (7-8.15), (58.5)	7.57 (7.13-7.93), (59.2)	0.882
Serum creatinine (mg/dL)	0.80 (0.72-0.93)	0.85 (0.73-0.99)	0.166
eGFR (CKD-EPI; ml/min/1.73m ²)	102 (91-109)	95 (83-104)	0.003
ALT (UI/L)	19 (14-26)	20 (16-26)	0.231
Albumin-to-creatinine ratio	4 (2-7)	4 (2-8)	0.775
Pharmacological treatment			
ACEi / ARB	24 (23)	38 (45)	0.001
Antiplatelet drugs	7 (7)	10 (12)	0.211

Data are shown as n (percentage) within each column, mean (±SD) or median (Q1-Q3).

p values for group comparisons are reported and shown in bold if statistically significant.

ACEi: angiotensin-converting enzyme inhibitor; ALT: alanine aminotransferase; ARB: angiotensin receptor blocker; CVD: cardiovascular disease; CSII: continuous subcutaneous insulin infusion; DBP: diastolic blood pressure; eGFR: estimated glomerular filtration rate; HDL: high-density lipoprotein; LDL: low-density lipoprotein; SBP: systolic blood pressure; T1D: type 1 diabetes

* Defined as <55 years in men and <65 years in women.

[†] Defined as HDL-cholesterol <40 mg/dL in men and <50 mg/dL in women.

Supplementary Table 3. ¹H-NMR-assessed advanced lipoprotein profile according to sex.

	Women (n=80)	Men (n=109)	p-value
VLDL-P number (nmol/L)			
Total	29.92 (23.75-33.70)	34.61 (29.19-46.85)	<0.001
Large	0.7875 (0.6155-0.9841)	1.023 (0.8302-1.3926)	<0.001
Medium	2.651 (1.815-3.946)	3.420 (2.532-5.039)	0.004
Small	25.58 (20.84-28.82)	29.36 (25.62-40.06)	<0.001
Ratio large/total	0.0265 (0.0248-0.0287)	0.0284 (0.0267-0.0317)	<0.001
VLDL-P composition (mg/dL)			
VLDL-C	7.918 (5.684-10.583)	11.233 (8.122-16.227)	<0.001
VLDL-TG	41.36 (32.62-46.19)	46.53 (39.31-63.25)	<0.001
Ratio VLDL-C/VLDL-TG	0.1917 (±0.0473)	0.2277 (±0.0440)	<0.001
VLDL-P size (nm)	41.97 (41.69-42.31)	42.06 (41.79-42.34)	0.400
LDL-P number (nmol/L)			
Total	1275.65 (±212.29)	1250.64 (±202.28)	0.412
Large	172.97 (155.95-192.46)	171.58 (153.21-189.08)	0.743
Medium	354.09 (289.93-452.56)	349.04 (272.03-402.25)	0.104
Small	710.05 (670.30-761.66)	718.19 (652-793.56)	0.686
Ratio small/total	0.5694 (±0.0569)	0.5872 (±0.0675)	0.058
LDL-P composition (mg/dL)			
LDL-C	126.82 (±22.87)	123.42 (±20.60)	0.286
LDL-TG	13.87 (11.97-16.65)	13.34 (11.54-15.50)	0.382
Ratio LDL-C/LDL-TG	9.128 (7.945-9.970)	9.247 (8.165-10.256)	0.496
LDL size (nm)	21.02 (±0.28)	20.95 (±0.34)	0.171
HDL-P number (μmol/L)			
Total	33.48 (30.31-37.63)	29.01 (26.69-31.60)	<0.001
Large	0.2741 (0.2554-0.3113)	0.2607 (0.2439-0.2826)	0.006
Medium	11.54 (10.24-12.66)	9.781 (8.730-10.73)	<0.001
Small	22.14 (±3.82)	19.44 (±3.94)	<0.001
Ratio small/total	0.6475 (±0.0342)	0.6536 (±0.0412)	0.283
HDL-P composition (mg/dL)			
HDL-C	68.59 (59.90-79.45)	57.09 (50.90-62.63)	<0.001
HDL-TG	17.66 (±3.94)	16.62 (±4.67)	0.108
Ratio HDL-C/HDL-TG	4.160 (±1.206)	3.695 (±0.982)	0.004
HDL size (nm)	8.277 (±0.060)	8.269 (±0.072)	0.440
Other atherogenic variables			
Non-HDL-P (nmol/L)	1232.5 (1149.3-1370)	1258.5 (1119.6-1385.7)	0.938
Ratio LDL-P/HDL-P	36.23 (31.73-43.38)	41.91 (35.76-51.08)	<0.001
Ratio total-P/HDL-P	37.10 (32.49-44.40)	43.10 (37.22-52.46)	<0.001
Ratio LDL-P/LDL-C (¹ H-NMR)	10.09 (9.84-10.24)	10.09 (9.89-10.40)	0.313

Data are shown as mean (±SD) or median (Q1-Q3).

p values for group comparisons are reported and shown in bold if statistically significant.

HDL: high-density lipoprotein, HDL-C: cholesterol content in HDL, HDL-P: HDL particles, HDL-TG: triglyceride content in HDL, LDL: low-density lipoprotein, LDL-C: cholesterol content in LDL, LDL-P: LDL particles, LDL-TG: triglyceride content in LDL, ¹H-NMR: nuclear magnetic resonance spectroscopy, Non-HDL-P: Non-HDL particles, total-P: total particles, VLDL: very low-density lipoprotein, VLDL-C: cholesterol content in VLDL, VLDL-P: VLDL particles, VLDL-TG: triglyceride content in VLDL

Supplementary Table 4. ¹H-NMR-assessed advanced lipoprotein profile according to statin therapy.

	Statin non-users (n=105)	Statin users (n=84)	p-value
VLDL-P number (nmol/L)			
Total	30.75 (25.118-41.87)	32.81 (29.02-42.29)	0.089
Large	0.9379 (0.6946-1.185)	0.9464 (0.7782-1.325)	0.217
Medium	3.119 (2.058-4.692)	3.253 (2.116-4.619)	0.746
Small	26.30 (22.12-33.63)	28.22 (25.56-36.31)	0.040
Ratio large/total	0.0278 (0.0257-0.0299)	0.0280 (0.0250-0.0300)	0.762
VLDL-P composition (mg/dL)			
VLDL-C	9.164 (6.582-13.633)	9.853 (7.716-14.612)	0.323
VLDL-TG	42.34 (34.51-57.73)	43.56 (37.79-59.50)	0.117
Ratio VLDL-C/VLDL-TG	0.2143 (±0.0477)	0.2101 (±0.0501)	0.557
VLDL-P size (nm)	42.07 (41.79-42.36)	41.97 (41.76-42.29)	0.275
LDL-P number (nmol/L)			
Total	1279.01 (±164.85)	1239 (±248.05)	0.206
Large	179.39 (165.57-191.07)	162.55 (148.67-182.26)	0.001
Medium	361.19 (214.20-440.67)	321.15 (264.38-399.07)	0.012
Small	727.81 (663.33-786.06)	704.15 (630.72-758.82)	0.172
Ratio small/total	0.5724 (±0.0565)	0.5887 (±0.0710)	0.080
LDL-P composition (mg/dL)			
LDL-C	127.31 (±16.66)	121.81 (±26.30)	0.098
LDL-TG	13.34 (12.12-15.98)	13.69 (11.42-15.39)	0.628
Ratio LDL-C/LDL-TG	9.523 (8.226-10.276)	8.950 (7.786-9.790)	0.032
LDL size (nm)	21.02 (±0.28)	20.94 (±0.35)	0.093
HDL-P number (μmol/L)			
Total	29.37 (27.46-34.02)	31.04 (29-25.34)	0.071
Large	0.2689 (0.2541-0.2900)	0.2616 (0.2448-0.2914)	0.379
Medium	10.41 (9.28-11.62)	10.44 (9.42-11.88)	0.653
Small	20.13 (±4.22)	21.15 (±3.91)	0.091
Ratio small/total	0.6462 (±0.0384)	0.6571 (±0.0377)	0.054
HDL-P composition (mg/dL)			
HDL-C	60.07 (52.65-69.50)	61.25 (54.87-70.87)	0.427
HDL-TG	16.30 (±4.69)	18 (±3.82)	0.008
Ratio HDL-C/HDL-TG	4.073 (±1.200)	3.665 (±0.926)	0.011
HDL size (nm)	8.281 (±0.067)	8.262 (±0.066)	0.050
Other atherogenic variables			
Non-HDL-P (nmol/L)	1292.3 (1178.2-1401)	1197.8 (1102.1-1346.6)	0.019
Ratio LDL-P/HDL-P	41.71 (35.59-48.35)	36.50 (32.05-45.02)	0.006
Ratio total-P/HDL-P	43.03 (36.19-49.62)	38 (32.80-46.02)	0.007
Ratio LDL-P/LDL-C (¹ H-NMR)	10.01 (9.85-10.21)	10.18 (9.97-10.40)	0.002

Data are shown as mean (±SD) or median (Q1-Q3).

p values for group comparisons are reported and shown in bold if statistically significant.

HDL: high-density lipoprotein, HDL-C: cholesterol content in HDL, HDL-P: HDL particles, HDL-TG: triglyceride content in HDL, LDL: low-density lipoprotein, LDL-C: cholesterol content in LDL, LDL-P: LDL particles, LDL-TG: triglyceride content in LDL, ¹H-NMR: nuclear magnetic resonance spectroscopy, Non-HDL-P: Non-HDL particles, total-P: total particles, VLDL: very low-density lipoprotein, VLDL-C: cholesterol content in VLDL, VLDL-P: VLDL particles, VLDL-TG: triglyceride content in VLDL

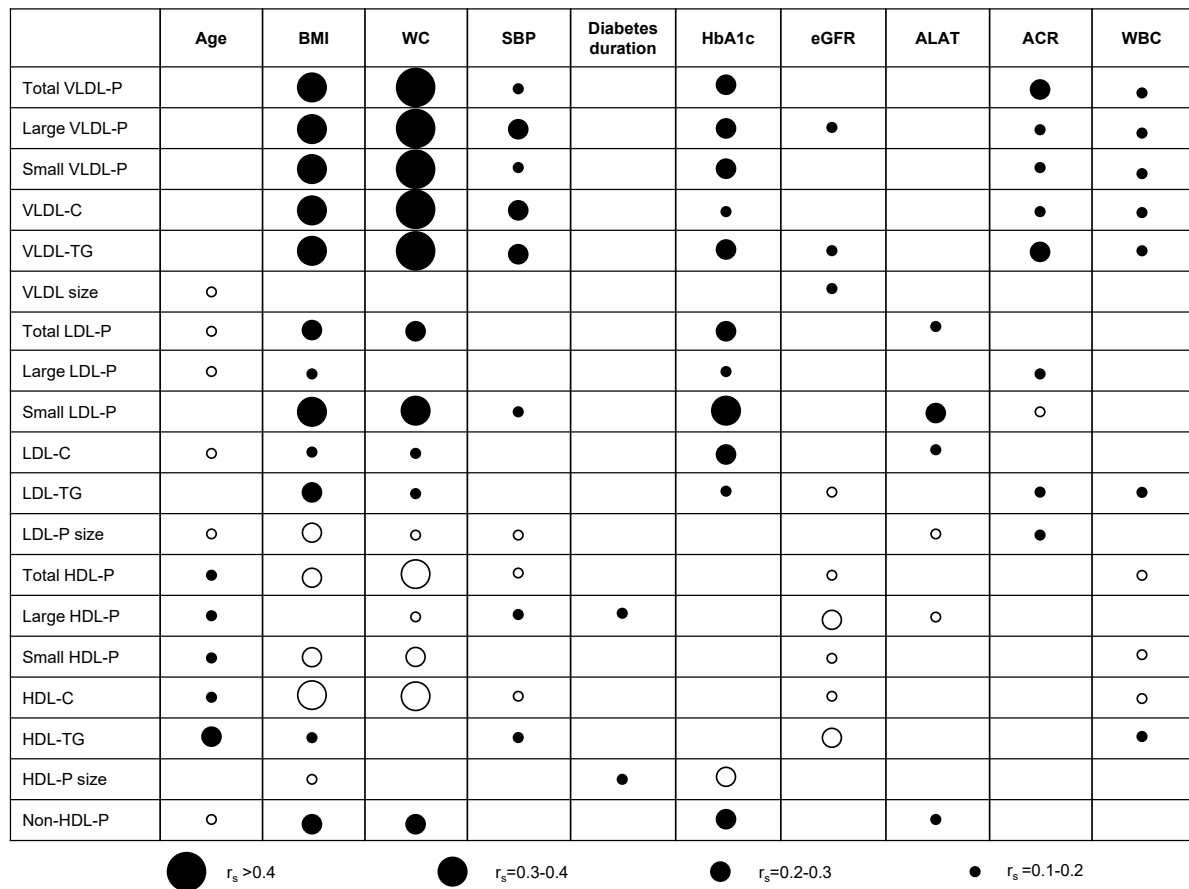
Supplementary Table 5. Age, sex, and statin treatment adjusted logistic regression analysis for associations between conventional and NMR-assessed advanced lipoprotein profiles with preclinical atherosclerosis.

Conventional lipid profile	Carotid plaque presence	
	OR (CI95%)	p-value
Total cholesterol (10 mg/dL increase)	0.887 (0.788-0.998)	0.046
LDL-cholesterol (10 mg/dL increase)	0.868 (0.750-1.006)	0.060
HDL-cholesterol (10 mg/dL increase)	0.792 (0.627-1.000)	0.050
Triglycerides (10 mg/dL increase)	1.035 (0.985-1.087)	0.170
Non-HDL-cholesterol (10 mg/dL increase)	0.928 (0.815-1.055)	0.254
Remnant cholesterol (5 mg/dL increase)	1.334 (1.055-1.687)	0.016
¹H-NMR -assessed advanced lipoprotein profile		
VLDL-P number (10 nmol/L increase)	1.141 (1.005-1.296)	0.042
VLDL-C (10 mg/dL increase)	1.650 (1.060-2.590)	0.027
VLDL-TG (10 mg/dL increase)	1.067 (0.986-1.154)	0.107
VLDL size (1 nm increase)	0.770 (0.391-1.514)	0.448
LDL-P number (100 nmol/L increase)	0.906 (0.756-1.085)	0.282
LDL-C (10 mg/dL increase)	0.877 (0.733-1.050)	0.153
LDL-TG (10 mg/dL increase)	0.753 (0.311-1.821)	0.529
LDL size (1 nm increase)	0.380 (0.131-1.100)	0.074
HDL-P number (10 μ mol/L increase)	0.486 (0.243-0.971)	0.041
Medium HDL-P (1 μ mol/L increase)	0.765 (0.621-0.942)	0.012
HDL-C (10 mg/dL increase)	0.682 (0.508-0.916)	0.011
HDL-TG (10 mg/dL increase)	1.309 (0.603-2.842)	0.497
HDL size (1 nm increase)	0.232 (0.002-28.53)	0.552
Non-HDL-P number (100 nmol/L increase)	0.940 (0.793-1.114)	0.474
Other atherogenic variables		
LDL-P / HDL-P ratio (0.5 increase)	1.005 (0.987-1.024)	0.583
Total-P / HDL-P ratio (0.5 increase)	1.007 (0.989-1.025)	0.443
Small-LDL-P / Total LDL-P ratio (0.1 increase)	1.867 (1.088-3.205)	0.023
LDL-P / LDL-C (¹ H-NMR) ratio (0.5 increase)	1.604 (1.007-2.557)	0.047
LDL-P / LDL-C (conventional) ratio (0.5 increase)	1.159 (1.047-1.283)	0.004
Non-HDL-P / Non-HDL-C (conventional) ratio (0.5 increase)	1.155 (1.007-1.324)	0.039
VLDL-P / Remnant-cholesterol ratio (0.5 increase)	1.315 (0.994-1.740)	0.055
Triglycerides / Remnant-cholesterol (both conventional) ratio (0.5 increase)	1.143 (0.935-1.396)	0.192
Total cholesterol / HDL-C (both conventional) ratio (0.5 increase)	1.097 (0.893-1.347)	0.377
LDL-C / HDL-C (both conventional) ratio (0.5 increase)	1.036 (0.812-1.322)	0.777
Triglycerides / HDL-C (both conventional) ratio (0.5 increase)	1.129 (1.004-1.269)	0.043

Odds Ratio with confidence intervals (95%) and p-values are reported. Statistically significant p-values are shown in bold.

HDL: high-density lipoprotein, HDL-C: cholesterol content in HDL, HDL-P: HDL particles, HDL-TG: triglyceride content in HDL, LDL: low-density lipoprotein, LDL-C: cholesterol content in LDL, LDL-P: LDL particles, LDL-TG: triglyceride content in LDL, ¹H-NMR: nuclear magnetic resonance spectroscopy, Non-HDL-P: Non-HDL particles, total-P: total particles, VLDL: very low-density lipoprotein, VLDL-C: cholesterol content in VLDL, VLDL-P: VLDL particles, VLDL-TG: triglyceride content in VLDL.

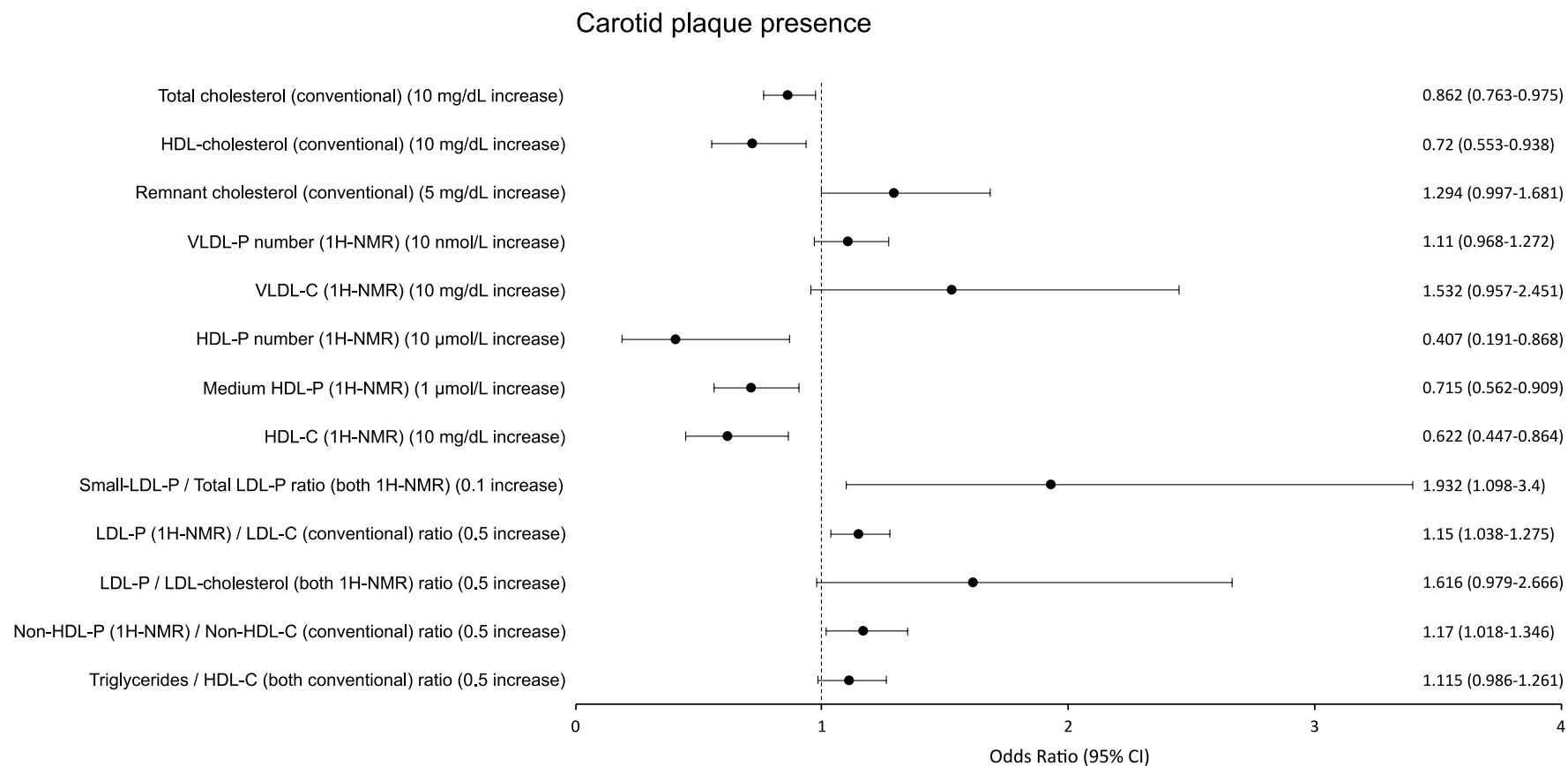
Supplementary Figure 1. Associations between NMR-assessed advanced lipoprotein profile and clinical and laboratory parameters in participants without statin treatment (n=105).



Solid and open circles indicate positive and negative relationships, respectively.

ACR: albumin-to-creatinine ratio; ALAT: alanine aminotransferase; BMI: body mass index; eGFR: estimated glomerular filtration rate; FLI: fatty liver index; HDL: high-density lipoprotein; HDL-C: cholesterol content in HDL; HDL-P: HDL particles; HDL-TG: triglyceride content in HDL; LDL: low-density lipoprotein; LDL-C: cholesterol content in LDL; LDL-P: LDL particles; LDL-TG: triglyceride content in LDL; NMR: nuclear magnetic resonance; SBP: systolic blood pressure; VLDL: very low-density lipoprotein; VLDL-C: cholesterol content in VLDL; VLDL-P: VLDL particles; VLDL-TG: triglyceride content in VLDL; WBC: white blood cells; WC: waist circumference.

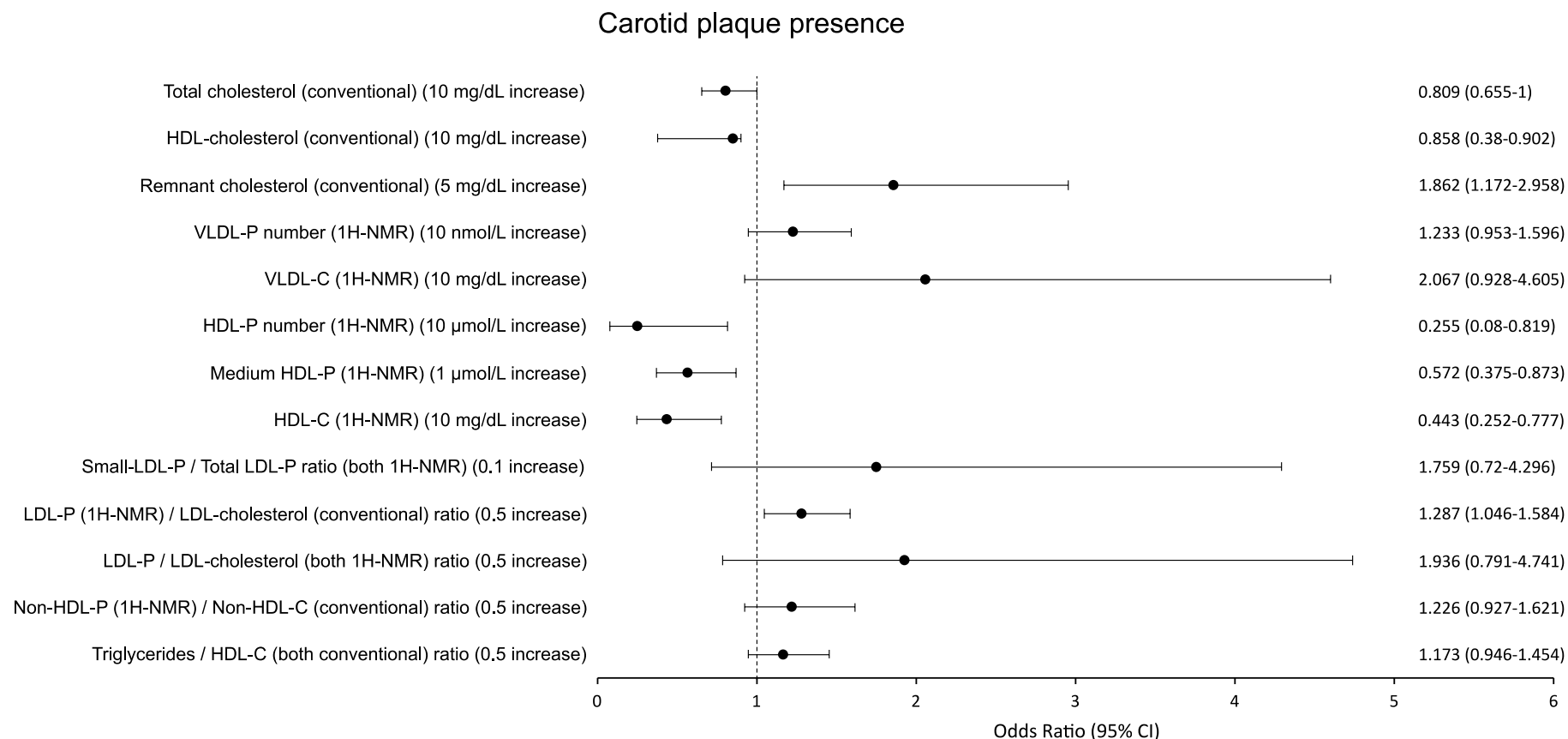
Supplementary Figure 2. Fully-adjusted logistic regression model analysing the relationships between conventional and ¹H-NMR-assessed advanced lipoprotein profile with the presence of carotid plaque.



Adjusted per sex, age, body mass index, diabetes duration, systolic blood pressure, presence of diabetic retinopathy, smoking status, glycated haemoglobin (HbA1c), urine-to-creatinine ratio, statin score and antihypertensive treatment (angiotensin-converting enzyme inhibitor or angiotensin receptor blocker). Odds Ratio with confidence intervals (95%).

HDL: high-density lipoprotein, HDL-C: cholesterol content in HDL, HDL-P: HDL particles, LDL-C: cholesterol content in LDL, LDL-P: LDL particles, ¹H-NMR: nuclear magnetic resonance spectroscopy, Non-HDL-C: cholesterol content in non-HDL, Non-HDL-P: non-HDL particles, VLDL-C: cholesterol content in VLDL, VLDL-P: VLDL particles.

Supplementary Figure 3. Fully-adjusted logistic regression model analysing the relationships between conventional and ¹H-NMR-assessed advanced lipoprotein profile with the presence of carotid plaque in those without statin therapy (n=105).



Adjusted per sex, age, body mass index, diabetes duration, systolic blood pressure, presence of diabetic retinopathy, smoking status, glycated haemoglobin (HbA1c), urine-to-creatinine ratio and antihypertensive treatment (angiotensin-converting enzyme inhibitor or angiotensin receptor blocker).
Odds Ratio with confidence intervals (95%).

HDL: high-density lipoprotein, HDL-C: cholesterol content in HDL, HDL-P: HDL particles, LDL-C: cholesterol content in LDL, LDL-P: LDL particles, ¹H-NMR: nuclear magnetic resonance spectroscopy, Non-HDL-C: cholesterol content in non-HDL, Non-HDL-P: non-HDL particles, VLDL-C: cholesterol content in VLDL, VLDL-P: VLDL particles.

ARTÍCULO ORIGINAL 6: PERFIL LIPIDÓMICO AVANZADO MEDIANTE RESONANCIA MAGNÉTICA NUCLEAR EN LA EVALUACIÓN DEL RIESGO CARDIOMETABÓLICO EN LA DIABETES TIPO 1: UN ANÁLISIS EXPLORATORIO

Objetivos: La ECV es la principal causa de mortalidad en la DTI. Sin embargo, se necesita de herramientas de práctica diaria para identificar a aquellos más propensos a sufrir estos eventos. Nuestro objetivo fue evaluar las relaciones entre el análisis lipidómico basado en ^1H -RMN y varias variables de riesgo de ECV (incluida la aterosclerosis carotídea preclínica) en individuos con DTI de alto RCV.

Métodos: Incluimos pacientes con DTI sin ECV, con: edad ≥ 40 años, ERD, o ≥ 10 años de evolución de la diabetes con otro FRCV. La presencia de placa (grosor íntima-media $\geq 1,5$ mm) se determinó mediante ecografía carotídea. El análisis lipidómico se realizó mediante ^1H -RMN.

Resultados: Incluimos a 131 participantes (49,6% mujeres, edad $46,4 \pm 10,3$ años, duración de la diabetes $27,0 \pm 9,5$ años, 47,3% en tratamiento con estatinas, 28,2% con placas). Las variables relacionadas con la glucosa (HbA1c), antropométricas (IMC y perímetro de cintura) y de resistencia a la insulina (índice de hígado graso [FLI] y eGDR) fueron las más asociadas con el análisis lipidómico por ^1H -RMN ($p < 0,01$). En cuanto a la aterosclerosis preclínica, la esfingomielina se asoció independientemente con la presencia de placa carotídea (para un aumento de $0,1$ mmol/L, OR $0,50$ [$0,28$ – $0,86$]), incluso después de ajustar por edad, sexo, hipertensión, uso de estatinas, media de HbA1c en 5 años y duración de la diabetes. Además, el ácido linoleico y los ácidos grasos omega-6 permanecieron independientemente asociados con una mayor carga de placa (≥ 3 placas) en modelos multivariados ($0,17$ [$0,03$ – $0,93$] y $0,27$ [$0,07$ – $0,97$], respectivamente; $p < 0,05$ para ambos).

Conclusiones: En nuestro estudio preliminar de individuos con DTI de alto RCV, varios parámetros lipidómicos determinados con ^1H -RMN, se asociaron independientemente con la aterosclerosis preclínica. Específicamente, los ácidos grasos omega-6 y el ácido linoleico parecen prometedores para identificar a aquellos con una mayor carga de placas.



ORIGINAL RESEARCH

Nuclear Magnetic Resonance-Based Lipidomics in the Assessment of Cardiometabolic Risk in Type 1 Diabetes: An Exploratory Analysis

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ABSTRACT

Introduction: Cardiovascular disease (CVD) is the leading cause of mortality in type 1 diabetes (T1D). However, there is a need for daily practice tools for identifying those more prone to suffer from these events. We aimed to assess the relationships between nuclear magnetic resonance (^1H NMR)-based lipidomic analysis and several CVD risk variables (including preclinical

carotid atherosclerosis) in individuals with T1D at high risk.

Methods: We included patients with T1D without CVD, with at least one of the following: age ≥ 40 years, diabetic kidney disease, or ≥ 10 years of evolution with another risk factor. The presence of plaque (intima-media thickness > 1.5 mm) was determined by standardized ultrasonography protocol. Lipidomic analysis was performed by ^1H NMR. Bivariate and multivariate-adjusted differences in ^1H NMR lipidomics were evaluated.

Supplementary Information The online version contains supplementary material available at <https://doi.org/10.1007/s13300-023-01372-x>.

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Results: We included $n = 131$ participants (49.6% female, age 46.4 ± 10.3 years, diabetes duration 27.0 ± 9.5 years, 47.3% on statins). Carotid plaques were present in 28.2% of the individuals ($n = 12$, with ≥ 3 plaques). Glucose (HbA1c), anthropometric (body mass index and waist circumference), and insulin resistance-related (fatty liver index and estimated glucose disposal rate) variables were those most associated with ^1H NMR-derived lipidomic analysis ($p < 0.01$ for all). Regarding preclinical atherosclerosis, sphingomyelin was independently associated with carotid plaque presence (for 0.1 mmol/L increase, OR 0.50 [0.28–0.86]; $p = 0.013$), even after adjusting for age, sex, hypertension, statin use, mean 5-year HbA1c and diabetes duration. Furthermore, linoleic acid and ω -6 fatty acids remained independently associated with higher plaque burden (≥ 3 plaques) in multivariate models (0.17 [0.03–0.93] and 0.27 [0.07–0.97], respectively; $p < 0.05$ for both).

Conclusion: In our preliminary study of individuals with T1D at high risk, several ^1H NMR-derived lipidomic parameters were independently associated with preclinical atherosclerosis. Specifically, ω -6 fatty acids and linoleic acid seem promising for identifying those with higher plaque burden.

Keywords: Type 1 diabetes; Preclinical atherosclerosis; Vascular ultrasonography; Nuclear magnetic resonance; Lipidomic analysis

Key Summary Points

Why carry out this study?

Cardiovascular disease is the leading cause of death among the type 1 diabetes population. However, the tools available in daily practice for identifying those more prone to suffer from these events are suboptimal.

This exploratory study aimed to assess the relationships between nuclear magnetic resonance-derived lipidomic variables and several cardiometabolic traits (including preclinical carotid atherosclerosis) among these individuals.

What was learned from the study?

Several lipidomic variables were independently associated with preclinical carotid atherosclerosis, even after adjusting for some confounders such as age, sex, hypertension, statin use, mean 5-year HbA1c and diabetes duration. Specifically, linoleic acid and ω -6 fatty acids seem promising in identifying those with higher plaque burden (≥ 3 carotid plaques).

Since the identification of individuals with T1D at higher risk for developing future CVD is challenging in daily practice, the lipidomic analysis seems promising in this field. Further studies with larger sample size and/or longitudinal designs are needed to confirm our preliminary results.

INTRODUCTION

Cardiovascular disease (CVD) is still the leading cause of death among the type 1 diabetes population (T1D) [1, 2]. Although glycaemic control has been strongly associated with this increased risk, even in those with glucose levels meeting goals, cardiovascular death is threefold

higher [3]. Thus, extra-glycaemic parameters are probably involved in this accelerated atherosclerosis. In addition to several conventional [4] and non-conventional [5] cardiovascular risk factors, circulating lipids are thought to play an important role in CVD pathogenesis [6, 7]. In fact, in a large cohort study, an increase of 1 mmol/L of low density lipoprotein-cholesterol (LDL-cholesterol) was associated with a 35–50% greater risk of overall CVD [8]. Further, our study group has recently shown that individuals with T1D had some subtle lipoprotein metabolism derangements that could be associated with atherosclerosis progression [9].

Lipidomic analysis is a new technique that identifies a broad range of lipid species, which could uncover some variables potentially associated with chronic complications in this population [10]. Preliminary data from our group also showed that some dietary-related lipid parameters could identify individuals with T1D more prone to suffer from CVD [11, 12]. However, the information regarding lipidomic-related variables with preclinical atherosclerosis, especially in Mediterranean countries, is scarce. Against this background, in this exploratory analysis, we aimed to assess the relationships between nuclear magnetic resonance (^1H NMR) lipidomics and several CVD risk variables (including preclinical carotid atherosclerosis), in primary prevention individuals with T1D at high risk.

METHODS

Study Participants

We performed a cross-sectional study in individuals with T1D followed at the diabetes unit of a tertiary hospital. All subjects were recruited from January 2016 to November 2018. We included participants with no previous history of CVD (coronary artery disease, ischemic stroke, peripheral artery disease and/or heart failure) and high CVD risk according to the main CVD prevention guidelines, as previously stated [13–15]: (a) age ≥ 40 years; (b) presence of any stage of diabetic kidney disease; (c) or

≥ 10 years of diabetes duration and at least one additional CVD risk factor. The following CVD risk factors were considered: family history of premature CVD (defined as < 55 years in men and < 65 years in women [16]), active smoking habit, hypertension, low high density lipoprotein-cholesterol (HDL-cholesterol) levels (< 40 mg/dL in men, < 45 mg/dL in women), high triglycerides levels (> 150 mg/dL), being already on statins, a former episode of preeclampsia/eclampsia, the presence of diabetic retinopathy, impaired hypoglycaemia awareness or a previous episode of severe hypoglycaemia in the last 2 years. Patients with clinical suspicion of latent autoimmune diabetes in adults (LADA), monogenic diabetes or prior pancreatic diseases (pancreatitis, surgery) were excluded.

The study protocol was conducted according to the principles of the Declaration of Helsinki and approved by the hospital Research Ethics Committees (HCB/2017/0977). Patients were included after giving their written informed consent.

Clinical and Laboratory Measures

Clinical data such as age, gender, family history of premature CVD in first-degree relatives, smoking habit (never, former or active), the presence of hypertension, as well as the use of certain medications (antihypertensive, lipid-lowering or antiplatelet) were registered. T1D-specific clinical information was also recorded: diabetes duration (years), current insulin therapy (continuous subcutaneous insulin infusion [CSII] or multiple daily insulin injection therapy [MDI]), presence of retinopathy and/or nephropathy, and history of severe hypoglycaemia or impaired hypoglycaemia awareness (Clarke test score > 3 points [17, 18]).

Anthropometric measures including height, weight, waist and hip circumference were also recorded and obtained with standard methods. Body mass index (BMI) was calculated as weight in kilograms divided by square height in metres. Blood pressure was registered using a blood pressure monitor after a few minutes of rest.

Laboratory data were obtained in the local laboratory using standardized assays to measure serum creatinine, urinary albumin-to-creatinine ratio (ACR), glycated haemoglobin (HbA1c; high-performance liquid chromatography and expressed in National Glycohemoglobin Standardization Program/Diabetes Control and Complications Trial units), fasting glucose and lipid profile (total cholesterol, triglycerides and HDL-cholesterol by direct methods; LDL-cholesterol by the Friedewald formula). The estimated glomerular filtration rate (eGFR) was obtained by the Chronic Kidney Disease-Epidemiology Collaboration equation (CKD-EPI) [19]. The HbA1c values available for each patient were used to calculate the mean HbA1c in the last 5 years. Insulin sensitivity was evaluated with the estimated glucose disposal rate (eGDR) formula, a well-validated score in T1D expressed in mg/kg/min [16, 20] and calculated as follows: $24.31 - 12.22 \times \text{waist-to-hip ratio} - 3.29 \times \text{hypertension}$ (0 = no, 1 = yes) $- 0.57 \times \text{HbA1c}$ (in %). Lastly, the fatty liver index (FLI) was calculated as described previously [21].

Diabetic kidney disease was considered if ACR was ≥ 30 mg/g or eGFR was < 60 ml/min/ 1.73 m^2 ; or the use of angiotensin-converting enzyme inhibitors (ACEi) or angiotensin II receptor blockers (ARB), with no history of hypertension or CVD. The diagnosis of diabetic retinopathy was obtained from medical records and was always verified by an ophthalmologist. Hypertension was defined as consecutive determinations of systolic blood pressure ≥ 140 mmHg and diastolic blood pressure ≥ 90 mmHg or being treated with antihypertensive drugs (except those on ACEi/ARB for diabetic nephropathy).

¹H NMR Lipidomic Analysis

¹H NMR lipidomic analysis was performed as previously stated [12]. Lipophilic extracts were obtained from two 100- μ L aliquots of freshly thawed plasma using the BUME method [22] with slight modifications. BUME was optimized for batch extractions with diisopropyl ether (DIPE) replacing heptane as the organic solvent.

This procedure was performed with a BRAVO liquid-handling robot which can extract 96 samples at once. The upper lipophilic phase was completely dried in Speedvac until evaporation of organic solvents and frozen at -80°C until ¹H NMR analysis. Lipid extracts were reconstituted in a solution of CDCl₃/CD₃OD/D₂O (16:7:1, v/v/v) containing tetramethylsilane (TMS) at 1.18 mM as a chemical shift reference and transferred into 5-mm NMR glass tubes. ¹H NMR spectra were measured at 600.20 MHz using an Avance III-600 Bruker spectrometer. A 90° pulse with water presaturation sequence (zgpr) was used. Quantification of lipid signals in ¹H NMR spectra was carried out with LipSpin [23], in-house software based on Matlab (MATLAB, version 7.10.0 (R2010a); Natick, Massachusetts: The MathWorks Inc.; 2010.). Resonance assignments were based on values in the literature [24].

Carotid B-mode Ultrasound Imaging

Bilateral carotid artery B-mode ultrasound imaging was performed following a standardized protocol, as described previously [14, 15]. Acuson X300 and Acuson X700 (Siemens) ultrasound systems equipped with a linear transducer (frequency range 5–10 MHz) were used. Carotid intima-media thickness (IMT) was measured offline by semiautomatic software by the same experienced researcher (A.J.A.). The plaque was visualized using B-mode and colour Doppler examinations in both longitudinal and transverse planes to evaluate the presence of circumferential asymmetry. Carotid (bulb, internal or common carotid) plaque was defined as focal wall thickening encroaching into the arterial lumen by at least 50% of the surrounding IMT value or with a thickness of at least 1.5 mm measured from the media adventitia interphase to the intima-lumen surface [25]. The mean and the mean-maximum of all the carotid territories were recorded, as well as the maximum height of the carotid plaque. When plaque was present, the maximum IMT equalled the highest plaque height.

Statistical Analysis

Sample size could not be calculated before the analyses because of the absence of previous data on the distribution of lipidomic parameters. As a post hoc analysis, we calculated the sample size to achieve an absolute difference of 10% between groups for the lipidomic parameters. Assuming a two-tailed alpha error of 0.025, we obtained a statistical potency of 91%.

Data are presented as mean $\pm$ standard deviation, median (interquartile range) or number (percentage). The normal distribution of continuous variables was assessed by the Kolmogorov–Smirnov test. Differences in clinical and laboratory variables according to pre-clinical carotid atherosclerosis status were assessed with appropriate parametric and non-parametric tests. Relationships between ^1H NMR lipidomic variables and several dichotomous cardiometabolic traits, as well as carotid atherosclerosis, were assessed with the Mann–Whitney U test. Spearman correlation analyses were further used to assess relationships between ^1H NMR-related variables and continuous cardiometabolic parameters.

As an exploratory analysis, we assessed the independent relationships between ^1H NMR lipidomics and carotid atherosclerosis (presence of at least one plaque; presence of ≥ 3 plaques; dependent variables). To this end, logistic binary regression models were constructed (and odds ratios [OR] and 95% confidence interval were reported, accordingly). Different models were assessed: (a) model 1: age- and sex-adjusted; (b) model 2: model 1 + presence of hypertension and statin use; and (c) model 3: model 2 + mean HbA1c in the last 5 years and T1D duration. The level of significance was set at $p < 0.05$. All the analyses were performed with the SPSS 26.0 statistical package (Chicago, IL).

RESULTS

Subject Characteristics

A total of 131 participants were included in the study. Overall, the proportion of men and

women was similar, the mean age was 46.4 years and the mean diabetes duration was 27.0 years. Regarding CVD risk factors, 27.5% were active smokers, 29% had hypertension and 47.3% and 33.6% were on statins and ACEi/ARB, respectively. Microvascular complications were present in 43.5% of the participants (37.4% had retinopathy and 13.7% had diabetic kidney disease). The characteristics are summarised in Table 1.

On vascular ultrasonography, 37 (28.2%) had at least one carotid plaque ($n = 12$, with three or more plaques). Those with plaques were older (52.4 ± 9.7 vs. 44.1 ± 9.6 years; $p < 0.001$), had an increased prevalence of hypertension (43.2 vs. 23.4%; $p = 0.024$), a higher clinical systolic blood pressure (132 ± 15 mmHg vs. 124 ± 15 mmHg; $p = 0.009$) and a longer diabetes duration (31.1 ± 11.5 vs. 25.4 ± 8.2 years; $p = 0.008$; Table S1 in the supplementary material). The same variables were also directly associated with the number of carotid plaques (none, 1–2 plaques, ≥ 3 plaques; p for trend < 0.05 for all comparisons), plus the use of ACEi/ARB (28.7 vs. 66.7%), and inversely associated with eGFR (99 ± 16 vs. 88 ± 14 ml/min/1.73 m²) and LDL-cholesterol (113 ± 21 vs. 87 ± 14 mg/dL; for none vs. ≥ 3 plaques; p for trend < 0.05 for all comparisons; Table S2 in the supplementary material).

^1H NMR-Based Lipidomic Analysis in Relation to Cardiometabolic Traits

Whereas no differences were observed concerning age (Fig. 1), several between-gender differences were found: higher levels of ^1H NMR triglycerides and ω -9 fatty acids, and lower levels of glycerophospholipids, phosphatidylcholine, sphingomyelin and ω -3 fatty acids were observed among male participants ($p < 0.05$; Table S3 in the supplementary material). Only minor differences were found regarding hypertension and smoking habit, whereas those on statins showed lower levels of linoleic and ω -6 fatty acids, and higher levels of sphingomyelin and arachidonic acid–eicosapentaenoic acid (ARA-EPA) ($p < 0.05$, Table S3). Among other

Table 1 Characteristics of the study participants ($n = 131$)

Clinical characteristics	
Female	65 (49.6)
Age (years)	46.4 $\pm$ 10.3
Premature CVD in first-degree relatives*	25 (19.1)
Active smoking habit	36 (27.5)
Cumulative smoking (pack-years)	0 (0–12.5)
Hypertension	38 (29.0)
SBP (mmHg)	126 $\pm$ 16
DBP (mmHg)	80 $\pm$ 8
BMI (kg/m ²)	25.9 $\pm$ 4.1
Waist circumference (cm)	90 $\pm$ 12
Women	85 $\pm$ 11
Men	95 $\pm$ 11
T1D duration (years)	27.0 $\pm$ 9.5
Diabetic kidney disease	18 (13.7)
Diabetic retinopathy	49 (37.4)
CSII therapy	55 (42.0)
Laboratory characteristics	
Fasting plasma glucose (mg/dL)	151 $\pm$ 66
Haemoglobin A1c (%)	7.5 $\pm$ 0.9
Mean haemoglobin A1c in the last 5 years (%)	7.7 $\pm$ 0.9
Serum creatinine (mg/dL)	0.82 $\pm$ 0.17
eGFR (CKD-EPI; ml/min/1.73 m ²)	97.8 $\pm$ 15.9
ALT (UI/L)	22 $\pm$ 11
Total cholesterol (mg/dL)	188 $\pm$ 32
HDL-cholesterol (mg/dL)	60 $\pm$ 16
LDL-cholesterol (mg/dL)	111 $\pm$ 24
Triglycerides (mg/dL)	70 (54–90)
Non-HDL cholesterol (mg/dL)	128 $\pm$ 28
Remnant cholesterol (mg/dL)	14 (11–18)

Table 1 continued

Total cholesterol/HDL-cholesterol ratio	3.30 $\pm$ 0.89
Fatty liver index	24.8 (11.5–54.7)
eGDR (mg/kg/min)	8.92 (6.65–10.21)
Pharmacological treatment	
Statins	62 (47.3)
ACEi/ARB	44 (33.6)
Antiplatelet drugs	8 (6.1)

Data are shown as n (percentage), mean $\pm$ SD or median (Q1–Q3)

ACEi angiotensin-converting enzyme inhibitor, ALT alanine aminotransferase, ARB angiotensin receptor blocker, BMI body mass index, CVD cardiovascular disease, CSII continuous subcutaneous insulin infusion, DBP diastolic blood pressure, eGDR estimated glucose disposal rate, eGFR estimated glomerular filtration rate, SBP systolic blood pressure, T1D type 1 diabetes

*Defined as < 55 years in men and < 65 years in women

adipose-related variables, FLI showed the strongest correlations with most of the ¹H NMR-related lipidomic parameters, especially with ¹H NMR triglycerides and ω -9 fatty acids ($r_s > 0.4$ for both; Fig. 1). Finally, white blood cell count (as a marker of an inflammation-related variable) was directly associated with ¹H NMR triglycerides, saturated fatty acids and ARA-EPA ($r_s = 0.2$ – 0.3).

Regarding T1D-specific risk factors, there were minor differences regarding diabetes duration and retinopathy status. However, HbA1c showed direct relationships with ¹H NMR triglycerides, esterified cholesterol, ω -6 and ω -9 fatty acids, and ARA-EPA ($p < 0.05$ for all comparisons, Fig. 1). Further, only weak direct correlations were found with ACR (as a marker of diabetic kidney disease; $r_s = 0.1$ – 0.2); and eGDR, as a marker of insulin sensitivity, was strongly and inversely associated with ¹H NMR triglycerides ($r_s = -0.415$).

	Age	BMI	WC	SBP	Diabetes duration	HbA1c	eGFR	ALAT	ACR	WBC	eGDR	FLI
Triglycerides	-0.053	0.340	0.518	0.250	-0.570	0.309	0.250	0.123	0.176	0.267	-0.415	0.655
Glycerophospholipids	0.056	-0.160	-0.008	-0.075	-0.070	0.026	-0.094	0.020	0.165	0.143	0.055	0.262
Phosphatidylcholine	0.082	0.011	-0.017	-0.066	-0.260	0.003	-0.110	-0.026	0.193	0.142	0.050	0.225
Sphingomyelin	0.093	-0.035	-0.126	-0.145	-0.011	-0.020	-0.168	-0.010	0.003	0.039	0.165	0.171
Lysophosphatidylcholine	-0.096	-0.161	-0.054	-0.061	-0.104	-0.092	0.033	0.120	0.043	-0.073	0.052	-0.018
Esterified cholesterol	-0.042	-0.027	-0.006	0.026	-0.111	0.212	-0.006	-0.040	0.140	0.054	-0.009	0.161
Free cholesterol	-0.018	-0.083	-0.079	-0.039	-0.087	0.013	0.035	-0.042	0.089	0.045	0.118	0.162
Linoleic acid	-0.095	0.005	0.069	0.099	-0.132	0.098	0.051	-0.018	0.192	0.159	-0.051	0.229
Saturated fatty acids	-0.070	0.048	0.110	0.083	-0.031	0.036	0.008	0.051	0.152	0.221	-0.097	0.245
w-3 fatty acids	0.171	-0.133	-0.228	-0.095	0.216	-0.112	-0.285	-0.139	0.040	-0.140	0.171	-0.129
w-6 fatty acids	-0.101	0.119	0.171	0.065	-0.090	0.192	0.057	-0.004	0.133	0.086	-0.112	0.349
w-7 fatty acids	-0.089	-0.029	0.013	0.030	-0.118	-0.045	0.129	-0.028	0.211	0.152	0.036	0.172
w-9 fatty acids	0.028	0.177	0.295	0.133	-0.054	0.193	-0.083	0.068	0.170	0.169	-0.239	0.483
DHA	0.072	0.009	0.042	0.035	0.046	0.044	-0.130	-0.008	0.069	0.034	-0.009	0.106
ARA-EPA	-0.034	0.115	0.183	0.071	-0.161	0.183	0.037	0.087	0.056	0.261	-0.144	0.357

Fig. 1 Associations between NMR lipidomics and clinical and laboratory parameters. Numbers in cells indicate Spearman's *R* correlation index and the colour of each cell indicates the strength of association of that index according to the colour code shown in the right column. *ACR*

albumin-to-creatinine ratio, *ALAT* alanine aminotransferase, *BMI* body mass index, *eGDR* estimated glucose disposal rate, *eGFR* estimated glomerular filtration rate, *FLI* fatty liver index, *SBP* systolic blood pressure, *WBC* white blood cells, *WC* waist circumference

¹H NMR-Based Lipidomic Analysis and Preclinical Carotid Atherosclerosis

Only levels of sphingomyelin were inversely associated with the presence of at least one carotid plaque ($p < 0.05$, Table 2), which maintained the statistical significance after adjusting for confounders such as age, sex, presence of hypertension, statin use, mean HbA1c in the last 5 years and diabetes duration (for 0.1 mmol/L increase, OR 0.50 [0.28–0.86]; $p = 0.013$; Table 3). When other variables associated with a higher plaque burden were assessed (presence of ≥ 3 plaques), inverse associations were found with esterified and free cholesterol, linoleic acid and ω -6 fatty acids ($p < 0.05$ for all comparisons; Table 2), which remained statistically associated in fully adjusted models (model 3; OR 0.055 (0.006–0.51),

0.009 (0.0–0.60), 0.17 (0.03–0.93) and 0.27 (0.07–0.97), for esterified cholesterol, free cholesterol, linoleic acid and ω -6 fatty acids, respectively; $p < 0.05$ for all; Table 3).

DISCUSSION

In our non-targeted and exploratory ¹H NMR lipidomic analysis, we found several lipid species that could be associated with adverse cardiometabolic traits in individuals with T1D. Besides between-gender differences, glycaemic control (evaluated by HbA1c), anthropometric (BMI and waist circumference) and insulin resistance-related (i.e. FLI and eGDR) variables were those most associated with NMR-assessed lipidomic analysis. Furthermore, some of these parameters (especially those related to ω -6 fatty acids) were also independently associated with

Table 2 NMR lipidomics according to preclinical carotid atherosclerosis status

	Whole cohort (<i>n</i> = 131)		Presence of carotid plaque		Presence of ≥ 3 plaques	
	No (<i>n</i> = 94)	Yes (<i>n</i> = 37)	No (<i>n</i> = 119)	Yes (<i>n</i> = 12)	No (<i>n</i> = 119)	Yes (<i>n</i> = 12)
Triglycerides	0.77 (0.51–1.08)	0.76 (0.51–1.07)	0.78 (0.50–1.43)	0.58 (0.40–1.01)	0.77 (0.52–1.10)	0.58 (0.40–1.01)
Glycerophospholipids	2.49 (2.30–2.72)	2.51 (2.29–2.69)	2.39 (2.15–2.85)	2.21 (2.12–2.57)	2.51 (2.27–2.74)	2.21 (2.12–2.57)
Phosphatidylcholine	2.40 (2.17–2.61)	2.41 (2.18–2.59)	2.35 (2.12–2.70)	2.26 (2.10–2.50)	2.41 (2.18–2.62)	2.26 (2.10–2.50)
Sphingomyelin	0.61 (0.55–0.67)	0.62 (0.56–0.69)	0.57 (0.54–0.66)*	0.58 (0.55–0.61)	0.62 (0.55–0.68)	0.58 (0.55–0.61)
Lysophosphatidylcholine	0.47 (0.41–0.53)	0.48 (0.41–0.55)	0.45 (0.41–0.51)	0.43 (0.40–0.46)	0.48 (0.42–0.55)	0.43 (0.40–0.46)
Esterified cholesterol	4.65 (4.38–5.11)	4.65 (4.43–5.09)	4.67 (3.98–5.28)	4.38 (3.94–4.70)*	4.67 (4.43–5.19)	4.38 (3.94–4.70)*
Free cholesterol	2.08 (1.89–2.35)	2.09 (1.94–2.35)	2.08 (1.73–2.28)	1.90 (1.67–2.08)**	2.11 (1.91–2.36)	1.90 (1.67–2.08)**
Linoleic acid	4.14 (3.74–4.59)	4.20 (3.80–4.58)	4.06 (3.59–4.67)	3.67 (3.46–4.20)*	4.20 (3.77–4.61)	3.67 (3.46–4.20)*
Saturated fatty acids	4.77 (4.18–5.51)	4.80 (4.20–5.44)	4.63 (4.12–5.76)	4.65 (4.48–5.78)	4.80 (4.17–5.51)	4.65 (4.48–5.78)
ω -3 fatty acids	0.24 (0.19–0.30)	0.24 (0.19–0.30)	0.25 (0.19–0.32)	0.22 (0.19–0.40)	0.25 (0.19–0.30)	0.22 (0.19–0.40)
ω -6 fatty acids	2.73 (2.43–3.34)	2.83 (2.49–3.37)	2.60 (2.28–3.27)	2.30 (1.57–2.82)*	2.82 (2.47–3.35)	2.30 (1.57–2.82)*
ω -7 fatty acids	2.62 (2.27–3.05)	2.63 (2.25–2.98)	2.54 (2.34–3.28)	2.53 (2.41–2.80)	2.63 (2.25–3.10)	2.53 (2.41–2.80)
ω -9 fatty acids	3.86 (3.09–4.79)	3.79 (3.21–4.55)	4.38 (2.73–5.57)	3.40 (2.15–4.83)	3.86 (3.17–4.77)	3.40 (2.15–4.83)
DHA	0.088 (0.064–0.118)	0.085 (0.065–0.114)	0.097 (0.060–0.139)	0.115 (0.048–0.151)	0.088 (0.065–0.114)	0.115 (0.048–0.151)
ARA-EPA	0.61 (0.51–0.82)	0.61 (0.50–0.82)	0.61 (0.51–0.81)	0.62 (0.57–0.75)	0.61 (0.50–0.82)	0.62 (0.57–0.75)

Data are shown as median (Q1–Q3). Data are presented as mmol/L

ARA arachidonic acid, DHA docosahexaenoic acid, EPA eicosapentaenoic acid

* $p < 0.05$; ** $p < 0.01$ between groups

Table 3 Associations between NMR lipidomics and carotid atherosclerosis: multiple regression analysis

	Model 1*		Model 2*		Model 3*	
	OR (95% CI)	<i>p</i> value	OR (95% CI)	<i>p</i> value	OR (95% CI)	<i>p</i> value
Presence of carotid plaque						
Triglycerides	1.09 (0.73–1.63)	0.674	1.09 (0.72–1.65)	0.693	1.09 (0.72–1.64)	0.697
Glycerophospholipids	0.65 (0.25–1.69)	0.373	0.68 (0.26–1.80)	0.435	0.67 (0.25–1.79)	0.429
Phosphatidylcholine	0.64 (0.23–1.76)	0.386	0.67 (0.24–1.86)	0.444	0.64 (0.23–1.79)	0.397
Sphingomyelin	0.52 (0.31–0.88)	0.015	0.51 (0.30–0.89)	0.018	0.50 (0.28–0.86)	0.013
Lysophosphatidylcholine	0.78 (0.02–40.42)	0.903	0.97 (0.02–53.45)	0.989	1.29 (0.02–79.02)	0.904
Esterified cholesterol	0.89 (0.45–1.74)	0.734	0.91 (0.46–1.79)	0.787	0.92 (0.45–1.88)	0.824
Free cholesterol	0.50 (0.15–1.74)	0.277	0.55 (0.16–1.94)	0.352	0.54 (0.15–1.92)	0.338
Linoleic acid	0.91 (0.53–1.55)	0.721	0.92 (0.54–1.58)	0.767	0.94 (0.55–1.61)	0.814
Saturated fatty acids	0.91 (0.65–1.28)	0.584	0.91 (0.65–1.28)	0.580	0.90 (0.64–1.27)	0.552
ω-3 fatty acids	1.83 (0.03–96.4)	0.765	2.18 (0.04–120.0)	0.704	1.48 (0.02–97.4)	0.855
ω-6 fatty acids	0.82 (0.50–1.35)	0.435	0.81 (0.49–1.35)	0.421	0.81 (0.49–1.35)	0.416
ω-7 fatty acids	1.40 (0.88–2.23)	0.153	1.44 (0.90–2.31)	0.125	1.47 (0.92–2.35)	0.109
ω-9 fatty acids	1.05 (0.87–1.28)	0.622	1.05 (0.87–1.28)	0.596	1.06 (0.87–1.28)	0.570
DHA	1.04 (0.94–1.14)	0.443	1.05 (0.95–1.15)	0.372	1.05 (0.95–1.16)	0.369
ARA-EPA	0.98 (0.25–3.89)	0.981	1.06 (0.26–4.38)	0.932	1.11 (0.26–4.70)	0.889
Presence of ≥ 3 carotid plaques						
Triglycerides	0.84 (0.27–2.66)	0.772	0.76 (0.21–2.75)	0.677	0.72 (0.15–3.40)	0.676
Glycerophospholipids	0.23 (0.03–1.62)	0.140	0.27 (0.04–1.93)	0.192	0.17 (0.01–2.32)	0.185
Phosphatidylcholine	0.16 (0.02–1.367)	0.094	0.17 (0.02–1.54)	0.114	0.14 (0.01–1.80)	0.130
Sphingomyelin	0.48 (0.21–1.10)	0.082	0.53 (0.21–1.32)	0.172	0.57 (0.20–1.59)	0.282
Lysophosphatidylcholine	0.96 (0.0–962.3)	0.991	3.05 (0.0–4726)	0.766	0.73 (0.0–1380)	0.934
Esterified cholesterol	0.12 (0.02–0.59)	0.009	0.10 (0.02–0.58)	0.010	0.055 (0.006–0.51)	0.011
Free cholesterol	0.019 (0.001–0.39)	0.010	0.025 (0.001–0.56)	0.020	0.009 (0.0–0.60)	0.027
Linoleic acid	0.36 (0.11–1.18)	0.091	0.33 (0.93–1.20)	0.092	0.17 (0.03–0.93)	0.041
Saturated fatty acids	1.06 (0.63–1.80)	0.827	1.05 (0.60–1.81)	0.875	1.23 (0.66–2.28)	0.512
ω-3 fatty acids	0.11 (0.0–71.7)	0.500	0.18 (0.0–189.8)	0.626	0.60 (0.0–724.8)	0.888
ω-6 fatty acids	0.38 (0.13–1.06)	0.063	0.38 (0.14–1.07)	0.068	0.27 (0.07–0.97)	0.045
ω-7 fatty acids	1.61 (0.73–3.51)	0.236	1.77 (0.79–3.95)	0.165	1.89 (0.74–4.79)	0.181
ω-9 fatty acids	0.68 (0.38–1.021)	0.192	0.69 (0.39–1.21)	0.194	0.63 (0.34–1.15)	0.132
DHA	0.99 (0.83–1.17)	0.881	0.98 (0.84–1.20)	0.981	1.01 (0.83–1.23)	0.911

Table 3 continued

	Model 1*		Model 2*		Model 3*	
	OR (95% CI)	<i>p</i> value	OR (95% CI)	<i>p</i> value	OR (95% CI)	<i>p</i> value
ARA-EPA	1.13 (0.08–15.91)	0.930	1.49 (0.09–23.83)	0.776	2.42 (0.12–49.46)	0.565

Logistic regression model (OR and 95% confidence interval) is presented. All the data were expressed for increments of 1 mmol/L, except for sphingomyelin (0.1 mmol/L increase) and DHA (0.01 mmol/L increase)

ARA arachidonic acid, DHA docosahexaenoic acid, EPA eicosapentaenoic acid

*Model 1: age- and sex-adjusted; model 2: model 1 adjusted for age, sex, presence of hypertension and statin treatment; model 3: model 2 + mean HbA1c in the last 5 years and diabetes duration

preclinical carotid atherosclerosis burden, a surrogate of future CVD events [26, 27]. To the best of our knowledge, this preliminary data represents one of the first contributions of the usefulness of ¹H NMR-based lipidomics in the assessment of CVD risk in Mediterranean individuals with T1D.

Identifying the individuals with T1D more prone to suffering from CVD is challenging. Although there are several clinical scores validated for estimating such cardiovascular risk in this population [28, 29], some of them often fail when applied in daily practice [15]. To overcome these limitations, the use of biomarkers has gained attention in the last few years. Thus, either imaging [30] or circulating biomarkers [31–33] seem promising, especially in those populations in which current tools perform rather poorly (such as T1D). In this regard, high-throughput lipidomic analysis could have the potential to unravel new pathways and biomarkers in the field of CVD in T1D.

The role of various lipids in the pathogenesis of T1D, their mediation in inflammation and the complications of the entity are controversial. Some, such as ω -3 fatty acids and their derivatives, have been shown to reduce inflammation and autoimmunity by different mechanisms while others, such as ω -9 fatty acids, show otherwise [34]. Sphingomyelin is the most abundant sphingolipid in tissues and blood and has an eminently structural function in cell membranes. It has been associated with an increase in proinflammatory cytokines and it is even suggested that it could be pathophysiologically related to the formation and progression of atheromatosis [35]. In this sense, the

inverse and independent association between sphingomyelin and the presence of carotid plaque in our sample could seem contradictory. Several reports have pointed out sphingomyelin as a crucial factor for glomerular and endothelial function [36]. In this sense, some previous studies from the Scandinavian population showed that this lipid variable was directly associated with worse kidney [37–39] and coronary heart disease outcomes [39]. Nevertheless, other studies using a more powerful lipidomic analysis (which could identify up to 106 different molecular lipid species) showed that several sphingomyelin subclasses were associated with worse kidney outcomes and cardiovascular mortality while other subclasses were associated with better outcomes [10, 38]. Furthermore, no significant relationships were found between any of the sphingomyelin subclasses and stroke [10]. Also, a prospective of the Multi-Ethnic Study of Atherosclerosis cohort [40], in the general population and primary prevention, found no association between sphingomyelin levels and incident CVD, and the association was even inverse in some models. In this regard, further studies should assess the relationships between the in-depth lipidomic analysis with the different manifestations of atherosclerosis.

Although there has been a lot of controversy regarding the role of ω -6 fatty acids, especially linoleic acid, in CVD prevention [41, 42], our findings showing inverse associations of these lipid species with atherosclerosis burden (≥ 3 carotid plaques) are reassuring. Our results are aligned with three recent meta-analyses in the general population; these include several

biomarkers of dietary linoleic acid intake (adipose tissue and blood) and report a strong association of this fatty acid with CVD protection [43–45]. In this regard, our study group has also shown that this lipid species (assessed through means of gas chromatography) was inversely associated with the prevalence of symptomatic carotid artery disease in the general population [46], as well as with carotid atherosclerosis burden among the T1D population [11]. These previous and our actual findings concur with the recommendations released by the American Heart Association (AHA) to replace foods rich in saturated fatty acids with those rich in ω -6 fatty acids to decrease CVD risk [47], not only for the general population but also for high-risk populations such as patients with T1D.

Several strengths and limitations should be acknowledged. One of the main strengths is the novelty in the population studied, as all the previous reports came from Scandinavian countries. Since Mediterranean individuals have very different genetic and behavioural backgrounds, the extrapolation of the existing results could be difficult. Furthermore, instead of intima-media thickness, we assessed the presence of carotid plaque, a better predictor of future CVD [26, 27]. Several strengths and limitations should be acknowledged. One of the main strengths is the novelty in the population studied, as all the previous reports came from Scandinavian countries. Since Mediterranean individuals have very different genetic and behavioural backgrounds, the extrapolation of the existing results could be difficult. Furthermore, instead of intima-media thickness, we assessed the presence of carotid plaque, a better predictor of future CVD [26, 27]. Additionally, in our research group, we are exploring the clinical utility of multiple parameters determined with ^1H NMR to identify those at higher cardiovascular risk to then be able to individualize the prevention strategy in each case. The great advantage of this technology is that several of these parameters can be simultaneously determined with a single sample (lipidomics, lipoprotein subclasses, inflammatory markers, metabolomics etc.), being able to have a broad perspective of various factors involved in the

pathophysiology of cardiovascular disease and lowering costs. However, the study also has limitations. First, causality cannot be established because of the cross-sectional nature of the study. Second, as a result of the limited sample and the high number of comparisons, the study must be considered preliminary data. All the associations found should be confirmed in future studies with a bigger sample and/or with longitudinal follow-up. Third, given the paucity of previous data about lipidomic analyses in T1D, the sample size could not be calculated beforehand. Fourth, the ankle-brachial index, as a surrogate marker of cardiovascular disease, would have been interesting to analyse together with the other clinical variables and their correlation with lipidomic parameters but, unfortunately, the data was not available. Fifth, some other studies have assessed various parameters by ^1H NMR such as lipoprotein subclass disturbances [48], but investigations analysing the use of this technique in lipidomic variables are scarce. Sixth, since the recruitment was mainly in 2017, the use of continuous glucose monitoring (CGM) or hybrid closed loop systems (HCL) was residual (< 5% for MCG and 0% for HCL). The use of CSII (started in our hospital in 2007–2008) improved glycaemic control in our sample (HbA1c 7.47% vs. 7.8% in multiple insulin dose group; *t* test *p* value 0.042 between groups), so on the basis of glycaemic control (mean HbA1c over the last 5 years), an overall improvement in the cardiovascular profile is to be expected in our study and therefore our findings should be interpreted with caution as they cannot be extrapolated to populations with lower use of this technology. Seventh, the finding of several significant associations has also been controversial for several of the parameters, so their applicability in real clinical practice should be analysed beforehand in studies with a larger sample. Finally, because of the aforementioned limitations, some of the relationships should be interpreted with caution. In this regard, the inverse associations between the free and esterified cholesterol with high atherosclerosis burden (≥ 3 plaques) could be misleading. On one hand, these two species were highly correlated with total and LDL-cholesterol in our sample ($r_s > 0.3$ – 0.4 , data not

shown), which in turn were lower in those harbouring more carotid plaques (Table S2). Although these associations persisted (but were blunted) when including statin treatment in multivariate models, selection bias cannot be ruled out. On the other hand, this finding could reflect the need for better biomarkers of CVD than classical ones. Previous studies have already shown that LDL-cholesterol is not the best predictor of future CVD events in the setting of high-risk patients with a high background cardioprotective treatment use [49].

CONCLUSION

In our exploratory study including individuals with T1D at high CVD risk, some variables obtained from the ^1H NMR-derived lipidomic analysis could be associated with preclinical atherosclerosis. Specifically, sphingomyelin levels were inversely associated with carotid plaques whereas the parameters related to ω -6 fatty acids, especially linoleic acid, could identify those individuals with a more advanced atherosclerosis burden. While the causal effect of these biomolecules in atherosclerosis should be confirmed in future studies, lipidomic analysis has the potential to unravel new pathways and biomarkers for CVD prevention among the T1D population.

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Noriega, Emilio Ortega, Marga Gimenez, Verónica Perea, Laura Boswell, Karla Mariaca, Carla Font, Alex Mesa, Clara Viñals, Jesús Blanco, Irene Vinagre, Adriana Pané, Laura Boswell, Enric Esmatjes, Ignacio Conget and Antonio J. Amor acquired and processed all clinical data; Antonio J. Amor performed the US measurements; Tonet Serés-Noriega and Antonio J. Amor contributed to data analysis and interpretation, wrote, reviewed and edited the manuscript. Antonio J. Amor and Verónica Perea contributed to the study concept and design. Marga Gimenez, Verónica Perea, Clara Viñals, Jesús Blanco, Irene Vinagre, Ignacio Conget and Antonio J. Amor supervised the study and participated in data analysis and interpretation. Antonio J. Amor designed the figures and had final responsibility for the decision to submit them for publication. Antonio J. Amor is the guarantor of this work and, as such, had full access to all the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis.

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Compliance with Ethics Guidelines. This study was performed in line with the principles of the Declaration of Helsinki. Approval was granted by the Ethics Committee of the hospital Clínic de Barcelona (HCB/2017/0977). All subjects were included after giving their written informed consent. All authors give consent for publication.

Prior Publication. The results of this work have not been published, partially or totally, in any other medium, either written or oral.

Data Availability. The data sets analyzed during the current study are available from the corresponding author upon reasonable request.

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Supplementary Material Corresponding to

**Nuclear magnetic resonance-based lipidomics in the assessment of
cardiometabolic risk in type 1 diabetes: an exploratory analysis**

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Table S1. Characteristics of the study participants according to the presence of carotid plaques.

	No plaque (n=94)	Presence of at least 1 plaque (n=37)	p-value
Clinical characteristics			
Female	48 (51.1)	17 (45.9)	0.598
Age (years)	44.1±9.6	52.4±9.7	<0.001
Premature CVD in first-degree relatives*	19 (20.2)	6 (16.2)	0.600
Active smoking habit	28 (29.8)	8 (21.6)	0.346
Cumulative smoking (Pack-years)	0 (0-12.5)	0 (0-20)	0.691
Hypertension	22 (23.4)	16 (43.2)	0.024
SBP (mmHg)	124±15	132±15	0.009
DBP (mmHg)	80±9	79±7	0.519
BMI (kg/m ²)	26.0±4.1	25.4±4.0	0.473
Waist circumference (cm)	90±12	89±12	0.701
Women	86±11	84±10	0.487
Men	95±11	94±12	0.795
T1D duration (years)	25.4±8.2	31.1±11.5	0.008
Diabetic kidney disease	13 (13.8)	5 (13.5)	0.962
Diabetic retinopathy	33 (35.1)	16 (43.2)	0.386
CSII therapy	40 (42.6)	15 (40.5)	0.834
Laboratory characteristics			
Fasting plasma glucose (mg/dL)	150±68	155±62	0.684
Haemoglobin A1c (%)	7.5±0.9	7.6±0.9	0.736
Mean Haemoglobin A1c in the last 5 years (%)	7.6±1.0	7.7±0.8	0.790
Serum creatinine (mg/dL)	0.82±0.17	0.82±0.16	0.993
eGFR (CKD-EPI; ml/min/1.73m ²)	99±16	94±16	0.072
ALT (UI/L)	23±11	22±16	0.742
Total cholesterol (mg/dL)	190±29	181±37	0.164
HDL-cholesterol (mg/dL)	61±17	58±15	0.337
LDL-cholesterol (mg/dL)	113±21	106±31	0.264
Triglycerides (mg/dL)	70 (53-88)	72 (54-122)	0.649
Non-HDL cholesterol (mg/dL)	129±24	124±36	0.399
Remnant cholesterol (mg/dL)	14 (10-18)	14 (11-25)	0.521
Total cholesterol / HDL-cholesterol ratio	3.29±0.82	3.31±1.06	0.910
Fatty liver index	24.3 (11.3-55.7)	30.5 (11.5-54.6)	0.929
eGDR (mg/kg/min)	9.38 (7.50-10.28)	7.69 (6.15-9.80)	0.057
Pharmacological treatment			
Statins	41 (43.6)	18 (48.6)	0.602
ACEi / ARB	27 (28.7)	17 (45.9)	0.060
Antiplatelet drugs	7 (7.4)	1 (2.7)	0.307

Data are shown as n (percentage) within each column, mean±SD or median (Q1-Q3). p-values between groups are shown.

ACEi: angiotensin-converting enzyme inhibitor; ALT: alanine aminotransferase; ARB: angiotensin receptor blocker; CVD: cardiovascular disease; CSII: continuous subcutaneous insulin infusion; DBP: diastolic blood pressure; eGDR: estimated glucose disposal rate; eGFR: estimated glomerular filtration rate; SBP: systolic blood pressure; T1D: type 1 diabetes

* Defined as <55 years in men and <65 years in women.

Table S2. Characteristics of the study participants according to the number of carotid plaques.

	No plaque (n=94)	Presence of 1-2 plaques (n=25)	Presence of ≥3 plaques (n=12)	P for trend
Clinical characteristics				
Female	48 (51.1)	12 (48.0)	5 (41.7)	0.533
Age (years)	44.1±9.6	49.9±9.4	57.7±8.4	<0.001
Premature CVD in first-degree relatives*	19 (20.2)	4 (16.0)	2 (16.7)	0.643
Active smoking habit	28 (29.8)	5 (20.0)	3 (25.0)	0.457
Cumulative smoking (Pack-years)	0 (0-12.5)	0 (0-5)	20 (0-40.6)	0.104
Hypertension	22 (23.4)	8 (32.0)	8 (66.7)	0.004
SBP (mmHg)	124±15	130±15	135±16	0.006
DBP (mmHg)	80±9	79±8	81±7	0.759
BMI (kg/m ²)	26.0±4.1	25.1±4.0	26.1±4.0	0.698
Waist circumference (cm)	90±12	89±11	91±15	0.867
Women	86±11	85±11	80±10	0.345
Men	95±11	92±10	98±13	0.829
T1D duration (years)	25.4±8.2	33.0±9.9	27.0±13.7	0.028
Diabetic kidney disease	13 (13.8)	3 (12.0)	2 (16.7)	0.917
Diabetic retinopathy	33 (35.1)	13 (52.0)	3 (25.0)	0.852
CSII therapy	40 (42.6)	10 (40.0)	5 (41.7)	0.876
Laboratory characteristics				
Fasting plasma glucose (mg/dL)	150±68	162±69	142±48	0.953
Haemoglobin A1c (%)	7.5±0.9	7.7±1.0	7.4±0.7	0.982
Mean Haemoglobin A1c in the last 5 years (%)	7.6±1.0	7.8±0.8	7.5±0.8	0.886
Serum creatinine (mg/dL)	0.82±0.17	0.81±0.16	0.86±0.16	0.744
eGFR (CKD-EPI; ml/min/1.73m ²)	99±16	96±17	88±14	0.027
ALT (UI/L)	23±11	21±10	23±7	0.865
Total cholesterol (mg/dL)	190±29	193±38	159±20	0.012
HDL-cholesterol (mg/dL)	61±17	59±14	57±16	0.323
LDL-cholesterol (mg/dL)	113±21	116±33	87±14	0.009
Triglycerides (mg/dL)	70 (53-88)	72 (58-131)	57 (45-89)	0.237
Non-HDL cholesterol (mg/dL)	129±24	134±38	102±19	0.024
Remnant cholesterol (mg/dL)	14 (10-18)	14 (11.5-26)	11.5 (9.2-17.7)	0.248
Total cholesterol / HDL- cholesterol ratio	3.29±0.82	3.46±1.10	3.00±0.94	0.640
Fatty liver index	24.3 (11.3-55.7)	27.2 (12.1-40.9)	40.3 (7.81-76.43)	0.890
eGDR (mg/kg/min)	9.38 (7.50-10.28)	8.46 (6.65-9.93)	6.36 (5.37-9.34)	0.078
Pharmacological treatment				
Statins	41 (43.6)	12 (48.0)	6 (50.0)	0.601
ACEi / ARB	27 (28.7)	9 (36.0)	8 (66.7)	0.015
Antiplatelet drugs	7 (7.4)	0 (0)	1 (8.3)	0.577

Data are shown as n (percentage) within each column, mean±SD or median (Q1-Q3).

p-values for trend among groups are shown.

ACEi: angiotensin-converting enzyme inhibitor; ALT: alanine aminotransferase; ARB: angiotensin receptor blocker; CVD: cardiovascular disease; CSII: continuous subcutaneous insulin infusion; DBP: diastolic blood pressure; eGDR: estimated glucose disposal rate; eGFR: estimated glomerular filtration rate; SBP: systolic blood pressure; T1D: type 1 diabetes

* Defined as <55 years in men and <65 years in women.

Table S3. NMR-lipidomics according to several clinical characteristics.

	Gender		Hypertension		Active smoking habit		Diabetic retinopathy		Use of statins	
	Female (n=65)	Male (n=66)	No (n=93)	Yes (n=38)	No (n=95)	Yes (n=36)	No (n=82)	Yes (n=49)	No (n=69)	Yes (n=62)
Triglycerides	0.64 (0.47-0.91)	0.89 (0.66-1.39)**	0.76 (0.51-1.04)	0.78 (0.56-1.14)	0.77 (0.52-1.06)	0.77 (0.51-1.38)	0.76 (0.51-0.96)	0.84 (0.52-1.14)	0.74 (0.49-1.09)	0.78 (0.53-1.09)
Glycerophospholipids	2.57 (2.32-2.77)	2.38 (2.16-2.69)*	2.55 (2.25-2.75)	2.39 (2.19-2.61)	2.41 (2.23-2.72)	2.59 (2.24-2.73)	2.55 (2.28-2.74)	2.39 (2.17-2.66)	2.42 (2.17-2.69)	2.56 (2.30-2.74)
Phosphatidylcholine	2.48 (2.28-2.68)	2.30 (2.12-2.56)*	2.43 (2.18-2.66)	2.35 (2.13-2.55)	2.35 (2.14-2.61)	2.52 (2.24-2.61)	2.43 (2.20-2.65)	2.34 (2.10-2.56)	2.33 (2.09-2.59)	2.42 (2.24-2.65)
Sphingomyelin	0.63 (0.58-0.70)	0.59 (0.53-0.66)**	0.62 (0.56-0.68)	0.59 (0.54-0.64)	0.61 (0.54-0.67)	0.62 (0.58-0.68)	0.61 (0.55-0.69)	0.60 (0.55-0.66)	0.60 (0.54-0.66)	0.64 (0.56-0.71)*
Lysophosphatidylcholine	0.47 (0.39-0.51)	0.48 (0.42-0.57)	0.48 (0.42-0.57)	0.44 (0.40-0.52)	0.47 (0.41-0.53)	0.48 (0.39-0.53)	0.49 (0.41-0.57)	0.46 (0.40-0.51)	0.48 (0.42-0.53)	0.46 (0.40-0.57)
Esterified cholesterol	4.71 (4.44-5.15)	4.58 (4.31-5.10)	4.71 (4.43-5.17)	4.54 (4.32-4.97)	4.67 (4.37-5.10)	4.61 (4.42-5.17)	4.67 (4.43-5.19)	4.61 (4.31-5.03)	4.71 (4.48-5.17)	4.55 (4.29-5.08)
Free cholesterol	2.10 (1.94-2.36)	2.03 (1.85-2.32)	2.13 (1.94-2.37)	1.98 (1.80-2.27)*	2.05 (1.87-2.27)	2.17 (1.98-2.38)	2.11 (1.95-2.36)	2.02 (1.86-2.34)	2.08 (1.94-2.35)	2.08 (1.85-2.34)
Linoleic acid	4.20 (3.77-4.52)	4.10 (3.72-4.71)	4.20 (3.74-4.66)	4.04 (3.71-4.53)	4.08 (3.69-4.52)	4.27 (3.78-4.80)	4.20 (2.92-4.62)	4.07 (3.63-4.58)	4.22 (3.79-4.76)	4.01 (3.64-4.47)*
Saturated fatty acids	4.80 (4.36-5.63)	4.74 (4.16-5.41)	4.75 (4.27-5.47)	4.85 (4.17-5.63)	4.76 (4.12-5.75)	4.78 (4.49-5.11)	4.81 (4.29-5.55)	4.75 (4.15-5.37)	4.69 (4.15-5.29)	4.89 (4.35-5.77)
w-3 fatty acids	0.27 (0.21-0.33)	0.21 (0.17-0.27)**	0.25 (0.19-0.30)	0.24 (0.19-0.32)	0.25 (0.19-0.32)	0.22 (0.18-0.28)	0.24 (0.19-0.32)	0.25 (0.19-0.29)	0.25 (0.19-0.32)	0.24 (0.19-0.30)
w-6 fatty acids	2.69 (2.46-3.19)	2.85 (2.29-3.48)	2.82 (2.49-3.32)	2.63 (2.29-3.46)	2.72 (2.39-3.32)	2.90 (2.46-3.60)	2.83 (2.42-3.37)	2.69 (2.42-3.33)	2.92 (2.58-3.54)	2.63 (2.28-3.10)*
w-7 fatty acids	2.63 (2.36-3.21)	2.56 (2.13-2.90)	2.65 (2.24-3.20)	2.60 (2.40-2.82)	2.59 (2.24-2.93)	2.66 (2.42-3.27)	2.64 (2.28-3.13)	2.55 (2.11-2.94)	2.59 (2.29-2.90)	2.63 (2.23-3.24)
w-9 fatty acids	3.54 (2.76-4.53)	4.01 (3.41-5.13)*	3.83 (3.15-4.66)	4.01 (2.88-4.94)	3.83 (3.06-4.77)	3.87 (3.15-4.94)	3.75 (2.84-4.76)	3.92 (3.24-5.01)	3.86 (2.97-4.73)	3.83 (3.12-4.81)
DHA	0.092 (0.065-0.119)	0.085 (0.061-0.114)	0.089 (0.066-0.114)	0.088 (0.054-0.129)	0.088 (0.065-0.115)	0.088 (0.060-0.121)	0.090 (0.065-0.121)	0.084 (0.060-0.111)	0.082 (0.061-0.114)	0.094 (0.067-0.124)
ARA-EPA	0.61 (0.48-0.80)	0.62 (0.51-0.82)	0.61 (0.51-0.87)	0.60 (0.50-0.78)	0.61 (0.51-0.82)	0.61 (0.48-0.95)	0.61 (0.52-0.83)	0.59 (0.48-0.79)	0.58 (0.48-0.73)	0.67 (0.55-0.90)*

Data are shown as median (Q1-Q3). Data are presented as mmol/L.

*p<0.05; **p<0.01 between groups

ARA: arachidonic acid; DHA: docosahexaenoic acid; EPA: eicosapentaenoic acid.

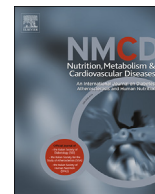
ARTÍCULO ORIGINAL 7: LA RAZÓN ENTRE NEUTRÓFILOS Y LINFOCITOS SE ASOCIA INDEPENDIENTEMENTE CON LA CARGA DE ATEROSCLEROSIS CAROTÍDEA EN INDIVIDUOS CON DIABETES TIPO 1

Objetivos: La desregulación entre la inmunidad innata y adaptativa se ha asociado a la ECV en la población general, pero en personas con DTI la evidencia es escasa. Nuestro objetivo fue estudiar la relación entre los biomarcadores inflamatorios y la aterosclerosis preclínica en esta población.

Métodos: Estudio transversal en individuos con DTI sin ECV y con: edad ≥ 40 años, ERD, o ≥ 10 años de duración de la diabetes con otro FRCV. Las placas carotídeas se evaluaron mediante ecografía. Se evaluaron la PCRus, la concentración de leucocitos circulantes, el RNL, la razón entre plaquetas y linfocitos circulantes y el índice sistémico de inflamación e inmunidad (SII), como marcadores inflamatorios. Se construyeron modelos ajustados multivariantes que incluían edad, sexo y otros FRCV para analizar sus asociaciones independientes con la carga de aterosclerosis.

Resultados: Incluimos a 602 sujetos (52,8% hombres, $48,7 \pm 10,2$ años de edad y $27,0 \pm 10,5$ años de duración de la diabetes). Se encontraron placas carotídeas en el 41,2% de los individuos (12,8%, ≥ 3 placas). El número de placas carotídeas (ninguna, 1-2, ≥ 3 placas) se asoció directamente con los leucocitos circulantes (6570 [5445-8050], 6640 [5450-8470] y 7310 [5715-8935] por mm^3 , respectivamente; p de tendencia = 0,021) y la RNL (1,63 [1,28-2,13], 1,78 [1,38-2,25] y 2,14 [1,58-2,92], respectivamente; p de tendencia $< 0,001$), pero solo la RNL permaneció directamente asociada en modelos ajustados (presencia de placas; OR 1,285 [1,040-1,587]; ≥ 3 placas, OR 1,377 [1,036-1,829]).

Conclusiones: La RNL se asoció de manera independiente y directa con la carga de placas carotídeas en individuos con DTI. Nuestros datos respaldan el papel de la inmunidad innata frente a la adaptativa en la aterosclerosis también en la población con DTI.



Neutrophil-to-lymphocyte ratio is independently associated with carotid atherosclerosis burden in individuals with type 1 diabetes

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KEYWORDS

Preclinical atherosclerosis; Carotid ultrasonography; Type 1 diabetes; Inflammation; Neutrophil-to-lymphocyte ratio

Abstract *Background and aims:* Recent studies have identified a relationship between innate versus. Adaptive immunity and cardiovascular disease (CVD) in the general population, but information on type 1 diabetes (T1D) is lacking. We aimed to study the relationship between inflammatory biomarkers and preclinical atherosclerosis in this population.

Methods and results: Cross-sectional study in T1D individuals without CVD and with ≥ 1 of the following: ≥ 40 years, diabetic kidney disease, or ≥ 10 years of diabetes duration with classical CVD risk factors. Carotid plaques were evaluated by ultrasonography. C-reactive protein, total leukocyte count, neutrophil-to-lymphocyte ratio (NLR), platelet-to-lymphocyte ratio and systemic immune-inflammation index were assessed as inflammatory markers. Multivariate-adjusted models including age, sex, and other CVD risk factors were constructed to test their independent associations with atherosclerosis burden. We included 602 subjects (52.8% men, 48.7 ± 10.2 years old and 27.0 ± 10.5 years of diabetes duration). Carotid plaques were found in 41.2% of the individuals (12.8%, ≥ 3 plaques). The number of carotid plaques (none, 1–2, ≥ 3 plaques), was directly associated with the leukocyte count (6570 [5445–8050], 6640 [5450–8470] and 7310 [5715–8935] per mm^3 , respectively; p for trend = 0.021) and the NLR (1.63 [1.28–2.13], 1.78 [1.38–2.25] and 2.14 [1.58–2.92], respectively; p for trend < 0.001), but only the NLR remained directly associated in fully-adjusted models (presence of plaques; OR 1.285 [1.040–1.587]; ≥ 3 plaques, OR 1.377 [1.036–1.829]).

Conclusions: The NLR was independently and directly associated with carotid plaque burden in T1D individuals. Our data support the role of innate versus. Adaptive immunity in atherosclerosis also among the T1D population.

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1. Introduction

Atherosclerosis is the formation of fibrofatty lesions in the innermost layer of arteries that can eventually reach the arterial lumen and increase the risk of developing cardiovascular disease (CVD) [1]. In individuals with type 1 diabetes (T1D), CVD remains the main cause of mortality and morbidity. Despite the improvement in glycemic control and a decline in the incidence of cardiovascular complications in the last years [2], these individuals are still at a two-to-eight-fold higher risk of developing cardiovascular events than the general population [2,3]. Thus, other extra-glycemic variables must play a role in the accelerated atherosclerosis usually seen in T1D individuals. Different biomarkers have been proposed to predict the development of atherosclerosis and CVD in the general population, with inflammation parameters being promising in this field [1,4–6]. However, the association between inflammation and CVD in T1D is still poorly understood.

T1D is a chronic disease caused by the immune-mediated destruction of beta cells [7]. Similarly, atherosclerosis is a long-standing process, in which inflammation and immunity also play an important role [1]. In the general population, inflammation markers have been shown to predict CVD, independently of other traditional risk factors [6]. Beyond these general inflammation parameters, other biomarkers reflecting the balance between innate immunity (i.e., neutrophil count and platelets) and adaptive immunity (i.e., lymphocyte count) seem promising for the identification of individuals more prone to present CVD [8]. Specifically, the combination of these measurements into ratios (e.g. the neutrophil-to-lymphocyte ratio (NLR), platelet-to-lymphocyte ratio (PLR), and the systemic immune-inflammation index (SII)), are thought to reflect the relative balance between innate and adaptive immunity even better [9]. In this regard, previous studies have shown that the NLR was strongly correlated with subclinical atherosclerosis in the general population [10]. Further, this biomarker is independently associated with overall CVD mortality [8]. Conversely, in T1D there are only preliminary data linking the NLR with microvascular damage [11,12], with very little data regarding macrovascular complications [13]. Additionally, no previous studies have specifically assessed the relationships between other ratios linking innate versus adaptive immunity with atherosclerosis in this population.

With this background, this study aimed to assess the independent relationships between several inflammatory biomarkers (especially focusing on the NLR; as a main innate vs. adaptive immunity marker) and preclinical carotid atherosclerosis burden in adults with T1D at high cardiovascular risk.

2. Methods

2.1. Study design and participants

We conducted a cross-sectional study in subjects with T1D from a specialized Diabetes Unit of a tertiary hospital

in Catalonia, Spain. We recruited consecutive subjects with the following inclusion criteria: no previous history of CVD (coronary artery disease, ischemic stroke, peripheral artery disease or heart failure) and any of the following: age ≥ 40 years; the presence of any stage of diabetic kidney disease (DKD); and/or ≥ 10 years of T1D duration with at least one additional cardiovascular risk factor. We considered the following as CVD risk factors: the presence of first-degree relatives with a history of premature CVD (<55 years in males and <65 years in females); active smoking habit, any degree of diabetic retinopathy (DR), hypertension, low high density lipoprotein (HDL)-cholesterol ($<40/45$ mg/dL, in males/females, respectively), triglycerides >150 mg/d, already on statin therapy, a former episode of preeclampsia/eclampsia, impaired hypoglycaemia awareness (Clarke test score >3 points [14,15]), or a previous episode of severe hypoglycaemia in the last two years.

The study protocol was conducted according to the principles of the Declaration of Helsinki and approved by the hospital Research Ethics Committee (HCB/2020/0195).

2.2. Clinical and laboratory measures

The clinical data registered were age, sex, family history of premature CVD in first-degree relatives, smoking habit, the use of antihypertensive (especially, angiotensin-converting enzyme inhibitors (ACEi) and angiotensin II receptor blockers (ARB), lipid-lowering, and antiplatelet drugs. Regarding diabetes-related variables, we recorded diabetes duration (years), the presence of chronic complications (DR and DKD), and the current insulin treatment (continuous subcutaneous insulin infusion or multiple daily injections) and with the daily insulin dose (measured in international units per kilogram of body weight).

Anthropometric measures included height, weight, waist and hip circumference, body mass index (BMI), and systolic and diastolic blood pressure after 3–4 min of rest. All these variables were obtained with standardized methods. DR was defined by fundus oculi and was always determined by an ophthalmologist. DKD was defined as an albumin-to-creatinine ratio ≥ 30 mg/g and/or an estimated glomerular filtration rate (eGFR) < 60 ml/min/1.73 m². The use of ACEi or ARB, with no history of hypertension or CVD, was also considered as DKD. Obesity was defined as a BMI ≥ 30 kg/m². Hypertension was defined as the use of antihypertensive drugs or a repeated clinical systolic blood pressure ≥ 140 mmHg or diastolic blood pressure ≥ 90 mmHg.

Laboratory parameters were measured in the local laboratory according to standard procedures. For further details, see Supplementary Files. The fatty liver index (FLI) was calculated with waist circumference, BMI, triglycerides, and gamma-glutamyl transferase, as described previously [16]. Finally, the estimated glucose disposal rate (eGDR) was also calculated to determine insulin sensitivity, expressed in mg/kg/min, and calculated as follows: $24.31 - 12.22 \times \text{waist-to-hip ratio} - 3.29 \times \text{hypertension}$ ($0 = \text{no}/1 = \text{yes}$) $- 0.57 \times \text{HbA1c (in \%)} [17]$.

2.3. Inflammatory laboratory measures

Inflammatory parameters were obtained in the local laboratory and included total leukocyte, neutrophil, lymphocyte, and platelet counts, as well as high-sensitive c-reactive protein (hsCRP). Cell count measurements were performed using flow cytometry (ADVIA 2120 [Siemens]) and were expressed as absolute counts per mm^3 . hsCRP was assessed by the immunoturbidimetric method (ADVIA 2400/1800 [Siemens]) and was expressed in mg/dL . The NLR and PLR were calculated as the division of the derived neutrophil count to lymphocyte count and platelet count to lymphocyte count, respectively. The SII was defined as the platelet count times the NLR.

2.4. Carotid B-mode ultrasound imaging

Predefined and standardized imaging protocols to evaluate carotid intima-media thickness (IMT) and the presence of plaque were carried out as described previously [18,19]. For further details, see Supplementary Files.

2.5. Statistical analyses

Data are presented as median and 25th and 75th percentiles, mean $\pm$ standard deviation, or number (percentage). Normal distribution was assessed by the Kolmogorov–Smirnov test. Comparisons of variables according to the presence and number of carotid plaques were performed using an unpaired Student's t-test or ANOVA for normally distributed continuous variables, or the Mann–Whitney U test or the Kruskal–Wallis H test for non-normally distributed variables. Proportions were compared using a chi-square test. The associations of all the inflammatory parameters with clinical, physical exam, laboratory or T1D-specific variables were assessed using R of Spearman or the unpaired Student's t-test and Mann–Whitney U test, as appropriate.

To analyse the independent associations of the different inflammatory parameters (leukocyte count, hsCRP, NLR, PLR and SII) with preclinical carotid atherosclerosis (presence of carotid plaque and presence of ≥ 3 plaques; dependent variables) we used logistic binary regression models. Model 1 only included age and sex as covariates. Model 2 further included other classical CVD risk factors (systolic blood pressure, smoking habit, low-density lipoprotein [LDL]-cholesterol, BMI, antiplatelet treatment, and statin use). Finally, model 3 included the same variables as model 2 plus other T1D-specific variables (diabetes duration, mean HbA1c in the last 5 years, albumin-to-creatinine ratio, and the presence of DR). Additionally, we assessed the differences in all the inflammatory variables according to the number of carotid plaques (none, 1–2, ≥ 3 plaques), after adjusting for the same variables as in model 3 (analysis of covariance, ANCOVA).

IBM SPSS Statistics version 23.0 (SPSS Inc.; Chicago, IL, USA) was used to perform the statistical analysis. The significance level was set as a p-value < 0.05 .

3. Results

3.1. Subject characteristics

A total of 602 subjects were included in this study, 52.8% were males, with a mean age of 48.7 ± 10.2 years, and a 27.0 ± 10.5 years of diabetes duration. Overall, 28.6% were active smokers, 24.6% had hypertension, and the mean BMI was 26.3 kg/m^2 . Regarding microvascular complications, 37.2% had DR and 10% had DKD. Two hundred forty-five participants (40.7%) were receiving statin therapy, 26.2% were on ACEi/ARB treatment and 8.6% were on antiplatelet drugs. Carotid ultrasound showed at least one carotid plaque in 41.2% of the individuals, and 12.8% ($n = 77$) had ≥ 3 plaques. The baseline clinical and laboratory characteristics according to the presence of carotid plaque or atherosclerotic burden (0, 1–2 or ≥ 3 plaques) are shown in [Supplementary Table S1](#) and [Table 1](#), respectively. Older age, longer T1D duration, the presence of hypertension, higher systolic blood pressure, and greater waist circumference were directly associated with the presence and number of carotid plaques ($p < 0.05$), with no differences according to sex, microvascular diabetic complications or hypoglycaemia unawareness. In addition, those with plaque or a higher number of plaques were more likely to use cardioprotective drugs (i.e., lipid-lowering, anti-hypertensives and antiplatelets drugs) and also show worse renal function (eGFR) and higher serum triglyceride levels ($p < 0.05$ for all comparisons).

3.2. Relationships between inflammatory parameters and clinical and laboratory characteristics

The associations between the different inflammatory parameters and the characteristics of the study subjects are shown in [Fig. 1](#) and [Supplementary Table S2](#). Minor differences were observed in sex (higher PLR in women; 12.00 vs. 11.08 ; $p < 0.01$), without changes regarding age. BMI and waist circumference showed a strong positive correlation with most of the inflammatory parameters and ratios (especially for hsCRP, with $r > 0.4$). Regarding classical CVD risk factors, the presence of hypertension and systolic blood pressure were directly associated with most of the variables ($p < 0.05$ for all comparisons, except for PLR); and active smokers showed remarkably higher levels of total leukocyte count (7810 vs. 6300 per mm^3 ; $p < 0.01$). Among laboratory parameters, triglycerides showed especially positive and HDL-cholesterol negative relationships with inflammatory parameters, with no differences regarding LDL-cholesterol. As expected, the FLI also showed a strong positive correlation with these parameters (especially for hsCRP; $r > 0.4$). Regarding T1D-specific characteristics, no differences were observed according to diabetes duration (and weak for HbA1c with total leukocyte count and hsCRP), but several markers of insulin-resistance did show a positive association with almost all the parameters (positive for insulin doses, negative for eGDR) as well as for DKD (leukocyte count; $p < 0.01$).

Table 1 Characteristics of the study participants according to the number of plaques.

	No plaque presence (n = 354)	Presence of 1–2 plaques (n = 171)	Presence of ≥ 3 plaques (n = 77)	p-value for trend
Clinical characteristics				
Female	177 (50)	77 (45)	30 (39)	0.061
Age (years)	45.4 $\pm$ 9.4	50.9 $\pm$ 8.8	59.5 $\pm$ 7.9	<0.001
Premature CVD in first-degree relatives	41 (11.6)	17 (9.9)	13 (16.9)	0.406
Current smokers	91 (25.7)	50 (29.2)	31 (40.3)	0.015
Hypertension	63 (17.8)	48 (28.1)	37 (48.1)	<0.001
SBP (mmHg)	126 $\pm$ 14	129 $\pm$ 15	136 $\pm$ 17	<0.001
BMI (kg/m ²)	26.2 $\pm$ 4	26.1 $\pm$ 4	26.7 $\pm$ 4.4	0.361
Waist circumference (cm)	91 $\pm$ 12	92 $\pm$ 12	96 $\pm$ 13	0.004
Female (cm)	86 $\pm$ 12	86 $\pm$ 13	90 $\pm$ 14	0.172
Male (cm)	95 $\pm$ 11	97 $\pm$ 10	99 $\pm$ 12	0.034
Diabetes duration (years)	25.8 $\pm$ 9.6	28.6 $\pm$ 11	29.5 $\pm$ 12.4	0.005
Diabetic kidney disease	35 (9.9)	15 (8.8)	10 (13)	0.618
Diabetic retinopathy	122 (34.5)	67 (39.2)	35 (45.5)	0.057
CSII therapy	121 (34.2)	47 (27.5)	20 (26)	0.073
Hypoglycaemia unawareness ^a	58 (16.5)	29 (17.3)	13 (16.9)	0.870
Laboratory characteristics				
Fasting plasma glucose (mg/dL)	156 $\pm$ 69	166 $\pm$ 62	161 $\pm$ 56	0.585
HbA1c (%)	7.6 $\pm$ 1	7.7 $\pm$ 0.9	7.6 $\pm$ 0.7	0.802
eGFR (CKD-EPI; ml/min/1.73m ²)	96 $\pm$ 14	93 $\pm$ 15	84 $\pm$ 16	<0.001
Total cholesterol (mg/dL)	190 $\pm$ 30	193 $\pm$ 31	186 $\pm$ 31	0.254
HDL-cholesterol (mg/dL)	63 $\pm$ 23	59 $\pm$ 15	63 $\pm$ 19	0.920
LDL-cholesterol (mg/dL)	112 $\pm$ 25	116 $\pm$ 26	105 $\pm$ 25	0.028
Triglycerides (mg/dL)	72 (59–97)	79 (63–107)	82 (66–116)	0.038
Pharmacological treatment				
Statins	124 (35)	68 (39.8)	53 (68.8)	<0.001
ACEi/ARB	75 (21.2)	48 (28.1)	35 (45.5)	<0.001
Antiplatelet drugs	19 (5.4)	16 (9.4)	17 (22.1)	<0.001

Data are shown as n (percentage), mean ($\pm$ SD) or median (Q1–Q3).

p values for group comparisons are reported.

ACEi: angiotensin-converting enzyme inhibitor; ARB: angiotensin receptor blocker; CVD: cardiovascular disease; CSII: continuous subcutaneous insulin infusion; eGFR: estimated glomerular filtration rate; SBP: systolic blood pressure.

Finally, whereas treatment with lipid-lowering or ACEi/ARB drugs was not associated with any change, subjects receiving antiplatelets showed lower levels of total leukocyte counts (6580 vs. 7395 per mm³), hsCRP (0.11 vs. 0.22 mg/dL) and NLR (1.67 vs. 2.13; $p < 0.05$ for all; [Supplementary Table S1](#)).

3.3. Association between inflammatory parameters and the presence of carotid atherosclerosis

Among the five inflammatory-related variables assessed, only NLR (1.63 [1.28–2.13] vs 1.86 [1.42–2.44] per mm³, $p = 0.001$) was higher for those harbouring versus those without plaque ([Supplementary Fig. 1](#)). Along the same line, after adjusting for age and sex (model 1) only total leukocyte count (odds ratio [OR] [95% confidence interval [CI]]: 1.134 [1.034–1.244], $p = 0.007$) and NLR (OR [95% CI]: 1.297 [1.067–1.577], $p = 0.009$) were independently associated with the presence of atherosclerosis ([Table 2](#)). However, after adjusting for various non-specific (model 2) and T1D-specific (model 3) CVD risk factors, the only association that remained independent was that of NLR (OR [95% CI]: 1.288 [1.052–1.577], $p = 0.014$ and 1.285 [1.040–1.066], $p = 0.020$, respectively; [Table 2](#)).

3.4. Association between inflammatory parameters and carotid atherosclerosis burden

The association of inflammatory parameters with the number of plaques was also assessed. The inflammatory parameters showing a stepped increase according to the number of carotid plaques (0, 1–2 or ≥ 3 plaques) were total leukocyte count (6570 [5445–8050], 6640 [5450–8470] and 7310 [5715–8935] per mm³; p for trend = 0.021) and NLR (1.63 [1.28–2.13], 1.78 [1.38–2.25] and 2.14 [1.58–2.92]; p for trend <0.001; [Fig. 2](#)). However, although in age- and sex-adjusted models high-sensitive C-reactive protein, total leukocyte count and NLR continued to be independently associated with carotid atherosclerosis burden, only the latter ratio showed direct associations when non-T1D-specific (model 2) and T1D-specific CVD risk factors (model 3) were taken into account (for the presence of ≥ 3 plaques; OR [95% CI]: 1.377 [1.036–1.829]; $p < 0.05$ for both comparisons; [Supplementary Table S3](#)). As a final analysis, all the previous inflammatory-related variables were adjusted for all the confounders (model 3) concerning the number of carotid plaques (none, 1–2 and ≥ 3 plaques; ANCOVA). Although all the variables showed a numerically stepped

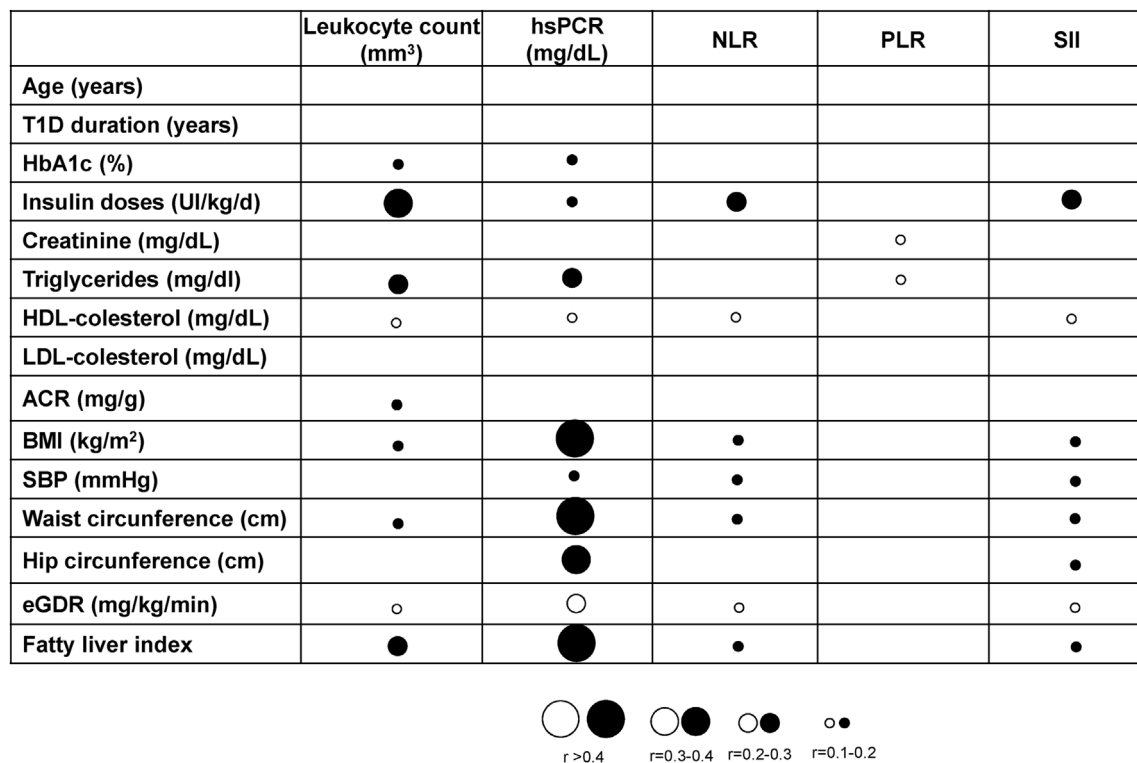


Figure 1 Associations between inflammatory parameters and the clinical and laboratory parameters. Solid and open circles indicate positive and negative correlations, respectively. hsCRP: high-sensitive c-reactive protein, NLR: neutrophil-to-lymphocyte ratio, PLR: platelet-to-lymphocyte ratio, SII: systemic immune-inflammation index; T1D: Type 1 diabetes, HbA1c: glycated hemoglobin, ACR: albumin-to-creatinine ratio, BMI: body mass index, SBP: systolic blood pressure, eGDR: estimated glucose disposal rate.

increase with the increase in the number of plaques (6881 vs. 7203, for total leukocyte count; 0.225 vs. 0.344 for hsCRP; 11.96 vs. 12.38 for PLR; and 4.61 vs. 5.35 for SII; for none vs. ≥ 3 plaques, respectively; p for trend > 0.1 for all comparisons; [Supplementary Fig. S2](#)), only NLR reached statistical significance (1.84 vs. 2.27 for none vs. ≥ 3 carotid plaques; respectively, p for trend = 0.002; [Fig. 3](#)).

4. Discussion

In our sample of individuals with T1D at primary prevention, circulating biomarkers assessing innate versus adaptive immunity were independently associated with preclinical atherosclerosis. Specifically, the NLR was strongly and directly associated with the presence and number of carotid plaque, even after adjusting for the main classical and T1D-related risk factors. To the best of our knowledge, this is the first study to assess the relationship between this easily-available inflammation-related parameter and the presence of carotid plaque assessed by ultrasonography, a surrogate of macrovascular complications in this population.

Inflammation has emerged as one of the main drivers of the atherosclerotic process in the general population [1,20,21]. Regarding the T1D population, previous studies have shown that these individuals have elevated classical inflammatory markers (i.e. leukocyte count or hsCRP) compared with their normoglycemic counterparts [22–24]. Our research group also found higher

inflammation-related parameters in 508 Mediterranean individuals with T1D vs. 347 normoglycemic controls [25], inflammatory surrogates were significantly elevated in both paediatric and adult T1D subjects, even after adjusting for the main confounders (i.e., adiposity) [22–24]. In line with these data, in our sample of individuals, adiposity-related (i.e., BMI or FLI) or insulin-resistance-related (i.e., eGDR and insulin dose) parameters were found to be the most strongly associated with these classical inflammatory variables ([Fig. 1](#)). Regarding the NLR (as a main marker of innate vs. adaptive immunity), a recent study also reported higher levels among 100 children with T1D vs. 100 healthy matched controls [12]. Interestingly, and in accordance with the previous [12] and other studies [13], in our sample of subjects with T1D, the association of the NLR with adiposity was less pronounced than with the leukocyte count or hsCRP ([Fig. 1](#)). Studies are needed to determine whether this ratio could be a better reflection of inflammation independently of one of the main confounders (i.e., obesity) in the T1D population. Altogether, this suggests that inflammation could be added as one of the drivers of the increased CVD risk in T1D, beyond hyperglycaemia.

In the general population, several studies have associated markers of innate versus adaptive immunity with the risk of atherosclerosis and/or future CVD [26,27]. Accordingly, Meg et al. demonstrated an independent association of the neutrophil count and the NLR with early markers of atherosclerosis, such as carotid-IMT [10]. In the

Table 2 Associations between inflammatory markers and the presence of carotid plaque by logistic regression analysis.

	Carotid plaque presence	
	OR (95% CI)	p-value
Model 1^a		
Leukocyte count (per 1000 mm ³ increase)	1.134 (1.034–1.244)	0.007
hsCRP (mg/dL)	1.515 (0.807–2.842)	0.196
Neutrophil-to-lymphocyte ratio	1.297 (1.067–1.577)	0.009
Platelets-to-lymphocyte ratio	1.012 (0.968–1.058)	0.602
SII (100 units increase)	1.061 (0.995–1.133)	0.072
Model 2^b		
Leukocyte count (per 1000 mm ³ increase)	1.101 (0.995–1.219)	0.063
hsCRP (mg/dL)	1.440 (0.751–2.760)	0.272
Neutrophil-to-lymphocyte ratio	1.288 (1.052–1.577)	0.014
Platelets-to-lymphocyte ratio	1.019 (0.973–1.067)	0.420
SII (100 units increase)	1.048 (0.981–1.119)	0.161
Model 3^c		
Leukocyte count (per 1000 mm ³ increase)	1.100 (0.993–1.219)	0.067
hsCRP (mg/dL)	1.440 (0.744–2.786)	0.279
Neutrophil-to-lymphocyte ratio	1.285 (1.040–1.587)	0.020
Platelets-to-lymphocyte ratio	1.018 (0.972–1.066)	0.450
SII (100 units increase)	1.048 (0.980–1.120)	0.169
Logistic regression models (odds ratio and 95% confidence interval) are presented.		
hsCR: high-sensitive C-reactive protein, SII: systemic immune-inflammation index, OR: odds ratio, CI: confidence interval.		
^a Model 1: Adjusted for age and sex.		
^b Model 2: Model 1 + systolic blood pressure, statin exposure, antiplatelet treatment, LDL-cholesterol, body mass index and smoking habit.		
^c Model 3: Model 2 + diabetes duration, 5-year mean HbA1c, diabetic retinopathy and albumin-to-creatinine ratio.		

Rotterdam study, Fany et al. showed that both inflammation-related parameters were independently associated with increased atherosclerosis burden or markers of vulnerable plaques in multiple vessels, including both coronary [28] and carotid territories [5]. Furthermore, both markers were independently and directly associated with hard CVD outcomes in these individuals [28]. The impact of the NLR was further analysed in 5 randomized controlled trials (CANTOS, JUPITER, SPIRE-1, SPIRE-2, and CIRT trials), including more than 60,000 participants. In all five studies, the baseline NLR predicted incident CVD events and total and cardiovascular death in a dose-dependent manner [27]. Interestingly, although lipid-lowering treatment did not modify NLR levels, canakinumab (an anti-inflammatory drug proven to

be associated with a decrease in CVD events in a large randomized controlled trial [29]) did decrease its levels [27]. Among the T1D population, the impact of the NLR on vascular complications, especially macrovascular events, has been scarcely studied to date. Regarding microvascular damage, only two small studies in paediatric subjects with T1D have found direct relationships [12]. Although this was outside the scope of our study, in our sample of adult subjects we also observed direct associations between the NLR and albumin-to-creatinine ratio (Fig. 1). The associations between markers of innate versus adaptative immunity and atherosclerosis in T1D have been even less assessed. In fact, only one small study in 76 subjects showed independent associations with aortic stiffness assessed by echocardiography [13]. The present study, which was performed in a larger sample ($n = 602$) and with a more validated surrogate of CVD than arterial stiffness (carotid plaques assessed by ultrasonography) [21], supports the role of the NLR on in atherosclerosis, and also in T1D. Specifically, we observed the direct and independent association of this ratio, not only with the presence but also with the number of carotid plaques in a dose-response manner (Fig. 2). Furthermore, it was the only inflammatory-related parameter which remained statistically associated with atherosclerosis burden after adjustment for both classical and T1D-related CVD risk factors (Table 2 and Fig. 3). Altogether, this suggests that the NLR, as a widely available proxy of innate versus adaptative immunity, could help identify individuals with more advanced atherosclerosis (and thus, more prone to present CVD) among the T1D population. Further studies assessing if this inflammatory ratio could also select individuals in which very specific treatments (i.e., anti-inflammatories or anti-platelet drugs) would be more effective are warranted.

Newer and more reliable biomarkers of CVD are needed in the setting of T1D, in which the existing tools (i.e., cardiovascular risk scales) perform rather poorly [30]. Regarding inflammation-related variables, the results of the present study agree with previous data from our research group [31,32]. We previously demonstrated that the novel quantification of glycoproteins by nuclear magnetic resonance was better than classical inflammatory-derived variables (i.e., leukocyte count and hsCRP) in identifying individuals with T1D and higher atherosclerosis burden [31,32]. Herein, we also demonstrated that even though the leukocyte count and the NLR were independently associated with carotid plaques in age- and sex-adjusted models, only the latter ratio remained statistically associated when other variables closely related to atherosclerosis (and inflammation) were taken into account (Table 2). Unlike nuclear magnetic resonance-assessed parameters, the NLR has no additional cost, since it is available in any routine blood test. Furthermore, this ratio could have other advantages over other classical parameters in identifying individuals at high risk of developing CVD. Firstly, neutrophils have long been known to play a crucial role in the pathogenesis of atherosclerosis. The classical model for atherosclerotic

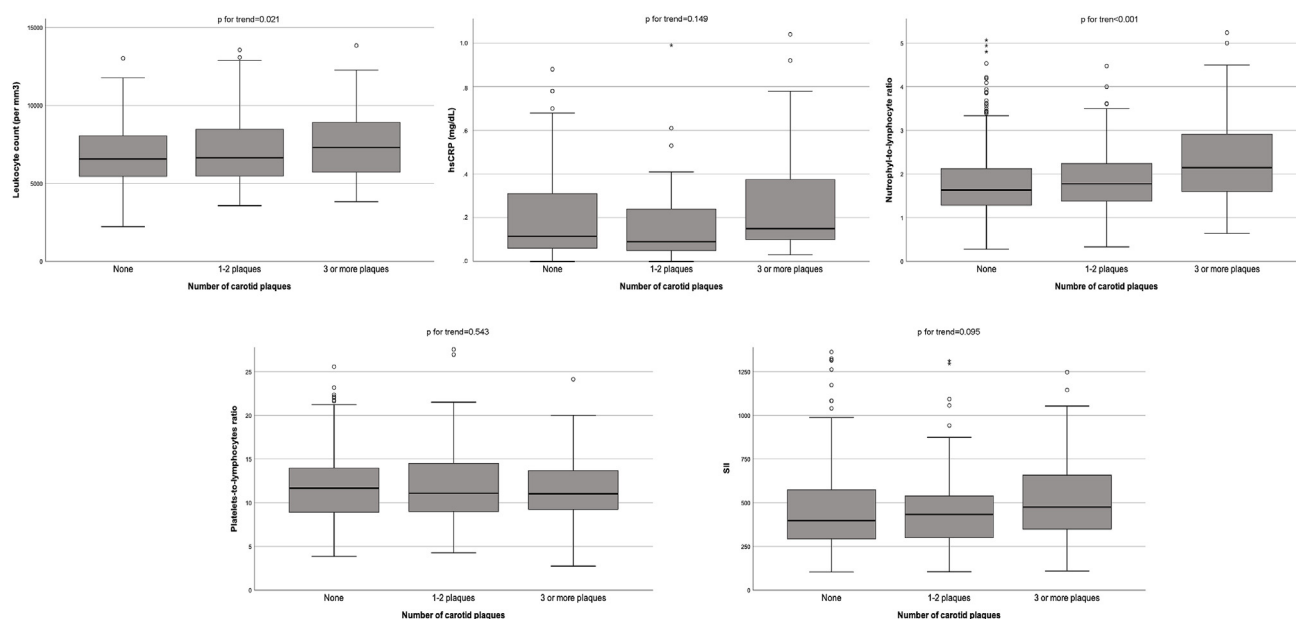


Figure 2 Box-plots of inflammatory-related variables according to the number of carotid plaques. P-for trend according to the number of plaques are shown. For abbreviations see Fig. 1.

plaque development begins with circulating monocytes adhering to the endothelium and migrating into the sub-endothelial space. These cells are later transformed into foam cells after the phagocytosis of oxidized LDL-cholesterol, ultimately forming much of the lipid core of unstable plaques [33]. In contrast, higher levels of lymphocytes (as a marker of adaptive immunity) have been largely associated with atheroprotection in several population-based studies [5,27]. Consequently, the NLR considers both pro- (i.e., neutrophils) and anti-atherogenic

(i.e., lymphocytes) parameters; unlike the total leukocyte count, which only accounts for the absolute number of white cells irrespective of their main action in atherogenesis. Moreover, the NLR could also be a reflection of an autonomic imbalance. In this sense, neutrophils have adrenergic receptors, whereas lymphocytes have cholinergic receptors [34]. Therefore, a higher NLR may point to a higher ratio of sympathetic/parasympathetic activity. An increased sympathetic tone is positively correlated with higher rates of oxygen consumption and increased production of proinflammatory cytokines, such as interleukin-6 and tumour necrosis factor- α , which have shown to be involved in the development of CVD [35,36].

The present study has some strengths and limitations. The main strength is its novelty. As far as we know, this is the first study to assess the relationships between markers of innate versus adaptive immunity (especially, the NLR) in the setting of carotid atherosclerosis in the T1D population. Furthermore, instead of arterial stiffness [13], we used carotid plaques visualized by ultrasonography, an exploration recommended by the last European guidelines on CVD prevention to improve risk classification [21], and a better independent predictor of CVD than IMT [37]. Finally, we carefully collected data on drug treatment (especially lipid-lowering and anti-platelet drugs) and other clinical and analytical variables (including BMI), which reduced the bias of our results. However, we have to acknowledge some limitations. First, this is a cross-sectional study, therefore no causal relationships can be inferred. Second, our population is based on a single-centre tertiary hospital, in which carotid ultrasound is included in the usual care of T1D subjects. Thus, our sample might be not representative of other geographical areas or medical centres. Secondly, we lack information

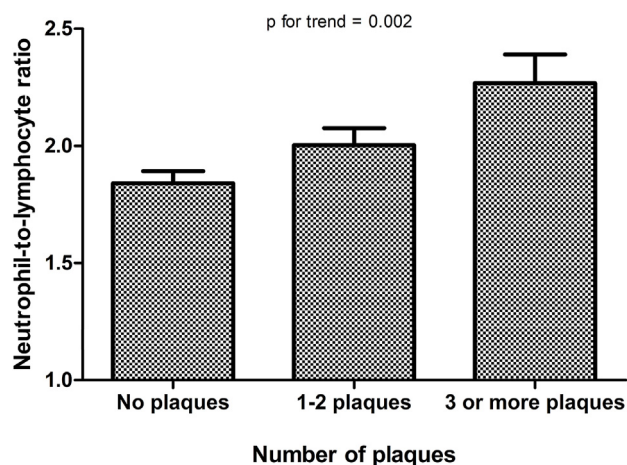


Figure 3 Fully-adjusted associations between neutrophil-to-lymphocyte ratio with the number of carotid plaques. Analysis of covariance adjusted for age, sex, systolic blood pressure, smoking habit, LDL-cholesterol, body mass index, antiplatelet treatment, statin use, diabetes duration, 5-year mean HbA1c, albumin-to-creatinine ratio, and diabetic retinopathy. NLR = 1.84 ± 0.052 , 2.00 ± 0.073 and 2.27 ± 0.122 for none, 1–2 and ≥ 3 carotid plaques; respectively.

about specific subpopulations of lymphocytes, which have been associated with a differential progression of sub-clinical atherosclerosis [38] or with features of vulnerable plaques [39]. Although it would have been very interesting from an academic point of view, we believe that the robustness of our study lies in the use of a widely available biomarker (easily obtainable from any blood test in our daily practice) to better define high-risk subjects. Finally, no reliable data were available on the presence of diabetic neuropathy, although this, and especially cardiovascular autonomic dysfunction, are important prognostic markers of cardiovascular disease [40,41].

In summary, the NLR, as a surrogate marker of innate versus adaptative immunity, was independently and directly associated with carotid atherosclerosis burden in individuals with T1D, even after taking into account several non-specific and T1D-specific CVD risk factors and cardioprotective treatment. These findings suggest that inflammation also plays an important role in the atherosclerotic process in T1D, beyond glycemic control. In this context, the NLR, as a non-expensive, non-invasive, widely available, and easy-to-calculate ratio, might help to identify individuals with higher plaque burden, although the underlying mechanism of its pro-atherogenic properties has yet to be elucidated. The inclusion of the NLR in clinical practice, at baseline and in the follow-up, may help to identify T1D subjects with a higher risk of CVD not identified by traditional CVD risk factors and tailor cardioprotective treatments accordingly. Notwithstanding, prospective and well-designed studies in different geographical areas are needed to validate this ratio.

Author contributions

All authors have discussed the results and commented on the final version of the manuscript. KM, TS, IC, LB, CF, AJA, AP, CV, IV, JB, EE, MG and AJA acquired and processed all clinical data; AJA and CV performed the US measurements; AM contributed to the study concept and design. AJA and KM participated in data analysis and interpretation; wrote, reviewed and edited the manuscript. IC, MG, VP, JB, CV, AP, AM, TS, EE and AJA supervised the study and participated in data analysis and interpretation. AJA and KM wrote the manuscript, designed the figures and had final responsibility for the decision to submit for publication. AJA is the guarantor of this work and, as such, had full access to all the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis.

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Declaration of competing interest

None.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.numecd.2023.09.017>.

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Supplemental Material

Corresponding to:

Neutrophil-to-lymphocyte ratio is independently associated with carotid atherosclerosis burden in individuals with type 1 diabetes

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Total Supplementary Tables: 3

Total Supplementary Figures: 2

Supplementary Material and Methods

Laboratory measures

All the participants performed at least 10–12 hours of overnight fasting without any concurrent stress (minor illness, strenuous exercise, etc.) the day before the blood draw. Plasma glucose concentrations were measured using the glucose oxidase method. The eGFR was calculated from the Chronic Kidney Disease-Epidemiology Collaboration equation (CKD-EPI) in ml/min/1.73m²[1]. Total cholesterol, triglycerides and HDL-cholesterol were measured directly; LDL-cholesterol was estimated using the Friedewald formula. HbA1c values were measured using a high-performance liquid chromatography method (expressed in the National Glycohemoglobin Standardization Program/ Diabetes Control and Complications Trial units). The HbA1c values were the average of all the values available from the previous 5 years.

Carotid B-mode ultrasound imaging

Bilateral carotid artery ultrasound was performed in all the participants, with a high-resolution B-mode ultrasound [Acuson X700 (Siemens) and Aplio a450 (Canon)] with an electric linear array 5–10 MHz transducer. Predefined and standardized imaging protocols to evaluate the carotid intima-media thickness (IMT) and plaque presence were carried out as described previously[2]. All the procedures were performed by experienced researchers (C.V. and A.J.A.), and the IMT measurements were made by the same researcher (A.J.A) using semiautomatic software. The carotid arteries were measured in longitudinal and transversal planes using a B-mode of US and colour Doppler to identify the possible presence of any asymmetry. Carotid plaques in the common carotid, bulb or internal carotid were defined as focal echo-structures encroaching into the arterial lumen by at least 50% of the surrounding IMT value, or when IMT was at least 1.5 mm as

measured from the media adventitia interface to the intima-lumen surface[3]. The mean IMT, mean-maximum IMT and the maximum height of carotid plaque were measured. If carotid plaques were present, the maximum IMT was equal to the highest plaque height. Peak systolic and end-diastolic velocities were used to evaluate carotid stenosis and the planimetric area was measured at a transversal view when significant plaques (IMT >2.5 mm) were found.

References

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Supplementary Table S1. Characteristics of the study participants according to the presence of carotid plaque.

	No plaque (n=354)	Plaque presence (n=248)	p-value
Clinical characteristics			
Female	177 (50)	107 (43)	0.097
Age (years)	45.4 ± 9.4	53.6 ± 9.4	<0.001
Premature CVD in first-degree relatives	41 (11.6)	30 (12.1)	0.847
Current smokers	91 (25.7)	81 (32.7)	0.063
Hypertension	63 (17.8)	85 (34.3)	<0.001
SBP (mmHg)	126 ± 14	131 ± 16	<0.001
BMI (kg/m ²)	26.2 ± 4	26.3 ± 4	0.793
Waist circumference (cm)	91 ± 12	93 ± 13	0.024
Female (cm)	86 ± 12	87 ± 13	0.603
Male (cm)	95 ± 11	98 ± 11	0.070
Diabetes duration (years)	25.8 ± 9.6	28.9 ± 11.4	0.001
Diabetic kidney disease	35 (9.9)	25 (10.1)	0.938
Diabetic retinopathy	122 (34.5)	102 (41.1)	0.096
CSII therapy	121 (34.2)	67 (27)	0.062
Hypoglycaemia unawareness	58 (16.5)	42 (17.1)	0.830
Laboratory characteristics			
Fasting plasma glucose (mg/dL)	156 ± 69	164 ± 60	0.131
HbA1c (%)	7.5 ± 1	7.6 ± 0.9	0.670
eGFR (CKD-EPI; ml/min/1.73m ²)	96 ± 14	90 ± 15	<0.001
Total cholesterol (mg/dL)	190 ± 30	191 ± 31	0.859
HDL-cholesterol (mg/dL)	63 ± 23	60 ± 16	0.187
LDL-cholesterol (mg/dL)	112 ± 25	112 ± 26	0.903
Triglycerides (mg/dL)	72 (59-97)	80 (64-110)	0.004
Pharmacological treatment			
Statins	124 (35)	121 (48.8)	0.001
ACEi/ARB	75 (21.2)	83 (33.5)	0.001
Antiplatelet drugs	19 (5.4)	33 (13.3)	0.001

Data are shown as n (percentage), mean (±SD) or median (Q1-Q3).

p values for group comparisons are reported

ACEi: angiotensin-converting enzyme inhibitor; ARB: angiotensin receptor blocker; CVD: cardiovascular disease; CSII: continuous subcutaneous insulin infusion; eGFR: estimated glomerular filtration rate; SBP: systolic blood pressure.

Supplementary Table S2. Univariate relationships between inflammatory parameters and other variables in the whole cohort (n=606).

	Leukocyte count (per mm ³)	hsCRP (mg/dL)	NLR	PLR	SII
Sex					
Female	6855 (5640-8340)	0.13 (0.05-0.32)	1.66 (1.30-2.32)	12.00 (9.27-15.25)	432.3 (298.8-612.6)
Male	6520 (5445-8057)	0.12 (0.06-0.25)	1.77 (1.36-2.23)	11.08 (8.75-13.50)**	409.8 (299.6-553.8)
Diabetic retinopathy					
Yes	6630 (5585-8497)	0.12 (0.05-0.29)	1.66 (1.33-2.20)	11.65 (9.04-14.33)	412.1 (289.8-566.5)
No	6700 (5465-8160)	0.13 (0.06-0.29)	1.75 (1.35-2.30)	11.47 (8.89-14.2)	423.6 (303.4-581.4)
Diabetic kidney disease					
Yes	7585 (6005-8722)**	0.13 (0.05-0.27)	1.92 (1.46-2.20)	10.63 (8.67-12.78)	447.2 (332.2-590.8)
No	6535 (5477-8152)	0.12 (0.06-0.29)	1.66 (1.33-2.25)	11.58 (8.93-14.45)	412.3 (296.4-574.1)
Hypertension					
Yes	7155 (5702-8562)**	0.14 (0.06-0.35)	1.89 (1.49-2.43)**	11.6 (9.02-14.67)	459.1 (328.0-639.2)**
No	6530 (5430-8057)	0.11 (0.06-0.26)	1.65 (1.30-2.15)	11.5 (8.90-14.05)	397.2 (285.6-563.6)
Statin therapy					
Yes	6950 (5495-8420)	0.14 (0.06-0.31)	1.77 (1.41-2.33)	11.39 (8.88-14.48)	435.7 (313.4-599.0)
No	6560 (5480-8030)	0.11 (0.06-0.26)	1.66 (1.31-2.16)	11.57 (9.00-14.02)	405.4 (286.5-564.6)
AAS therapy					
Yes	6580 (5465-8152)**	0.11 (0.06-0.28)*	1.67 (1.31-2.18)**	11.56 (8.93-14.07)	412.1 (289.5-568.0)
No	7395 (6125-9667)	0.22 (0.11-0.35)	2.13 (1.57-2.52)	11.49 (9.28-14.80)	454.0 (350.2-666.6)
ACEi / ARB					
Yes	7125 (5655-8517)	0.15 (0.06-0.35)	1.86 (1.44-2.33)	11.39 (9.04-14.34)	454.1 (323.8-647.2)
No	6505 (5435-8072)	0.11 (0.06-0.26)	1.67 (1.31-2.17)	11.53 (8.92-14.12)	398.5 (286.4-560.1)
Active smoking habit					
Yes	7810 (6245-9290)**	0.10 (0.05-0.37)	1.81 (1.42-2.40)	10.48 (8.05-13.10)**	420.8 (312.6-611.6)
No	6300 (5300-7682)	0.13 (0.06-0.27)	1.69 (1.31-2.19)	12.05 (9.29-14.76)	417.7 (291.6-564.6)

Data are shown as median (Q1-Q3); *p<0.05; **p<0.01

ACEi: angiotensin-converting enzyme inhibitor; ARB: angiotensin receptor blocker; hsCRP: high-sensitive C-reactive protein; NLR: Neutrophile-to-lymphocyte ratio; PLR: platelet-to-lymphocyte ratio; SII: systemic immune-inflammation index.

Supplementary Table S3. Associations between inflammatory markers and carotid atherosclerosis burden by logistic regression analysis.

	≥3 plaques	
	OR (95% CI)	p-value
Model 1^a		
Leukocyte count (per 1000 mm ³ increase)	1.163 (1.015-1.331)	0.029
hsCRP (mg/dL)	2.444 (1.105-5.407)	0.027
Neutrophil-to-lymphocyte ratio	1.363 (1.064-1.747)	0.014
Platelets-to-lymphocyte ratio	0.993 (0.924-1.066)	0.836
SII (100 units increase)	1.083 (0.997-1.177)	0.059
Model 2^b		
Leukocyte count (per 1000 mm ³ increase)	1.030 (0.884-1.201)	0.702
hsCRP (mg/dL)	1.856 (0.782-4.402)	0.161
Neutrophil-to-lymphocyte ratio	1.353 (1.030-1.777)	0.030
Platelets-to-lymphocyte ratio	1.018 (0.942-1.099)	0.656
SII (100 units increase)	1.068 (0.970-1.176)	0.179
Model 3^c		
Leukocyte count (per 1000 mm ³ increase)	1.027 (0.881-1.197)	0.734
hsCRP (mg/dL)	1.878 (0.757-4.663)	0.174
Neutrophil-to-lymphocyte ratio	1.377 (1.036-1.829)	0.028
Platelets-to-lymphocyte ratio	1.025 (0.947-1.109)	0.544
SII (100 units increase)	1.072 (0.972-1.181)	0.163

Logistic regression models (Odds Ratio and 95% confidence interval) are presented.

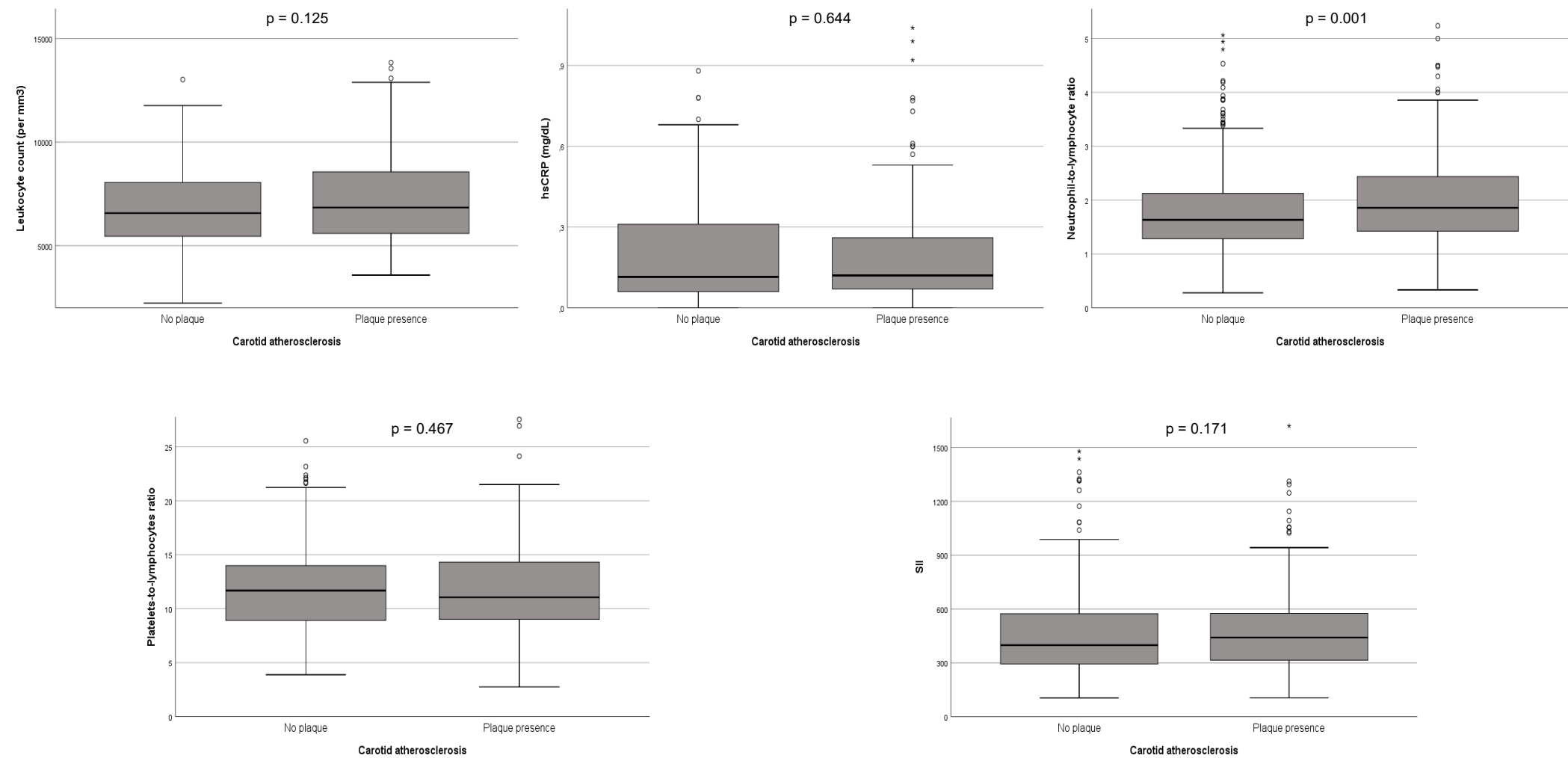
^aModel 1: Adjusted for age and sex.

^bModel 2: Model 1 + systolic blood pressure, statin exposure, antiplatelet treatment, LDL-cholesterol, body mass index and smoking habit.

^cModel 3: Model 2 + diabetes duration, 5-year mean HbA1c, diabetic retinopathy and albumin-to-creatinine ratio.

hsCRP: high-sensitive C-reactive protein, SII: systemic immune-inflammation index, OR: odds ratio, CI: confidence interval.

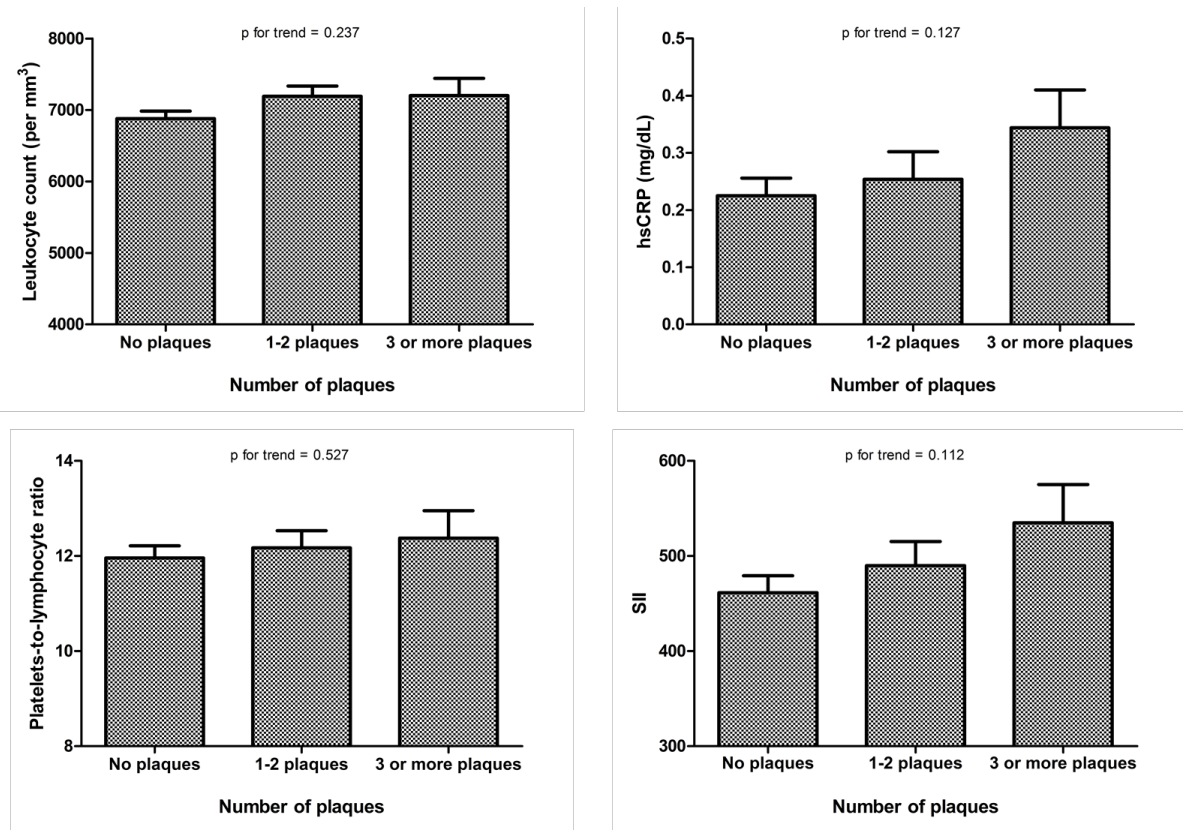
Supplementary Figure S1. Box-plots of inflammatory-related variables according to the presence of carotid plaque.



p-values between groups comparison are reported.

hsCRP: high-sensitive c-reactive protein; SII: systemic immune-inflammation index.

Supplementary Figure S2. Fully-adjusted associations between other inflammation-related variables and the number of carotid plaques



Analysis of covariance adjusted for age, sex, systolic blood pressure, active smoking habit, LDL-cholesterol, body mass index, antiplatelet treatment, statin use, diabetes duration, mean HbA1c in the last 5 years, albumin-to-creatinine ratio, and presence of retinopathy.

Leukocyte count = 6881±103, 7192±144 and 7203±240 per mm³, for none, 1-2 and ≥3 carotid plaques; respectively.

hsCRP = 0.225±0.031, 0.254±0.048 and 0.344±0.066 for none, 1-2 and ≥3 carotid plaques; respectively.

Platelet-to-lymphocyte ratio = 11.96±0.256, 12.17±0.363 and 12.38±0.578 for none, 1-2 and ≥3 carotid plaques; respectively.

SII = 461.3±17.9, 489.8±25.3 and 534.9±40.3 for none, 1-2 and ≥3 carotid plaques; respectively.

hsCRP: high-sensitivity c-reactive protein; SII: systemic immune-inflammation index.

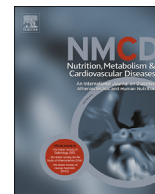
ARTÍCULO ORIGINAL 8: LA CUANTIFICACIÓN DE LAS GLICOPROTEÍNAS DETERMINADAS MEDIANTE RESONANCIA MAGNÉTICA NUCLEAR SE ASOCIA CON LA ATEROSCLEROSIS CAROTÍDEA PRECLÍNICA EN PACIENTES CON DIABETES TIPO 1

Objetivos: Las glicoproteínas desempeñan un papel clave en los procesos inflamatorios y cardiometabólicos. Su implicación en la aterosclerosis en la DTI es desconocida. Evaluamos las relaciones entre los marcadores inflamatorios clásicos, las glicoproteínas medidas por ^1H -RMN y la aterosclerosis preclínica en estos pacientes.

Métodos: Se seleccionaron pacientes con DTI sin ECV con: edad ≥ 40 años, ERD o ≥ 10 años de evolución con otro FRCV. La presencia de placa (grosor íntima-media $> 1,5$ mm) se determinó por ecografía. Se determinaron las concentraciones de PCRus y leucocitos circulantes (marcadores clásicos de inflamación) y de glicoproteínas medidas por ^1H -RMN (GlycA, GlycB, GlycF y las razones altura/anchura [A/A] de GlycA y GlycB).

Resultados: Se incluyeron 189 pacientes (58% hombres, edad 47,0 [40,7-55,2] años). El 35% presentó placas (22%, ≥ 2 placas). No hubo asociación entre PCRus o leucocitos con la aterosclerosis. Sin embargo, en modelos ajustados por edad y sexo, GlycA, GlycF y las razones A/A de GlycA y GlycB aumentaron gradualmente con el número de placas (0, 1, ≥ 2 placas) solo en pacientes sin estatinas ($p < 0,05$), sin asociación en pacientes que recibieron este medicamento (p de interacción $< 0,05$; en ≥ 2 placas). Finalmente, en modelos ajustados por otros FRCV clásicos y específicos de DTI, las razones A/A de GlycA y GlycB permanecieron asociadas con la placa carotídea (OR 1,39 [1,12-1,90] y OR 6,89 [1,85-25,62], respectivamente).

Conclusiones: En individuos con DTI sin tratamiento hipolipemiante, las glicoproteínas medidas por ^1H -RMN estuvieron independientemente asociadas con la presencia y la cantidad de aterosclerosis carotídea, a diferencia de otros marcadores inflamatorios clásicos. Se necesitan estudios adicionales para determinar su utilidad como biomarcadores de ECV.



Quantification of glycoproteins by nuclear magnetic resonance associated with preclinical carotid atherosclerosis in patients with type 1 diabetes

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KEYWORDS

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Atherosclerosis;
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ultrasonography

Abstract *Background and aims:* Glycoproteins play a key role in inflammatory and cardiometabolic processes. Their implication in atherosclerosis in type 1 diabetes (T1D) is unknown. We assessed the relationships between classic inflammatory markers, glycoproteins measured by nuclear magnetic resonance (¹H-NMR), and preclinical atherosclerosis in these patients.

Methods and results: We selected patients with T1D, without cardiovascular disease (CVD), with: age ≥ 40 years, nephropathy (micro/macrolalbuminuria), or ≥ 10 years of evolution with another risk factor. The presence of plaque (intima-media thickness > 1.5 mm) was determined by ultrasonography. Concentrations of high-sensitive C-reactive protein (hsCRP), circulating leukocytes (classical inflammation markers) and ¹H-NMR-glycoproteins (GlycA, GlycB, GlycF, and the height/width [H/W] ratios of GlycA and GlycB) were determined. We included 189 patients (58% male, age 47.0 [40.7–55.2] years). Thirty-five percent presented plaques (22%, ≥ 2 plaques). There was no association between hsCRP or leukocytes and atherosclerosis. However, in age- and sex-adjusted models, GlycA, GlycF, and the H/W ratios of GlycA and GlycB gradually increased with the number of plaques (0, 1, ≥ 2 plaques) only in patients without statins ($p < 0.05$), with no association in patients receiving this drug (p for interaction < 0.05 ; in ≥ 2 plaques). Finally, in models adjusted for other classical and T1D-specific risk factors, GlycA and GlycB H/W ratios remained associated with carotid plaque (OR 1.39 [1.12–1.90] and OR 6.89 [1.85–25.62], respectively).

Conclusion: In T1D individuals without lipid-lowering treatment, ¹H-NMR-glycoproteins were independently associated with the presence and amount of carotid atherosclerosis, unlike other classical inflammatory markers. Further studies are needed to ascertain their utility as CVD biomarkers.

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Introduction

Cardiovascular disease (CVD) accounts for up to 47% and 39% of all deaths in females and males in the general population in Europe [1], and it is the leading cause of death among patients with type 1 diabetes (T1D) [2]. Landmark studies have shown a reduction in cardiovascular events in this population either under intensive insulin therapy [3] or receiving lipid-lowering drugs [4]. In addition, a chronic inflammatory state has been observed regardless of weight or glycaemic control in young subjects with T1D [5], and this can represent an additional cardiovascular risk factor [6].

Several variables have been independently associated with atherosclerosis. Thus, in patients with prediabetes or early-onset diabetes, either anthropometric variables such as the waist to hip ratio [7], or laboratory parameters such as the triglyceride to high-density lipoprotein (HDL) cholesterol ratio [8] or glycated haemoglobin (HbA1c) levels [9] have been directly associated with carotid and coronary atherosclerosis. In this sense, chronic inflammation also plays a key role in the process of atherosclerosis [10], and targeted anti-inflammatory treatment has been shown to reduce cardiovascular events in patients with a previous myocardial infarction and elevated inflammatory markers [11]. Historically, the degree of inflammation has been estimated using classical markers such as circulating leukocytes or high-sensitive C-reactive protein (hsCRP). Novel protein glycan-derived markers of systemic inflammation, such as N-acetylglucosamine/galactosamine (GlycA) and sialic acid (GlycB), measured by nuclear magnetic resonance (^1H NMR) spectroscopy, have shown to be correlated with hsCRP [12], and their utility as inflammation parameters under various conditions has been described [13,14]. GlycA has also been associated with an increased risk of death in patients with cardiovascular disease and atherogenic dyslipemia [15].

The implication of these new NMR-derived inflammatory markers in atherosclerosis of patients with T1D is still unknown. With this background, the aim of this study was to assess the relationships between classical inflammatory markers (circulating leukocytes and hsCRP), glycoproteins measured by ^1H NMR, and carotid atherosclerosis (i.e. carotid plaque, with a predictive role in several heart diseases in other populations [16,17]) in the primary prevention of CVD in high-risk T1D patients.

Methods

Subjects

This was a cross-sectional study in subjects with T1D without established CVD (coronary artery disease, ischaemic stroke, peripheral artery disease, or heart failure) recruited from a specialised Diabetes Unit of the Hospital Clinic in Barcelona, Spain. All participants met the following inclusion criteria: age ≥ 40 years; presence of diabetic nephropathy at any stage, irrespective of the age of the subject and diabetes duration; and/or duration of

T1D ≥ 10 years with at least one additional CVD risk factor. We considered the following additional CVD risk factors: the presence of diabetic retinopathy, active smoking habit (excluding former-smokers), hypertension (defined as systolic blood pressure ≥ 140 mmHg and diastolic blood pressure ≥ 90 mmHg, or being treated with antihypertensive drugs), family history of premature CVD in first-degree relatives (defined as any CVD occurring before 55 years of age in men and before 65 years of age in women), low HDL-cholesterol (< 40 mg/dL in males, < 45 mg/dL in females), triglycerides > 150 mg/d, the presence of severe hypoglycaemia events (defined as an episode of confirmed hypoglycaemia that requires external assistance for recovery) or hypoglycaemia unawareness (defined as score > 3 in the Clarke Test, with the validated Spanish version [18]) and women with a history of preeclampsia/eclampsia. Patients with pancreatic disease such as pancreatitis or pancreatic surgery as well as those with clinical suspicion of latent autoimmune diabetes in adults (LADA) were excluded.

All patients provided informed consent, and the study was approved by the Ethical Committee of the hospital (number of approval HCB/2017/0977).

Clinical and laboratory determinations

Both demographic characteristics (gender, age) and clinical data including the duration of T1D, history of microvascular diabetes complications, family history of premature CVD in first-degree relatives, medical treatment (multiple-dose insulin, continuous subcutaneous insulin infusion [CSII], antihypertensive agents, lipid-lowering drugs and anti-platelets) were recorded in all subjects. High-intensity statin therapy was defined as doses that achieve a reduction of low-density lipoprotein (LDL) cholesterol $\geq 50\%$.

Diabetic retinopathy was defined by fundus oculi and was always confirmed by a specialised ophthalmologist. Diabetic nephropathy was evaluated according to the albumin-to-creatinine ratio, considering < 30 mg/g as normal and ≥ 30 mg/g as diabetic nephropathy (confirmed on at least two out of three consecutive determinations). The use of angiotensin-converting enzyme inhibitors or angiotensin II receptor blockers, with no history of hypertension or CVD, was also considered as diabetic nephropathy.

Anthropometric data (weight, height, and waist and circumference) were also recorded. Weight was determined using a calibrated electronic scale with the patient wearing light clothes and barefoot. The body mass index (BMI) was calculated as weight in kilograms divided by square height in meters. Waist circumference was measured at the midpoint between the lowest rib and the iliac crest.

Laboratory parameters were determined in fasting blood and first morning urine spot samples. None of the participants had any stress the day before the blood was drawn (minor illness, strenuous exercise, etc.). Lipid profile (including total cholesterol, triglycerides, and HDL-cholesterol, glucose, creatinine, and the albumin-to-creatinine ratio) were analysed with standardised assays. LDL-cholesterol was determined by the Friedewald

formula. Non-HDL cholesterol was calculated as the subtraction of HDL-cholesterol from total cholesterol. The estimated glomerular filtration rate (eGFR) was assessed by the Chronic Kidney Disease Epidemiology Collaboration equation. HbA1c values (Tosoh G8 Automated HPLC Analyzer -Tosoh Bioscience Inc., South San Francisco, CA, USA - DCCT aligned, normal range 4–6% [20–42 mmol/mol]) were also registered. The leukocyte count and hsCRP were assessed by flow cytometry and immunoturbidimetric assay, respectively. Insulin sensitivity was assessed with estimated glucose disposal rate (eGDR) formula, a well-validated score in T1D expressed in mg/kg/min [19] and calculated as follows: $24.31 - 12.22 \times \text{waist-to-hip ratio} - 3.29 \times \text{hypertension}$ (0 = no, 1 = yes) $- 0.57 \times \text{HbA1c}$ (in %). Finally, the fatty liver index (FLI) was calculated as described previously [20].

Carotid B-mode ultrasound imaging

Carotid ultrasound images were obtained using high-resolution B-mode ultrasound (Acuson X300 or Acuson X700 [Siemens]) with an electric linear array 5–10 MHz transducer. Standardised and predefined imaging protocols to appraise carotid intima-media thickness (IMT), and the presence of carotid plaque was determined as described previously [19,21]. Carotid plaques were visualised using B-mode and colour Doppler in transverse and longitudinal planes to consider circumferential asymmetry. Carotid plaques were defined as focal echo-structures trespassing into the arterial lumen by at least 50% of the surrounding IMT value, or when IMT was at least 1.5 mm as measured from the media-adventitia interface to the intima–lumen surface [22]. Two skilled endocrinologists (A.J.A and E.O.) performed all the procedures, and IMT measurements were made by the same researcher (A.J.A) using semiautomatic software. The mean and mean-maximum IMT of all carotid segments (common carotid, bulb, and internal carotid) were recorded, in addition to the maximum height of carotid plaque. If carotid plaques were present, the maximum IMT was equal to the highest carotid plaque height. Peak systolic and end-diastolic velocities were used to assess carotid stenosis, and the planimetric area was measured at a transversal view when significant carotid plaques (IMT >2.5 mm) were found.

Glycoprotein analysis by ¹H NMR

Before ¹H NMR analysis, a dissolution at pH 7.4 with 200 µL of serum, 50 µL of deuterated water and 300 µL of 50 mM phosphate buffer solution (PBS) was made. The spectra of ¹H NMR were recorded at 310 K in a Bruker Avance III 600 spectrometer at a proton frequency of 600.20 MHz (14.1 T) [14]. In short, the region of the spectrum at which the glycoproteins resonate (2.15–1.90 ppm) was analysed using many functions according to the chemical shift: GlycA, GlycB and, GlycF. The total area was determined and converted into a concentration according to the number of sugar–protein bonds for each function. We also calculated the position, height,

bandwidth, area and their ratios. GlycA and GlycB concentrations established the amount of acetyl groups of protein bonded to N-acetylgalactosamine, N-acetylneuraminic and N-acetylglucosamine acid. The GlycF area emerges from the concentration of –COCH₃ acetyl groups of N-acetylgalactosamine, N-acetylneuraminic and N-acetylglucosamine not bonded to proteins (free fraction). Height/width ratios of GlycA and GlycB (H/W) were associated with the shape of the peaks developed by the ¹H NMR signals expressing the aggregation state or flexibility of the sugar–protein bonds [13,14].

Statistical analysis

Descriptive analysis of the data was carried out. The normal distribution of continuous variables was evaluated with the Kolmogorov–Smirnov test. Results for quantitative normal variables are expressed as mean ± standard deviation, quantitative variables with non-normal distribution as median (first quartile–third quartile), and qualitative variables as frequency and percentage. The comparison of binomial qualitative variables with a normal distribution was made by means of the Chi-square test, quantitative variables with the Student's T test or ANOVA, and quantitative variables with non-Gaussian distribution by means of the Mann–Whitney U test or the Kruskal–Wallis H test. Correlations between quantitative variables were analysed with R of Spearman.

To search for independent relationships between pre-clinical carotid atherosclerosis (presence of carotid plaque or presence of ≥2 plaques; dependent variable) and inflammatory markers (either classical or ¹H-NMR-glycoproteins; independent variable), we constructed logistic binary regression models. Age and sex were included in all models, in addition to classical CVD risk factors (BMI, systolic blood pressure, LDL-cholesterol and smoking habit) and T1D-specific factors (diabetes duration, HbA1c, presence of diabetic retinopathy and albumin-to-creatinine ratio). Additionally, we assessed the differences in ¹H-NMR-glycoproteins according to the number of carotid plaques (none, 1, ≥2 plaques) in age- and sex-adjusted models (analysis of covariance, ANCOVA). All the analyses were performed in the whole sample, as well as after dividing the participants according to statin treatment. The P-value for interaction between ¹H-NMR-glycoproteins and statin use was also provided for some of the tests.

All the analyses were performed using IBM® SPSS® Statistics v.25 (IBM Corporation, Armonk, New York) statistical software. The significance level was set as a p-value < 0.05.

Results

Subject characteristics

A total of 189 subjects were included (58% males, median age 47.0 [40.7–55.2] years, median duration of diabetes 26.5 [20.6–33.4] years). Overall, 27.5% were current

smokers, 30.7% had hypertension, 38% diabetic retinopathy (25 out of 72 were proliferative) and 11% had nephropathy. Eighty-four of the 189 subjects were on statin treatment (16.7% with high-intensity statin therapy).

In the whole cohort, 35% had at least one carotid plaque (22% with two or more plaques), with no between-gender differences ($p > 0.120$ for both). There was a stepped increase in age, the presence of hypertension (and systolic hypertension) and triglyceride values, and a decrease in eGFR, as the number of carotid plaques increased ($p < 0.05$ for all values). Conversely, there were no differences in smoking status or statin use. The rest of the characteristics of the patients according to preclinical atherosclerosis status are shown in Table 1.

The characteristics of the study participants according to statin treatment were also compared. Statin users ($n = 105$) were older (49 [42.2–57.6] vs. 45 [39.9–52.7]

years, $p = 0.004$), had a higher prevalence of hypertension (42% vs. 22%, $p = 0.003$), had lower eGFR values (94.9 [83.0–104.3] vs. 102.0 [90.6–108.9] ml/min/1.73 m², $p = 0.033$), and, as expected, had lower LDL-cholesterol levels (103 [91–124] vs. 118 [105–132] mg/dL, $p = 0.002$). There were no differences in other classical CVD risk factors or other T1D-specific variables (Supplemental Table S1).

Relationships between ¹H-NMR-glycoproteins and clinical and laboratory parameters

As expected, all the ¹H-NMR-glycoproteins showed strong correlations with classical inflammatory variables (i.e., leukocyte count and hsCRP; $r = 0.3$ – 0.45 , $p < 0.01$; Table 2). Additionally, direct and strong associations were found with several anthropometric parameters (BMI, $p < 0.05$ for

Table 1 Characteristics of the study participants according to the presence of carotid plaque.

	No plaque presence (n = 123)	Presence of 1 plaque (n = 25)	Presence of ≥2 plaques (n = 41)	p-value
Clinical characteristics				
Male	66 (54)	17 (68)	26 (63)	0.293
Age (years)	43.8 (38.6–50.9)	48.4 (42.7–58.9) ^a	55.2 (47.3–61.1) ^a	<0.001
Premature CVD in first-degree relatives*	19 (15)	1 (4)	5 (12)	0.298
Current smokers	35 (29)	4 (16)	13 (32)	0.354
Cumulative smoking (Pack-years)	0 (0–10)	0 (0–10)	5 (0–24.75)	0.106**
Hypertension	29 (24)	6 (24)	23 (56) ^{a,b}	<0.001
SBP (mmHg)	123 (116–132)	130 (123–138) ^a	133 (118–145) ^a	0.003
DBP (mmHg)	80 (±9)	81 (±6)	80 (±10)	0.812
BMI (kg/m ²)	26.27 (±4.17)	26.51 (±2.87)	26.07 (±3.95)	0.908
Waist circumference (cm)				
Female (cm)	86.0 (±1.5)	88.9 (±4.6)	85.13 (±3.1)	0.746
Male (cm)	96.5 (±1.4)	97.6 (±2.3)	96.9 (±2.3)	0.930
T1D duration (years)	25.0 (20.6–31.4)	30.0 (18.4–34.7)	31.0 (21.4–38.5) ^a	0.038
Diabetic nephropathy	13 (11)	1 (4)	7 (17)	0.248
Diabetic retinopathy	46 (37)	9 (36)	17 (42)	0.874
CSII therapy	51 (42)	9 (36)	15 (37)	0.791
Laboratory characteristics				
Fasting plasma glucose (mg/dL)	140 (102–188)	175.5 (153–204) ^a	128 (105–174) ^b	0.029
Haemoglobin A1c (% (mmol/mol))	7.5 (7.0–7.9), (58)	7.4 (7.1–7.8), (57)	7.9 (7.1–8.2), (63)	0.435
Serum creatinine (mg/dL)	0.81 (0.72–0.93)	0.86 (0.77–1)	0.85 (0.71–0.98)	0.358
eGFR (CKD-EPI; ml/min/1.73m ²)	102 (90–109)	95 (82–105)	94 (81–103) ^a	0.030
ALT (U/L)	19 (15–24)	21 (18–29)	18 (15–27)	0.157
Total cholesterol (mg/dL)	189 (172–214)	183 (165–204.5)	188 (167–207)	0.908
HDL-cholesterol (mg/dL)	58 (49–70)	54 (45–64)	54 (47–65)	0.319
LDL-cholesterol (mg/dL)	112 (100–131)	113.5 (93–130)	115 (94–126)	0.915
Triglycerides (mg/dL)	69 (55–92)	85 (67–127) ^a	81 (61–108)	0.024
Non-HDL cholesterol (mg/dL)	129 (113–149)	135 (115–155)	130 (120–143)	0.810
Pharmacological treatment				
Statins	49 (40)	14 (56)	21 (51)	0.205
ACEi/ARB	32 (26)	8 (32)	22 (53) ^a	0.005
Antiplatelet drugs	9 (7)	2 (8)	6 (15)	0.360

Data are shown as n (percentage), mean (±SD) or median (Q1–Q3).

p values for group comparisons are reported.

ACEi: angiotensin-converting enzyme inhibitor; ALAT: alanine aminotransferase; ARB: angiotensin receptor blocker; CVD: cardiovascular disease; CSII: continuous subcutaneous insulin infusion; DBP: diastolic blood pressure; eGFR: estimated glomerular filtration rate; SBP: systolic blood pressure; T1D: type 1 diabetes.

*Defined as <55 years in men and <65 years in women.

**Difference between presence of ≥2 plaques vs. the others two groups $p < 0.05$.

Bold text indicates a statistically significant difference.

^a $p < 0.05$ vs. no plaque presence.

^b $p < 0.05$ vs. presence of 1 plaque.

Table 2 Correlations between NMR-glycoproteins and other clinical and laboratory variables in the whole cohort (n = 189).

	Area GlycA	Area GlycB	Area GlycF	H/W GlycA	H/W GlycB
General clinical characteristics					
Age (years)	r = -0.171 p = 0.018	r = -0.140 p = 0.054	r = -0.128 p = 0.080	r = -0.121 p = 0.096	r = -0.136 p = 0.062
BMI (kg/m ²)	r = 0.299 p < 0.001	r = 0.145 p = 0.046	r = 0.199 p = 0.006	r = 0.366 p < 0.001	r = 0.304 p < 0.001
Waist circumference (cm)	r = 0.311 p < 0.001	r = 0.110 p = 0.135	r = 0.233 p = 0.001	r = 0.319 p < 0.001	r = 0.255 p < 0.001
SBP (mmHg)	r = 0.129 p = 0.077	r = 0.094 p = 0.196	r = 0.100 p = 0.172	r = 0.105 p = 0.150	r = 0.075 p = 0.302
T1D-specific characteristics					
Diabetes duration (years)	r = -0.103 p = 0.16	r = -0.158 p = 0.03	r = -0.095 p = 0.191	r = -0.074 p = 0.312	r = -0.120 p = 0.099
Haemoglobin A1c (%)	r = 0.317 p < 0.001	r = 0.145 p = 0.046	r = 0.184 p = 0.011	r = 0.313 p < 0.001	r = 0.244 p = 0.001
Creatinine-to-albumin ratio (mg/g)	r = 0.230 p = 0.001	r = 0.125 p = 0.087	r = 0.192 p = 0.008	r = 0.228 p = 0.02	r = 0.149 p = 0.041
eGDR (mg/kg/min)	r = -0.289 p < 0.001	r = 0.137 p = 0.062	r = -0.222 p = 0.002	r = -0.280 p < 0.001	r = -0.214 p = 0.003
Lipid profile					
LDL-cholesterol (mg/dL)	r = 0.120 p = 0.1	r = -0.008 p = 0.913	r = 0.116 p = 0.113	r = 0.104 p = 0.156	r = 0.103 p = 0.160
HDL-cholesterol (mg/dL)	r = -0.382 p < 0.001	r = -0.290 p < 0.001	r = -0.268 p < 0.001	r = -0.309 p < 0.001	r = -0.231 p = 0.001
Triglycerides (mg/dL)	r = 0.640 p < 0.001	r = 0.275 p < 0.001	r = 0.528 p < 0.001	r = 0.523 p < 0.001	r = 0.408 p < 0.001
Other characteristics					
hsCRP (mg/dL)	r = 0.416 p < 0.001	r = 0.314 p < 0.001	r = 0.276 p = 0.001	r = 0.482 p < 0.001	r = 0.394 p < 0.001
Leukocyte count (per mm ³)	r = 0.419 p < 0.001	r = 0.309 p < 0.001	r = 0.290 p < 0.001	r = 0.417 p < 0.001	r = 0.336 p < 0.001
Creatinine (mg/dL)	r = 0.107 p = 0.15	r = 0.108 p = 0.139	r = 0.113 p = 0.123	r = 0.088 p = 0.231	r = 0.007 p = 0.928
ALAT (UI/L)	r = 0.036 p = 0.62	r = -0.05 p = 0.5	r = 0.073 p = 0.316	r = 0.023 p = 0.753	r = 0.029 p = 0.690
Fatty liver index	r = 0.584 p < 0.001	r = 0.289 p = 0.002	r = 0.444 p < 0.001	r = 0.560 p < 0.001	r = 0.411 p < 0.001

Spearman's Rho correlation coefficients and p-value between pairs of variables are provided.

ALAT: alanine aminotransferase; eGDR: estimated glucose disposal rate; hsCRP: high-sensitive C reactive protein; SBP: systolic blood pressure.

Bold text indicates a statistically significant difference.

all), markers of fatty liver content (FLI, $p < 0.01$), glycaemic control (HbA1c; $p < 0.05$), and T1D-specific surrogates of insulin sensitivity (eGDR, $p < 0.01$). Regarding the conventional lipid profile, ¹H-NMR-parameters showed direct associations with triglyceride levels ($r = 0.3$ – 0.64 , $p < 0.001$ for all) and indirect associations for HDL-cholesterol ($r = 0.2$ – 0.38 ; $p < 0.01$), with no significant correlation with LDL-cholesterol (Table 2). No differences were found according to gender, other classical CVD risk factors (smoking habit or hypertension), or according to some specific treatments (CSII use or antiplatelet treatment; Supplemental Table S2). Finally, no major differences were found when the cohort was divided according to statin treatment, although some of the relationships were blunted in patients receiving this lipid-lowering treatment (especially in BMI, HbA1c or HDL-cholesterol), whereas the associations with classical inflammatory parameters were more pronounced (Supplemental Tables S3 and S4).

¹H-NMR-glycoproteins and classical inflammatory parameters and preclinical carotid atherosclerosis

In age- and sex-adjusted models, the area of GlycA and GlycF, and H/W ratios of GlycA and GlycB showed a stepped increase (p for trend < 0.05 , for all comparisons) as the number of carotid plaques increased, only in participants without statin treatment ($n = 105$; Fig. 1). In addition, when other classical CVD risk factors and T1D-specific variables were further included in the regression analysis, some of the previous ¹H-NMR-parameters remained statistically associated in this subgroup of participants, either with the presence of carotid plaques (OR 1.39 [1.12–1.90] and OR [6.89 1.85–25.62]) or with presence of ≥ 2 plaques (OR 1.46 [1.04–2.04] and OR 6.69 [1.53–29.20]; for H/W ratio of GlycA and H/W ratio of GlycB, respectively; $p < 0.05$ for all comparisons; Table 3). No significant relationships were found among individuals under statin treatment (p for interaction < 0.05 , with ≥ 2

plaques). Classical inflammatory variables (leukocyte count or hsCRP) were not related to preclinical atherosclerosis, either in the age- and sex-adjusted (data not shown) or in the fully-adjusted models (Table 3).

Discussion

In our sample of patients with T1D at high-risk of CVD, $^1\text{H-NMR}$ -glycoproteins were associated with the presence and the number of carotid plaques, only in the subgroup of participants without statin treatment. Specifically, in this subgroup of patients, GlycA and GlycB H/W ratios showed a direct association with atherosclerosis, independently of other classical and T1D-specific risk factors. Conversely, classical biomarkers of inflammation were not associated, even after adjusting for multiple variables. To the best of our knowledge, this is the first study to assess the relationship of $^1\text{H-NMR}$ -glycoproteins, a new biomarker of inflammation, with preclinical carotid atherosclerosis in T1D patients.

Inflammation is thought to have a crucial role in the atherosclerotic process [10]. In fact, a strong direct relationship has been shown between classical inflammation markers and atherosclerotic CVD events in apparently healthy populations [23]. Several studies have described higher levels of classical inflammatory markers among T1D patients, a population with elevated CVD risk, compared to

their non-diabetic counterparts [5,24,25]. In studies in both paediatric [5,24] and adult [25] T1D patients, inflammatory surrogates were significantly elevated, even after adjusting for the main confounders (such as markers of adiposity or glycaemic control) [5]. Taking these results into account, inflammation could be added as one of the drivers of the increased CVD risk among the T1D population, even in individuals with optimal glycaemic control [26]. Along this line, two independent studies have shown that several classical inflammatory markers were associated with the presence [27] and progression [28] of subclinical carotid atherosclerosis, independently of glycaemic control or other CVD risk factors. In our selected sample of T1D patients at high-risk, neither hsCRP nor the leukocyte count were associated with the presence or the number of carotid plaques (Table 3). Although our results seem to be contradictory to previous reports, several considerations should be taken into account. First, the geographical origin (Mediterranean region) of our sample of participants was fairly different from the previous two studies (North America and Canada [28] and Japan [27]). Second, our sample of participants was more contemporary than the latter studies, reflecting the more updated management of T1D. Finally, and most importantly, some variables closely related to inflammation and CVD risk were not included in the multivariate models of the previous studies. In this regard,

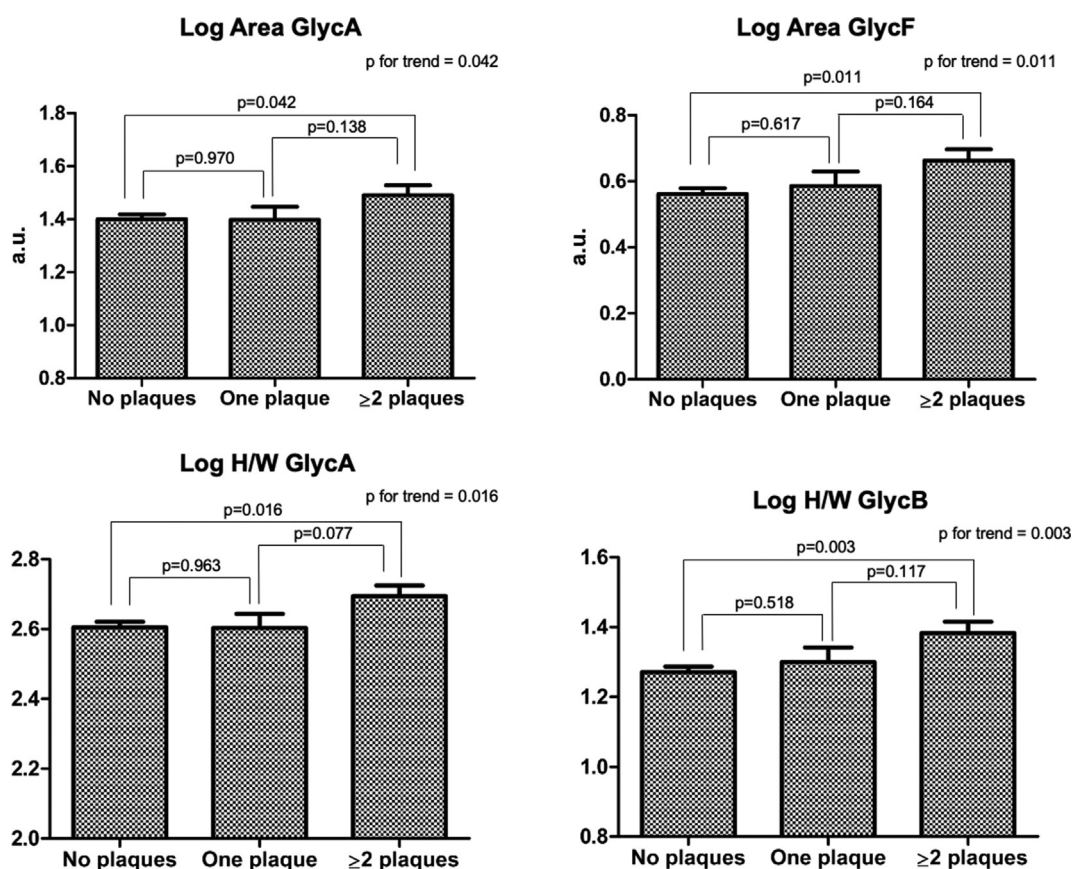


Figure 1 Age- and sex-adjusted $^1\text{H-NMR}$ -glycoproteins according to the number of carotid plaques in individuals without statin treatment (n = 105). Between-group (no plaque, one plaque and ≥ 2 plaques) comparisons and p-value for trends were reported. H/W: height/width.

Table 3 Associations between classical inflammation markers and NMR-glycoproteins with carotid atherosclerosis by multiple regression analysis.

	Carotid plaque		≥2 plaques	
	Statin non-users (n = 105)	Statin users (n = 84)	Statin non-users (n = 105)	Statin users (n = 84)
	OR (95% CI)	OR (95% CI)	OR (95% CI)	OR (95% CI)
hsCRP	0.04 (0.00–6.46)	1.69 (0.51–5.60)	0.01 (0.00–1.87)	0.06 (0.00–3.79)
Leukocyte count	1.01 (0.74–1.37)	1.01 (0.82–1.47)	1.07 (0.75–1.52)	0.99 (0.72–1.37)
Area GlycA	1.63 (0.81–3.28)	1.08 (0.73–1.59)	1.86 (0.89–3.88)	0.47 (0.18–1.26)
Area GlycB	0.54 (0.05–5.30)	0.39 (0.07–2.18)	1.11 (0.09–13.29)	0.14 (0.01–1.37)
Area GlycF	6.43 (0.90–45.95) [‡]	1.08 (0.42–2.79)	5.13 (0.81–32.70)	0.24 (0.02–2.61)
H/W GlycA ratio	1.39 (1.12–1.90)*	1.02 (0.81–1.29)	1.46 (1.04–2.04)*	0.84 (0.62–1.13)
H/W GlycB ratio	6.89 (1.85–25.62)†	0.86 (0.36–2.09)	6.69 (1.53–29.20)*	0.40 (0.13–1.19)

Logistic regression models adjusted per age, sex, body mass index, systolic blood pressure, diabetes duration, LDL-cholesterol, current smoking, Haemoglobin A1c, diabetic retinopathy and creatinine-to-albumin ratio. Odds ratio (OR) and 95% confidence intervals are reported.

* $p < 0.05$; † $p < 0.01$; ‡ $p = 0.063$.

Interactions with plaque presence: Area GlycF*statin use = 0.141; H/W GlycA ratio*statin use = 0.307; H/W GlycB ratio*Statin use = 0.056.

Interactions with ≥2 plaques: Area GlycF*statin use = 0.079; H/W GlycA ratio*statin use = 0.025; H/W GlycB ratio*statin use = 0.004.

Bold text indicates a statistically significant difference.

both microvascular complications and statin treatment were associated with inflammation [24,25,29] and, in turn, with atherosclerotic CVD [30,31]. The results of the previous studies could be limited because they did not include these parameters in the analysis of the association of inflammation with carotid atherosclerosis.

Newer markers are needed for the assessment of inflammation in relation to CVD risk in T1D. In our study, classical inflammation biomarkers showed a strong correlation with all the ¹H-NMR-glycoprotein parameters (Table 2). This is in agreement with the data of Lorenzo et al. [12], who, in a cross-sectional study, also found strong correlation of GlycA and GlycB with CRP in participants with and without type 2 diabetes. Furthermore, both GlycA and CRP were independently associated with parameters of insulin resistance [12]. The relationships between ¹H-NMR-glycoproteins and several obesity-related variables were also consistently described in other studies [32]. Interestingly, in our sample of patients with T1D, markers closely related to insulin resistance (BMI, waist circumference and FLI), as well as specific markers of insulin sensitivity in this population (eGDR) were also more associated with ¹H NMR variables. This ensures the robustness of this new ¹H-NMR-related biomarker in T1D individuals, a high-risk population not previously studied with this new technique.

Some studies have assessed the role of ¹H-NMR-glycoprotein in CVD beyond classical inflammatory markers. In a prospective study (median follow-up 17.2 years) of apparently healthy women, baseline GlycA and CRP values were similarly associated with future CVD events [33]. Interestingly, the association of GlycA with CVD was independent of CRP, at least in the first 6 years of the follow-up [33]. In the same line, 3 other studies in primary prevention populations (PREVEND, MESA and JUPITER) also showed direct relationships between baseline GlycA levels and future CVD, independently of CRP levels as well [34–37]. However, information regarding a T1D population, either in primary or secondary prevention, was lacking. Our results show, for the first time, a direct association between this

new inflammation biomarker and preclinical carotid atherosclerosis in T1D patients without lipid-lowering treatment. In addition, this association was present both when analysing the presence of carotid plaque and a more advanced preclinical atherosclerosis parameter (≥2 plaques), and after adjusting for other covariates closely related to atherosclerosis (Table 3). Further studies are needed to confirm the benefits of these novel ¹H-NMR-glycoproteins over classical inflammation markers in the assessment of CVD risk in the T1D population, especially in contemporary cohorts. Meanwhile, some data in different populations have already suggested that ¹H-NMR-parameters could have advantages over the classical hsCRP [32]. Since ¹H-NMR-glycoproteins integrate more multiple inflammatory pathways by capturing the global signal of several proteins, they can better capture the degree of systemic inflammation in T1D [38].

In addition to their well-known lipid lowering effects, there is growing evidence about the additional pleiotropic mechanisms of statins that may play a role in their anti-atherosclerotic effect [39,40]. One of these mechanisms is the anti-inflammatory effect through modulation of B and T lymphocytes among other immune cells [41] known to reduce hsCRP levels [29]. However, data about the influence of statins on ¹H-NMR-glycoproteins are very scarce. In a post-hoc analysis of the JUPITER Trial, GlycA levels were not significantly reduced after the initiation of rosuvastatin, but information regarding other ¹H-NMR-glycoprotein variables, other statins, or the effect on other populations (especially in patients with diabetes) is lacking. Our participants did not differ in classical inflammatory markers or ¹H-NMR-glycoprotein levels (not only GlycA, but others) when they were analysed according to statin treatment (Supplemental Table S1). However, ¹H-NMR-glycoprotein determinations before the initiation of statins were not available, and thus, this kind of analysis could not be performed. Furthermore, the patients receiving statins were older, with a higher prevalence of hypertension, and tended to be heavier than non-users (all of these variables are associated with inflammation). Thus,

it cannot be ruled out that statin treatment was actually initiated in patients with higher baseline markers of inflammation, which were blunted after this lipid-lowering treatment. Additionally, the reasons why ^1H -NMR-glycoproteins were only independently associated with carotid atherosclerosis in the subset of patients without statin treatment in our sample (p for interaction <0.05 for ≥ 2 plaques; Table 3) are not clear. Since this specific lipid-lowering drug has shown powerful effects both on atherosclerosis [30] and inflammation (at least measured in conventional biochemical analysis) [29,41], this could preclude any significant finding in statistical models when analysed together. Notwithstanding, and taking into account that GlycA levels were lowered with some other drugs targeting some inflammatory pathways (anti-TNF α [42] or some monoclonal antibodies [43], the effect of statins on ^1H -NMR-glycoproteins in the setting of T1D should be specifically addressed in further studies.

The main strength of our study is its novelty. Thus, as far as we know, this is the first study to assess the relationships between this novel inflammatory marker (^1H -NMR-glycoproteins) in the setting atherosclerosis in a T1D population. Since the factors underlying the excess of CVD risk among this population are not fully elucidated, efforts to evaluate newer biomarkers should be welcomed. In addition, the use of ultrasound for the identification of carotid plaques provides greater robustness to our results, as it is a better independent predictor of CVD than IMT [44]. Furthermore, we carefully collected data on drug treatment and other clinical and analytical variables, which reduced the bias of our results. On the other hand, our study has several limitations. First, the cross-sectional design and the lack of a control group does not allow any conclusion to be drawn regarding causality in our main findings. Secondly, since the study was carried out in a single tertiary hospital and only primary prevention T1D patients at high-risk were included, extrapolation of the results to other T1D patients at lower risk or in other settings should be made with caution. Thirdly, the strict selection criteria of the participants and the cross-sectional design of the study could have led to unexpected results regarding variables associated with atherosclerosis. Thus, we did not find an association between active smoking habit and the number of carotid plaques. However, when cumulative smoking was assessed as pack-years, a significant difference was actually found in those with ≥ 2 plaques vs. patients with none or only one plaque ($p < 0.01$, Table 1). Furthermore, this is in line with the data of the FinnDiane study, in which the information on dose-dependent effect of smoking was more strongly associated with incident CVD (especially with stroke) than information of whether the patients were active/former/never smokers [45]. Another unusual finding was the lack of association of statin treatment with atherosclerosis. This could be due to the inherent selection bias of this lipid-lowering treatment in the cross-sectional studies. Thus, our participants under this drug were older, had a greater prevalence of hypertension, with a trend to being heavier, and with more advanced kidney disease. Consequently, it

cannot be ruled out that statins (with their well-known effect of atheroprotection) could have counteracted the advanced preclinical atherosclerosis that these patients should actually have had. In fact, several cross-sectional studies assessing carotid atherosclerosis in T1D individuals from our same geographical area did not find an association between statin treatment and carotid plaque [46]. In fact, neither was an association between statin treatment and carotid plaque found in several cross-sectional studies assessing carotid atherosclerosis in T1D individuals from our same geographical area. Finally, our study could not evaluate the effect of statins on ^1H -NMR-glycoproteins in the T1D population, or the reason for the differential effect of this new inflammatory marker according to the use of these lipid-lowering drugs. Further studies are needed to fully address this unsolved question and assess if our findings were due to the patients included or is a differential effect on T1D overall.

In summary, in our sample of participants with T1D at high cardiovascular risk, ^1H -NMR-glycoproteins, but not classical markers of inflammation, were shown to be associated with the presence and amount of preclinical atherosclerosis only in the subgroup not taking statins. This association was maintained in some of the ^1H -NMR-related parameters measured (i.e., GlycA and GlycB H/W ratios), even after adjusting for several variables closely related to atherosclerosis and inflammation. Due to the growing prevalence of obesity observed in the T1D population in the last years [47], and taking into account that excess body fat is closely related to inflammation, newer inflammations markers could be useful in the identification of patients at risk of a CVD event. Notwithstanding, future studies with a larger sample, with a control group, and specifically designed to evaluate the usefulness of ^1H -NMR-glycoproteins as biomarkers of inflammation in the assessment of CVD risk in the T1D population are needed.

Declaration of competing interest

None declared.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.numecd.2021.03.021>.

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Supplementary material

Corresponding to:

Quantification of glycoproteins by nuclear magnetic resonance associated with preclinical carotid atherosclerosis in patients with type 1 diabetes

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Supplemental Tables: 4

Supplemental Table S1. Characteristics of the study participants according to statin use.

	Statin non-users (n=105)	Statin users (n=84)	p-value
Clinical characteristics			
Male	60 (57)	49 (58)	0.870
Age (years)	45 (39.9-52.7)	49 (42.2-57.6)	0.004
Premature CVD in first-degree relatives†	12 (11)	13 (16)	0.417
Current smokers	29 (28)	23 (27)	0.971
Hypertension	23 (22)	35 (42)	0.003
SBP (mmHg)	124 (114.5-135)	127 (119.25-139.75)	0.388
DBP (mmHg)	80.43 (±8.19)	80.29 (±9.78)	0.913
BMI (kg/m ²)	25.80 (±3.70)	26.83 (±4.21)	0.075
Waist circumference (cm)§			
Female (cm)	84.69 (±12.36)	87.94 (±9.59)	0.203
Male (cm)	95.29 (±10)	98.55 (±12.13)	0.130
T1D duration (years)	25.72 (20.04-33.31)	27.71 (21.87-33.54)	0.425
Diabetic nephropathy	10 (10)	11 (13)	0.440
Diabetic retinopathy	37 (35)	35 (42)	0.368
CSII therapy	37 (35)	38 (45)	0.164
Laboratory characteristics			
Fasting plasma glucose (mg/dL)	143 (102.50-190.25)	147.50 (118-192.50)	0.660
Haemoglobin A1c (% mmol/mol)	7.5 (7-8.15), (58)	7.57 (7.13-7.93), (59)	0.833
Serum creatinine (mg/dL)	0.8 (0.715-0.93)	0.845 (0.73-0.99)	0.526
eGFR (CKD-EPI; ml/min/1.73m ²)	102.0 (90.6-108.9)	94.9 (83.0-104.3)	0.033
ALT (UI/L)	19 (14-26)	20 (16.25-26)	0.404
Total cholesterol (mg/dL)	192 (175-214)	182 (163-200)	0.004
HDL-cholesterol (mg/dL)	56 (47-67.50)	57 (49.25-70)	0.909
LDL-cholesterol (mg/dL)	118 (105-132)	103 (91-124)	0.002
Triglycerides (mg/dL)	73 (56.50-101)	75.5 (62-100.75)	0.526
Non-HDL cholesterol (mg/dL)	137 (120-150.50)	124 (107.50-141)	0.033
Pharmacological treatment			
Statins	-	-	-
ACEi / ARB	24 (23)	38 (45)	0.001
Antiplatelet drugs	7 (7)	10 (12)	0.213
Inflammation markers			
hsCRP	0.15 (0.06-0.26)	0.095 (0.05-0.4)	0.396
Leukocyte count	6.59 (5.53-8.42)	6.80 (5.55-8.38)	0.614
Area GlycA	4.01 (3.63-4.46)	4.05 (3.67-4.55)	0.833
Area GlycB	1.58 (1.36-1.73)	1.60 (1.39-1.79)	0.833
Area GlycF	1.75 (1.61-1.94)	1.73 (1.59-1.95)	0.505
H/W GlycA	13.58 (12.44-14.77)	13.75 (12.43-15.56)	0.833
H/W GlycB	3.66 (3.32-4.03)	3.63 (3.30-4.12)	0.935

Data are shown as n (percentage), mean (±SD) or median (Q1-Q3).
p values for group comparisons are reported

ACEi: angiotensin-converting enzyme inhibitor; ALT: alanine aminotransferase; ARB: angiotensin receptor blocker; CVD: cardiovascular disease; CSII: continuous subcutaneous insulin infusion; DBP: diastolic blood pressure; eGFR: estimated glomerular filtration rate; SBP: systolic blood pressure; T1D: type 1 diabetes; hsCRP: high sensitivity C-reactive protein; H/W GlycA: height/width ratio of GycA; H/W GlycB: height/width ratio of GycB

† Defined as <55 years in men and <65 years in women

Supplemental Table S2. Univariate relationships between ¹H-NMR-glycoproteins and other clinical and laboratory categorical variables in the whole cohort (n=189).

	Area GlycA	Area GlycB	Area GlycF	H/W GlycA	H/W GlycB
Sex					
Male	4.09 (3.68-4.61)	1.57 (1.37-1.74)	1.77 (1.61-1.96)	13.66 (12.40-15.03)	3.63 (3.32-4.03)
Female	4 (3.63-4.41)	1.60 (1.41-1.79)	1.72 (1.58-1.88)	13.63 (12.53-14.91)	3.65 (3.31-4.08)
Active smoking habit					
Yes	4.14 (3.71-4.77)	1.60 (1.40-1.71)	1.85 (1.58-2.06)	14.43 (12.53-15.66)	3.79 (3.34-4.08)
No	4 (3.63-4.43)	1.58 (1.37-1.77)	1.72 (1.60-1.91)	13.35 (12.42-14.7)	3.62 (3.30-4.04)
Hypertension					
Yes	4.11 (3.68-4.56)	1.63 (1.37-1.83)	1.75 (1.59-1.97)	13.77 (12.25-15.88)	3.74 (3.29-4.12)
No	4 (3.63-4.49)	1.57 (1.39-1.71)	1.73 (1.61-1.94)	13.41 (12.48-14.84)	3.62 (3.34-4.04)
Premature CVD in first-degree relatives					
Yes	3.99 (3.70-4.51)	1.63 (1.42-1.84)	1.78 (1.63-1.93)	14.03 (12.34-14.70)	3.66 (3.35-3.96)
No	4.04 (3.63-4.50)	1.59 (1.37-1.74)	1.73 (1.60-1.94)	13.55 (12.44-15.09)	3.63 (3.3-4.10)
Diabetic retinopathy					
Yes	4.06 (3.57-4.66)	1.59 (1.35-1.76)	1.73 (1.58-1.98)	13.65 (12.44-15.63)	3.67 (3.30-4.04)
No	4 (3.67-4.41)	1.58 (1.39-1.75)	1.74 (1.62-1.93)	13.66 (12.44-14.84)	3.62 (3.33-4.08)
CSII use					
Yes	4.03 (3.61-4.38)	1.57 (1.36-1.75)	1.77 (1.59-1.96)	13.37 (12.38-14.70)	3.62 (3.32-3.97)
No	4.02 (3.73-4.56)	1.60 (1.40-1.74)	1.72 (1.61-1.94)	13.67 (12.45-15.44)	3.70 (3.31-4.14)
Antiplatelet therapy					
Yes	4 (3.59-4.77)	1.59 (1.34-1.75)	1.78 (1.60-1.97)	14.70 (12.72-17.06)	3.77 (3.52-4.49)
No	4.02 (3.68-4.49)	1.59 (1.38-1.75)	1.73 (1.60-1.94)	13.51 (12.42-14.81)	3.62 (3.29-4.04)

Data are shown as median (Q1-Q3); CVD: cardiovascular disease; CSII: continuous subcutaneous insulin infusion. CVD: cardiovascular disease.

Premature CVD in first-degree relatives is defined as <65 in women and <55 in men.

There were no significant differences between groups (p-value >0.05 for all comparison tests).

Supplemental Table S3. Univariate relationships between NMR-glycoproteins and other clinical and laboratory variables in participants without statins (n=105).

	Area GlycA	Area GlycB	Area GlycF	H/W GlycA	H/W GlycB
General clinical characteristics					
Age (years)	r=-0.247 p=0.011	r=-0.236 p=0.015	r=-0.187 p=0.056	r=-0.160 p=0.102	r=-0.184 p=0.060
BMI (kg/m ²)	r=0.371 p<0.001	r=0.164 p=0.094	r=0.335 p<0.001	r=0.404 p<0.001	r=0.335 p<0.001
Waist circumference (cm)	r=0.322 p=0.001	r=0.146 p=0.138	r=0.307 p=0.002	r=0.309 p=0.001	r=0.267 p=0.006
SBP (mmHg)	r=0.177 p=0.071	r=0.194 p=0.048	r=0.109 p=0.267	r=0.130 p=0.186	r=0.063 p=0.523
T1D-specific characteristics					
Diabetes duration (years)	r=-0.094 p=0.341	r=-0.165 p=0.093	r=-0.147 p=0.133	r=-0.059 p=0.548	r=-0.113 p=0.250
Haemoglobin A1c (%)	r=0.319 p=0.001	r=0.248 p=0.011	r=0.172 p=0.079	r=0.340 p<0.001	r=0.274 p=0.005
Creatinine-to-albumin ratio (mg/g)	r=0.284 p=0.003	r=0.166 p=0.091	r=0.263 p=0.007	r=0.291 p=0.003	r=0.225 p=0.021
eGDR	r=-0.294 p=0.002	r=-0.170 p=0.084	r=-0.273 p=0.005	r=-0.311 p=0.001	r=-0.250 p=0.010
Lipid profile					
LDL-cholesterol (mg/dL)	r=0.249 p=0.011	r=0.130 p=0.189	r=0.195 p=0.047	r=0.244 p=0.012	r=0.196 p=0.046
HDL-cholesterol (mg/dL)	r=-0.432 p<0.001	r=-0.355 p<0.001	r=-0.397 p<0.001	r=-0.391 p<0.001	r=-0.341 p<0.001
Triglycerides (mg/dL)	r=0.612 p<0.001	r=0.317 p=0.001	r=0.487 p<0.001	r=0.505 p<0.001	r=0.378 p<0.001
Other characteristics					
hsCRP (mg/dL)	r=0.351 p=0.002	r=0.147 p=0.204	r=0.215 p=0.062	r=0.407 p<0.001	r=0.374 p=0.001
Leukocyte count (per mm ³)	r=0.382 p<0.001	r=0.255 p=0.009	r=0.275 p=0.005	r=0.376 p<0.001	r=0.304 p=0.002
Creatinine (mg/dL)	r=0.019 p=0.844	r=-0.046 p=0.639	r=0.056 p=0.568	r=0.002 p=0.986	r=-0.094 p=0.340
ALT (UI/L)	r=-0.034 p=0.734	r=-0.103 p=0.298	r=-0.010 p=0.917	r=-0.058 p=0.557	r=-0.066 p=0.503
Fatty liver index	r=0.586 p<0.001	r=0.287 p=0.023	r=0.510 p<0.001	r=0.529 p<0.001	r=0.360 p=0.004

ALT: alanine aminotransferase; eGFR: estimated glomerular filtration rate; SBP: systolic blood pressure; hsCRP: high sensitivity C-reactive protein.

Supplemental Table S4. Univariate relationships between NMR-glycoproteins and other clinical and laboratory variables in participants with statin treatment (n=84).

	Area GlycA	Area GlycB	Area GlycF	H/W GlycA	H/W GlycB
General clinical characteristics					
Age (years)	r= -0.109 p=0.323	r= -0.094 p=0.398	r= -0.049 p=0.658	r= -0.095 p=0.393	r= -0.106 p=0.338
BMI (kg/m ²)	r=0.242 p=0.026	r=0.124 p=0.260	r=0.099 p=0.370	r=0.342 p=0.001	r=0.285 p=0.009
Waist circumference (cm)	r=0.300 p=0.006	r=0.053 p=0.637	r=0.191 p=0.084	r=0.328 p=0.002	r=0.234 p=0.033
SBP (mmHg)	r=0.074 p=0.502	r= -0.036 p=0.743	r=0.109 p=0.324	r=0.076 p=0.490	r=0.096 p=0.383
T1D-specific characteristics					
Diabetes duration (years)	r= -0.118 p=0.284	r= -0.147 p=0.184	r= -0.036 p=0.746	r= -0.083 p=0.452	r= -0.119 p=0.280
Haemoglobin A1c (%)	r=0.292 p=0.007	r=0.002 p=0.983	r=0.189 p=0.085	r=0.273 p=0.012	r=0.190 p=0.084
Creatinine-to-albumin ratio (mg/g)	r=0.155 p=0.160	r=0.077 p=0.487	r=0.104 p=0.347	r=0.154 p=0.163	r=0.035 p=0.755
eGDR	r= -0.253 p=0.021	r= -0.061 p=0.583	r= -0.176 p=0.111	r= -0.203 p=0.065	r= -0.143 p=0.196
Lipid profile					
LDL-cholesterol (mg/dL)	r=0.031 p=0.778	r= -0.105 p=0.343	r=0.006 p=0.960	r= -0.004 p=0.969	r=0.003 p=0.978
HDL-cholesterol (mg/dL)	r= -0.309 p=0.004	r= -0.201 p=0.066	r= -0.109 p=0.322	r= -0.214 p=0.051	r= -0.090 p=0.417
Triglycerides (mg/dL)	r=0.679 p<0.001	r=0.231 p=0.034	r=0.596 p<0.001	r=0.554 p<0.001	r=0.444 p<0.001
Other characteristics					
hsCRP (mg/dL)	r=0.466 p<0.001	r=0.447 p<0.001	r=0.297 p=0.017	r=0.527 p<0.001	r=0.387 p=0.002
Leukocyte count (per mm ³)	r=0.468 p<0.001	r=0.378 p<0.001	r=0.312 p=0.004	r=0.467 p<0.001	r=0.373 p<0.001
Creatinine (mg/dL)	r=0.188 p=0.086	r=0.244 p=0.025	r=0.192 p=0.081	r=0.152 p=0.167	r=0.121 p=0.272
ALT (UI/L)	r=0.110 p=0.319	r= -0.002 p=0.985	r=0.189 p=0.085	r=0.108 p=0.328	r=0.145 p=0.188
Fatty liver index	r=0.572 p<0.001	r=0.278 p=0.053	r=0.428 p=0.002	r=0.584 p<0.001	r=0.467 p=0.001

ALT: alanine aminotransferase; eGFR: estimated glomerular filtration rate; SBP: systolic blood pressure; hsCRP: high sensitivity C-reactive protein.

VIII. Discusión

1. Las herramientas generales para predecir eventos cardiovasculares no resultan útiles en personas con DTI

En nuestro segundo trabajo se objetivó la pobre concordancia entre la escala genérica propuesta por la ESC/EASD-2019 y la ecuación de RCV específica Steno-Risk [464]. Además, se demostró la incapacidad de la primera, y la capacidad de la segunda, para identificar correctamente a aquellos con presencia y/o mayor carga de aterosclerosis carotídea subclínica. Adicionalmente, el rendimiento de la ecuación Steno-Risk fue independiente del RCV estimado por ESC/EASD-2019, otras variables lipídicas no incluidas en la propia ecuación y el uso de tratamientos cardioprotectores, lo cual refuerza el potencial de esta herramienta en la población adulta con DTI representativa de nuestro entorno.

Otros estudios han objetivado las limitaciones de las escalas de riesgo genéricas en la predicción de eventos cardiovasculares en esta población. Hace prácticamente dos décadas, *Zgibor JC et al*, demostraron la carencia de calibración de dos de las herramientas más utilizadas por aquel entonces, las ecuaciones no específicas UKPDS [67] y Framingham [465], en la predicción de coronariopatía isquémica en la cohorte del estudio EDC [50]. La muestra de la cohorte del estudio EDC analizada, pese a ser representativa de aquella época, se considera de alto o muy alto RCV (diagnóstico de la DTI en la infancia, > 20 años de evolución, HbA1c media > 10%). En este sentido, el no tener en cuenta la presencia de complicaciones microvasculares ni la edad prematura de presentación de los eventos de ECV, evidenció la infraestimación del RCV real arrojado por ambas ecuaciones (UKPDS y Framingham), sobre todo entre aquellos con un mayor riesgo [50].

En el mismo sentido, un estudio, realizado en una muestra no seleccionada de adultos con DTI (n = 575), similar a la de nuestro estudio, a excepción de una edad y duración de la DTI diez años inferior, se objetivó una pobre concordancia entre la escala de la ESC/EAS de 2019 [304] (la clasificación, en el caso de la DTI, es la misma que la de la ESC/EASD-2019 [37]) y la ecuación Steno-Risk [466]. Resulta destacable que, de aquellos con una edad inferior a los 35 años, el 30% clasificó como de riesgo muy alto según ESC/EAS 2019, sin embargo, mediante la

ecuación Steno-Risk la práctica mayoría (99%) reclasificaron como de riesgo moderado, apuntando a la mayor inexactitud de la escala genérica entre los más jóvenes. Asimismo, en los mayores de 50 años, cerca del 30% de los clasificados como de muy alto riesgo según ESC/EAS 2019 mostraron riesgo moderado con la ecuación Steno-Risk [466]. Nuestros datos son acordes a lo expuesto, mostrando que el 60% de los clasificados como de muy alto riesgo según ESC/EASD-2019, presentaban riesgo bajo/moderado con Steno-Risk [464].

En un segundo estudio del mismo grupo italiano, se analizó el rendimiento predictivo de ambas herramientas de manera retrospectiva en $n = 456$ adultos con DTI. Tras 8,5 años de seguimiento medio, hubo 24 eventos no fatales de ECV, mayoritariamente IAM y revascularización coronaria. La escala ESC/EAS 2019 clasificó a más del 90% de los que presentaron un evento como de muy alto riesgo, mientras que la ecuación Steno-Risk tan solo clasificó correctamente a cerca del 40% [467]. Sin embargo, el 85% de los que no presentaron eventos, fueron clasificados correctamente como de riesgo bajo según Steno-Risk, mientras que la escala genérica no fue capaz de discriminar de forma precisa ($< 15\%$ clasificó como riesgo moderado). En esta cohorte, teniendo en cuenta las limitaciones por disponer de tan pocos eventos cardiovasculares, el riesgo muy alto de ESC/EAS 2019 se mostró altamente sensible pero poco específico (92% y 64%, respectivamente), mientras que el riesgo bajo/moderado de Steno-Risk alcanzó un mejor rendimiento global (sensibilidad 88%, especificidad 85%). Nuestros datos, con una prevalencia de placas carotídeas superior al 40%, muestran una sensibilidad y especificidad muy pobres ($< 60\%$) en la detección de aterosclerosis para el riesgo muy alto de ESC/EASD-2019 [464]. Además, más de la mitad de los participantes clasificados como de muy alto riesgo según ESC/EASD-2019 no albergaba placas carotídeas [464]. De hecho, al analizar a aquellos discordantes (mayor riesgo estimado por Steno-Risk que por ESC/EASD-2019), la ecuación específica logró identificar a los que presentaban aterosclerosis. Estos resultados disonantes pueden deberse principalmente a que el bajo número de eventos del estudio mencionado haya podido comprometer la potencia estadística, impidiendo valorar correctamente el rendimiento de la ecuación Steno-Risk [467].

Otro pequeño estudio de la zona norte de Italia (n = 223), halló una correlación positiva entre el RCV estimado por Steno-Risk y el GIM carotídeo máximo, así como una prevalencia veinte veces superior de placas carotídeas entre aquellos con un riesgo estimado alto vs. bajo por esta ecuación [468]. Estas asociaciones también se vieron con la presencia de retinopatía y neuropatía diabéticas, lo cual no es de extrañar teniendo en cuenta la estrecha relación existente entre las complicaciones micro y macrovasculares en personas con DTI [469]. Finalmente, también se encontró una asociación positiva entre el riesgo estimado por Steno-Risk y la ECV, aunque tan solo hubo tres eventos no fatales y las conclusiones deben considerarse exploratorias [468].

A pesar de la escasez de estudios que hayan valorado el rendimiento de las ecuaciones de RCV en la predicción de eventos cardiovasculares, se dispone de algunos datos con otras variables subrogadas de ECV y que apuntan en la misma dirección que nuestro estudio [464]. Previamente, nuestro grupo demostró una asociación positiva y progresiva entre el riesgo estimado por Steno-Risk, y el GIM carotídeo, la presencia y el número de placas ateroscleróticas en personas con DTI [470]. Adicionalmente, el riesgo alto según Steno-Risk, en contraposición a otros FRCV (e.g. tabaquismo activo, mayor edad o HTA), fue el parámetro que se asoció en mayor medida con la carga aterosclerótica (presencia de 2 o más placas carotídeas) [470]. En la misma línea, *Llauradó G et al*, evaluaron a 179 adultos con DTI en prevención primaria objetivando una relación directamente proporcional y significativa entre la rigidez arterial, determinada mediante la velocidad de la onda del pulso aórtica, y el riesgo estimado por Steno-Risk [471]. Otro pequeño estudio europeo, mostró resultados similares (correlación positiva con el riesgo estimado por Steno-Risk) al evaluar la rigidez arterial mediante el estándar oro (velocidad de la onda de pulso carótida-femoral), sin hallar diferencias significativas acorde a la clasificación de RCV de las guías ESC/EAS 2019 [472]. Finalmente, el único estudio no europeo disponible fue llevado a cabo en Australia en 85 adultos con DTI y no tratados con estatinas [473]. Con una edad media de 35 años, cerca del 80% presentaron un CAC score de 0 unidades de Agatston (UA). La mayoría de los sujetos con un alto riesgo estimado mediante Steno-Risk presentaron un CAC

score mayor a 0 (95%). Sin embargo, tan solo un tercio de los que clasificaron como de muy alto RCV según ESC/EAS 2019 presentaba un CAC score positivo [473]. Además, Steno-Risk demostró un buen rendimiento diagnóstico en la detección de un CAC score positivo [473].

Aunque la poca evidencia disponible parezca decantarse por la mayor utilidad de la ecuación específica Steno-Risk, resulta llamativo que de varios de los estudios mencionados y de nuestros datos, entre el 40 - 50% de los que presentaban aterosclerosis o ECV, fueron clasificados como de riesgo moderado por Steno-Risk [464,467,473]. Este hecho reafirma el propósito de la presente tesis, el cual no es solamente evidenciar la ineficacia de la estrategia general, si no proponer nuevos marcadores que permitan estrechar ese gris llamado “riesgo moderado”, el cual no permite dirigir la estrategia terapéutica en cada caso. A este respecto, el uso combinado de las técnicas de cribado de la aterosclerosis subclínica (e.g. ecografía carotídea, CAC score o estimación de la rigidez arterial), pueden ser de gran utilidad.

2. La dieta mediterránea y sus beneficios cardiovasculares

Los principales hallazgos del artículo correspondiente al objetivo 2a fueron que: 1) una mayor adherencia a la dieta mediterránea restringida en calorías, se asoció con la presencia y número de placas carotídeas en pacientes adultos con DTI y, 2) al valorar cada ítem del cuestionario PREDIMED-Plus, aquellos relacionados con el mayor consumo de frutos secos, fruta, legumbres y preferencia de carne blanca en vez de roja, se mantuvieron asociados de manera significativa en los modelos multivariantes [474].

En población sin DTI, una mayor adherencia a la dieta mediterránea se ha asociado con una menor incidencia de ECV en prevención primaria y secundaria. Se dispone de dos grandes ECA cardiovasculares a este respecto, por un lado, el estudio PREDIMED [129], en prevención primaria, y por el otro lado, el estudio CORDIOPREV [126], en prevención secundaria. En el caso del estudio PREDIMED

(n = 7447), la dieta mediterránea suplementada con aceite de oliva virgen extra (AOVE) o con nueces, demostró una reducción relativa de ECV (combinado de IAM, ictus o muerte cardiovascular) del 30% en comparación a una dieta baja en grasas, tras un seguimiento de cerca de cinco años [129]. En el estudio unicéntrico CORDIOPREV (n = 1002), tras siete años de seguimiento, se objetivó un beneficio, similar en magnitud, al seguir una dieta mediterránea vs. baja en grasas [125]. Por otro lado, el ECA PREDIMED-PLUS, todavía en curso, pretende evaluar el efecto en la ECV, no solamente del patrón dietético, si no de una intervención múltiple enfocada en la pérdida ponderal (dieta mediterránea restringida en calorías, incremento de la actividad física y terapia conductual vs. recomendaciones dietéticas estándar basadas en la dieta mediterránea no restringida en calorías y manejo cardiovascular habitual) en personas con sobrepeso u obesidad [475].

Adicionalmente, los análisis de un subgrupo de la cohorte PREDIMED, demostraron enlentecimiento de la progresión del GIM carotídeo en el grupo que siguió una dieta mediterránea enriquecida en nueces, mientras que el grupo control (dieta baja en grasas), mostró progresión [476]. Un subestudio del ECA CORDIOPREV objetivó beneficios similares en el GIM carotídeo en el grupo de intervención [477]. Por último, un estudio transversal basado en la cohorte del estudio ILERVAS (n = 8116), en prevención primaria, edad 45-70 años y sin diabetes, demostró una asociación negativa y significativa entre la adherencia a la dieta mediterránea y la presencia de aterosclerosis carotídea y/o femoral [478].

En la población con DTI, la evidencia es muy escasa y no se dispone apenas de estudios con variables clínicas cardiovasculares. Un estudio realizado en base al registro nacional iraní, en más de 1800 participantes adultos con DTI, demostró una relación consistente entre el grado de adherencia a la dieta mediterránea y la incidencia de ECV y de complicaciones microvasculares (ERD, retinopatía y neuropatía) [154]. Por otro lado, una mayor adherencia a la dieta mediterránea se ha relacionado con mejores resultados de diversos parámetros subrogados de ECV. Por ejemplo, en el estudio prospectivo CACTI (n = 652 adultos con DTI), la adherencia a la dieta mediterránea o a la dieta DASH, se asociaron negativamente con la adiposidad pericárdica, un marcador relacionado con la ECV [152]. Sin

embargo, no hubo diferencias en cuanto a progresión del CAC score tras seis años de seguimiento. Los análisis de la cohorte del estudio *SEARCH for Diabetes in Youth Study*, llevado a cabo en personas con DTI de 10 a 30 años de edad, no halló ninguna relación entre la adherencia a varios tipos de patrones de alimentación considerados cardiosaludables, entre ellos la dieta mediterránea, y los parámetros de rigidez arterial [479]. Cabe destacar que la adherencia a la dieta mediterránea de ambos estudios [152,479] fue inferior a la reportada en nuestro estudio y nuestro ámbito [474,480,481]. Además, el distinto contexto geográfico (EEUU) implica una diferencia en las costumbres, tipos y calidad de los alimentos y el uso de cuestionarios adaptados que complican la comparabilidad con nuestros resultados. Por ejemplo, en el estudio derivado de la cohorte del estudio *SEARCH* [479], no se dispuso de información acerca del consumo de AOVE, siendo uno de los componentes principales de la dieta mediterránea. Por último, algunos estudios en personas con DTI han asociado la mayor adherencia al patrón de alimentación mediterráneo con beneficios en cuanto al control glucémico [148,482,483] o en el peso y perímetro de cintura [484].

De los hábitos individuales, el consumo de legumbres, nueces, carne blanca en vez de roja y fruta, fueron los únicos que se asociaron a una menor prevalencia y/o carga de aterosclerosis carotídea [474]. Los beneficios cardiovasculares de la mayoría de estos hábitos han sido ampliamente reportados en la población general [485–489]. En personas con DTI del estudio FinnDiane, el consumo de más de dos raciones de nueces semanales se relacionó con un menor perímetro de cintura, HbA1c e IMC [490]. También, en el estudio EURODIAB, el bajo consumo de fibra dietética, ácidos grasos poliinsaturados, proteína vegetal y el alto consumo de colesterol, todos ellos relacionados con una pobre adherencia a los hábitos mencionados, se asociaron a peores niveles de diversos biomarcadores de disfunción endotelial e inflamatorios [491].

En resumen, por la cantidad y calidad de la evidencia científica, la dieta mediterránea se erige como uno de los mejores patrones de alimentación cardiosaludable en la población general. Los estudios en DTI, incluyendo el de la presente tesis, sugieren la utilidad clínica de este abordaje. No obstante, las

distintas metodologías utilizadas para valorar la adherencia dietética, las diferentes variables resultado empleadas y los diversos ámbitos geográficos, costumbres y culturas culinarias, no permiten extraer conclusiones estandarizadas. En cualquier caso, no parece haber motivo fisiopatológico que contrarreste los amplios beneficios cardiovasculares observados en la población sin DTI. Además, el patrón de alimentación mediterráneo es oriundo de la cuenca mediterránea y de nuestro territorio, casando perfectamente con nuestras costumbres y con la disponibilidad de los alimentos tradicionales. Es por ello que, ante la ausencia de alternativas contrastadas, se debe considerar la recomendación de la dieta mediterránea en las personas con DTI de nuestro entorno.

3. La importancia de los factores obstétricos

Del cuarto artículo de la presente tesis derivan varios hallazgos relevantes [492]. En primer lugar, la paridad se asoció a una mayor aterosclerosis carotídea en contraposición a la nuliparidad. En segundo lugar, el antecedente de preeclampsia se relacionó con la presencia y número de placas carotídeas sin demostrar diferencias significativas con los hombres con DTI. En tercer lugar, la ecuación Steno-Risk no identificó correctamente a aquellas mujeres con preeclampsia previa que albergaban placas de ateroma. Finalmente, la inclusión de la paridad o de la preeclampsia a Steno-Risk no mejoró la discriminación de la ecuación en cuanto a la identificación de las que presentaban aterosclerosis carotídea [492]. Estos resultados van en la línea de lo esperado y es consecuente con lo objetivado en estudios previos realizados en población sin DTI.

En población sin DTI, la paridad y, más específicamente, la multiparidad, se ha asociado en múltiples estudios con la ECVA. Un metaanálisis de estudios prospectivos ($n > 150\,000$), pese a una elevada heterogeneidad, indicó una asociación positiva en forma de J entre el número de partos y la incidencia de ECV [182]. Esta relación también se dio en el subgrupo de mujeres con diabetes [182]. Otro estudio en más de 3000 mujeres asiáticas, demostró una similar asociación directa y progresiva entre el número de partos y la aterosclerosis carotídea [493].

El mecanismo por el cual la paridad confiere un mayor RCV es meramente especulativo y se ha relacionado con el efecto acumulado de algunas posibles alteraciones secundarias a la gestación, como la sobre activación del eje renina-angiotensina-aldosterona, la resistencia periférica a la insulina, cambios en el metabolismo lipídico, inmunomodulación o disfunción endotelial, entre otros [494–497]. En este sentido, un estudio de aleatorización mendeliana sugiere una posible relación causal entre la multiparidad y la ECV [183].

Las evidencias previas en mujeres con DTI, sin embargo, no son tan esclarecedoras. En un estudio de la cohorte del TID-ECR, se comparó prospectivamente a mujeres embarazadas en el momento del reclutamiento (n = 28) con nulíparas (n = 469), sin hallar diferencias en la incidencia de complicaciones micro y/o macrovasculares tras cinco años de seguimiento [498]. A este respecto, el bajo número de eventos cardiovasculares (0 vs. 2, respectivamente), debe tenerse en cuenta a la hora de interpretar los resultados. Del mismo modo, un análisis transversal en más de 1500 mujeres brasileñas con DTI, se observó una mayor prevalencia de ECV a medida que se incrementaba el número de partos [499]. No obstante, esta asociación no se mantuvo en los modelos multivariantes. Destacar que se trataba de una muestra muy joven (edad media de 25 años) y con una baja prevalencia de complicaciones micro y macrovasculares, lo cual podría haber incrementado la probabilidad de error de tipo II [499]. En conclusión, se requieren de estudios prospectivos con mayor número de eventos cardiovasculares para poder discernir el impacto de la paridad en esta población.

A pesar de no conocer con precisión los mecanismos fisiopatológicos que originan la preeclampsia, se han propuesto relaciones bidireccionales entre la misma y la disfunción cardiovascular [500–504]. En este sentido, en población general, el riesgo de ECV es de entre 1,5 – 2,7 veces superior en las mujeres con antecedentes de preeclampsia [177,178,505,506]. En mujeres con DTI, en un trabajo preliminar de nuestro grupo, se objetivó la asociación positiva e independiente entre la preeclampsia y la presencia de placas carotídeas, así como el efecto aditivo de la misma y de la DTI en la aterosclerosis subclínica [192]. En la

misma dirección, un estudio reciente basado en el Swedish NDR, siguió durante 13 años a más de 65 000 mujeres primíparas con diabetes ($n = 9230$ con DTI) [191]. Es remarcable que una de cada cinco participantes con DTI presentó algún trastorno hipertensivo del embarazo (preeclampsia, eclampsia o HTA gestacional). Adicionalmente, el antecedente de alguno de estos trastornos se asoció marginalmente con un 20% mayor incidencia de ECV, sin diferencias en la mortalidad por cualquier causa [191].

Uno de los principales objetivos de nuestro estudio, fue evaluar el rendimiento en la identificación de aterosclerosis carotídea de la adición de los factores obstétricos (paridad y preeclampsia) a la ecuación Steno-Risk [492]. Pese a objetivar la escasa aplicabilidad de la ecuación entre las mujeres con antecedente de preeclampsia, la capacidad discriminatoria no fue significativamente mejor al añadir dichos factores. Ello podría deberse a que el RCV conferido por la paridad y la preeclampsia sea parcialmente mediado por otros FRCV ya presentes en la ecuación Steno-Risk. De hecho, grandes estudios en personas sin DTI confirman la asociación independiente de la preeclampsia con la ECV, pero la nula o ínfima mejoría al añadirse a los modelos predictivos [507,508].

Para finalizar, comentar que la ECV es, al igual que en los hombres, la principal causa de mortalidad en las mujeres y que, a pesar de ello, sigue siendo menos investigada, reconocida, diagnosticada y tratada [98,509]. En este sentido, valorar la presencia de factores específicos de la mujer puede ser de ayuda en la lucha por reducir la inequidad.

4. La complejidad del metabolismo lipídico en la DTI

Como se ha comentado en apartados previos, las personas con DTI presentan alteraciones cualitativas y cuantitativas las cuales pueden pasar desapercibidas si tan solo se determina el perfil convencional (colesterol total, cLDL, cHDL y triglicéridos séricos) [203]. En este sentido, en nuestro quinto artículo, objetivamos que una mayor discrepancia entre las lipoproteínas

aterogénicas medidas por ^1H -RMN y el colesterol convencional contenido en las mismas (razón partículas no-HDL / c-no-HDL y partículas LDL / cLDL), se asoció independientemente de múltiples factores, como otros lípidos convencionales o el tratamiento con estatinas, con la presencia de placas carotídeas [510]. Además, varios parámetros avanzados relacionados con las partículas de HDL y su contenido, se asociaron de manera inversa.

Para un valor determinado de cLDL, un mayor número de partículas de LDL (o mayor discrepancia) refleja, generalmente, la presencia de una mayor cantidad de partículas pequeñas y densas, características de la dislipemia aterogénica [511]. Las partículas de LDL pequeñas y densas son más proclives a padecer modificaciones que aumentan su tiempo de estancia en sangre y que fomentan el proceso de la aterosclerosis, considerándose más aterogénicas al compararse partícula por partícula [512–514]. Además, estas partículas suelen estar deplecionadas de colesterol libre, lo cual incrementa aún más la razón entre el número de partículas y el colesterol contenido en las mismas [515]. Los valores discrepantes entre las partículas aterogénicas y su contenido en colesterol pueden presentarse en personas sanas, aunque es más notorio cuando existe enfermedad metabólica asociada, como la hiperglucemia o la obesidad [516,517]. Esta discrepancia se ha propuesto como uno de los posibles contribuyentes del RCV residual. En población sin diabetes, por ejemplo, datos del *Women's Health Study* ($n = 27\,533$, seguimiento mediano = 17,2 años), demostraron que la incidencia de ECV coronaria fue de entre dos y tres veces superior en aquellas participantes con valores discordantes (c-no-HDL, apolipoproteína B y partículas de LDL determinadas mediante ^1H -RMN elevados, pero cLDL directo disminuido) vs. los que presentaban valores concordantemente bajos [518]. En el mismo sentido, un estudio de la cohorte *Framingham Offspring* ($n = 3066$), mostró que la incidencia de ECV vino determinada por los niveles de partículas de LDL (^1H -RMN), mientras que las diferencias en el cLDL calculado tuvieron escaso efecto [515]. Estos resultados están también respaldados por datos de más de 6000 sujetos de la cohorte MESA, donde se objetivó que los niveles de partículas de LDL (^1H -RMN) se asociaron con la incidencia de ECV y el GIM carotídeo, independientemente de

los niveles de cLDL [519]. A este respecto, en el estudio JUPITER, añadir el perfil avanzado de lipoproteínas por movilidad iónica a los FRCV clásicos, incluyendo los lípidos convencionales, incrementó levemente la capacidad pronóstica de ECV en ambos grupos (rosuvastatina vs. placebo) [520]. Otros estudios han relacionado estas discrepancias con la presencia de placas coronarias [521], progresión del CAC score [209,522] o ictus isquémico [523].

En el caso de las personas con DTI, un trabajo previo de nuestro entorno, objetivó un mayor número de partículas de LDL por unidad de cLDL convencional (razón partículas de LDL / cLDL) y una mayor discrepancia (partículas de LDL > 1000 nmol/L pero cLDL < 100 mg/dL) en el grupo con DTI vs. control sin diabetes [245]. Desafortunadamente, no se dispone de estudios que hayan analizado estas discrepancias con algún resultado cardiovascular en esta población. Sin embargo, *Llauradó et al*, analizaron el perfil avanzado de lipoproteínas mediante ¹H-RMN en 84 pacientes adultos con DTI y HbA1c media de 7,9%, objetivando que algunos parámetros relacionados con las VLDL y con las HDL, se asociaron, positiva y negativamente, con la rigidez arterial [246]. Además, en un pequeño subgrupo de la cohorte del estudio EDC, con una HbA1c > 10%, el número de partículas de VLDL también se asoció positivamente con la enfermedad coronaria [247]. Nuestros resultados concuerdan con lo anterior, pues al analizar por separado a aquellos con un peor control glucémico (HbA1c > 8%, similar a la del estudio mencionado), la mayoría de parámetros relacionados con las VLDL se asociaron a la presencia de placas carotídeas [510]. En contraposición, un estudio basado en la cohorte del DCCT/EDIC, analizó las relaciones del perfil lipoproteico basal, determinado por resonancia magnética, y la progresión del GIM carotídeo cerca de 19 años después, mostrando resultados poco concluyentes [256].

Finalmente, otros dos estudios, a pesar de no analizarlo directamente, parecen apoyar la utilidad de las discrepancias entre partículas aterogénicas y su contenido de colesterol. Por un lado, el tratamiento intensivo de la diabetes en el estudio DCCT, se relacionó con una disminución de las partículas de LDL pequeñas y aumento de las grandes determinadas por resonancia magnética, lo que probablemente se hubiese traducido en una disminución de la discrepancia

(partículas de LDL / cLDL), si se hubiese determinado [248]. Por otro lado, un estudio antiguo detectó una disminución de las partículas de LDL pequeñas y densas medidas por ultracentrifugación tras optimizar el control glucémico en una pequeña muestra de pacientes con diabetes “insulinodependiente” [524].

En resumen, y a la espera de estudios cardiovasculares prospectivos, los parámetros que indican una mayor discrepancia entre las partículas aterogénicas y el colesterol contenido en ellas, podría ser de utilidad para desentrañar parte del RCV residual en personas con DTI. Su implementación puede ser de especial relevancia entre aquellos con niveles de cLDL convencional “normal” o “bajo”, en los que hasta el 40% puede presentar un número discrepantemente elevado de partículas aterogénicas [245].

En nuestro sexto estudio se exploraron las asociaciones de múltiples especies lipidómicas con la presencia de aterosclerosis subclínica [525]. Los ácidos grasos omega-6, incluyendo el ácido linoleico, el colesterol libre y esterificado, y los niveles de esfingomielina, se asociaron inversamente con la presencia y/o carga de aterosclerosis carotídea [525]. Cabe destacar que la intención del estudio, en una pequeña muestra de 131 pacientes, fue eminentemente exploratoria. Es por eso que se decidió no ajustar las pruebas de significación por múltiples comparaciones, para reducir la probabilidad de error de tipo II. Por lo tanto, los hallazgos inconsistentes y conflictivos de varios de los parámetros lipidómicos analizados deben interpretarse bajo esta perspectiva.

La relación inversa de los niveles de esfingomielina fue inesperada, sobre todo siendo el único parámetro que se asoció de manera sólida con la presencia de placas carotídeas en todos los modelos [525]. La esfingomielina realmente comprende un conjunto de subespecies, las esfingomielinas, que representan los esfingolípidos más abundantes de las membranas celulares. El rol de las esfingomielinas es amplio y comprende desde funciones estructurales hasta de regulación de la homeostasis del colesterol [526]. En este sentido, una mayor proporción de esfingomielina en la membrana celular de las partículas de LDL, predispone a que se agreguen entre ellas en el interior de la capa íntima arterial, lo

cual se ha relacionado con la aterosclerosis y muerte cardiovascular [527–529]. En cuanto a la utilidad clínica de este marcador, los estudios muestran resultados poco esclarecedores, al hallar asociaciones directas, indirectas o no asociación, de las diferentes subespecies de esfingomielinas con la ECV [268,530–533]. La determinación de esfingomielina en las membranas eritrocitarias, en vez de en el plasma, podría ser de mayor utilidad a este respecto [534].

En el caso de la DTI, en el estudio FinnDiane, los niveles de esfingomielina séricos (^1H -RMN) se asociaron longitudinalmente con el empeoramiento de la función renal y el riesgo de ECV coronaria [283,535]. Al contrario, un estudio danés, llevado a cabo en 669 adultos con DTI, analizó mediante espectrometría de masas múltiples subespecies lipídicas y de esfingomielinas [282]. Varias de estas subespecies se relacionaron con un mejor pronóstico renal (FGe y albuminuria) y una menor mortalidad por cualquier causa, sin que su adición a diversos FRCV mejorase el área bajo la curva en la predicción de la mortalidad [282]. Otro estudio posterior del mismo grupo, también reportó asociaciones, positivas o negativas, de diferentes esfingomielinas con la ECV [281]. Por lo tanto, el papel de las esfingomielinas en las complicaciones microvasculares y en la ECV en personas con DTI es una incógnita pendiente de resolver. El mejor abordaje de cara a futuros estudios, probablemente se base en el uso de técnicas que puedan distinguir las distintas clases de esfingomielina, no solamente en el plasma, sino también en las membranas celulares, donde parecen ejercer sus principales funciones aterogénicas.

La asociación indirecta de los ácidos grasos omega-6 y del ácido linoleico con la carga aterosclerótica (≥ 3 placas), en algunos de los modelos de regresión, van en la línea de lo publicado anteriormente [525]. El ácido linoleico es el principal representante de los ácidos grasos omega-6 y el más abundante en la dieta y en el plasma. Su rol en la salud cardiovascular, no obstante, no está libre de controversia [536]. Varios metaanálisis apuntan hacia el beneficio cardiovascular del consumo dietético de ácido linoleico [537–539]. Por el contrario, cuando el consumo de omega-6 es a través de suplementación y no de alimentos, no se objetivan dichas asociaciones [540]. Incluso, el consumo excesivo de omega-6

proveniente mayoritariamente de aceites vegetales refinados, frecuentes en la dieta americana, se ha reportado como perjudicial [541]. En este sentido, alimentos ricos en omega-6, como los frutos secos, contienen múltiples compuestos bioactivos beneficiosos (e.g. fibra o polifenoles) que podrían mediar parte de los beneficios observados, en acuerdo con los resultados de nuestro tercer trabajo [474].

En la misma dirección, nuestro grupo analizó diversos biomarcadores de ingesta de ácidos grasos en la membrana eritrocitaria, en una muestra de adultos con DTI [286]. Un hallazgo destacable fue que los niveles de ácido linoleico fueron progresivamente inferiores a medida que aumentaba el número de placas carotídeas y se asociaron de manera independiente con la aterosclerosis [286]. Por otro lado, nuestro grupo reportó anteriormente resultados inconsistentes en una pequeña muestra de mujeres con DTI (n = 50), en el cual se halló una relación directa de los ácidos grasos omega-7 (¹H-RMN) con la presencia de aterosclerosis carotídea [285]. La significación clínica de esta asociación, no obstante, debe interpretarse con cautela, dado el dudoso papel de este tipo de ácidos grasos en la ECV [542,543].

En conclusión, diversos parámetros del perfil avanzado de lipoproteínas y lipidómico podrían ser de utilidad clínica en personas con DTI. Sin embargo, para poder dictaminarlo, se necesitan salvar una serie de obstáculos. En primer lugar, las técnicas para determinar estos parámetros son diversas y no están estandarizadas, por lo que la comparabilidad entre ellas no siempre es factible. En este sentido, un estudio comparó cuatro métodos para determinar las subclases de lipoproteínas y demostró la escasa correlación entre ellas en la determinación, principalmente, de las partículas de menor tamaño [544]. En segundo lugar, estos parámetros, sobre todo los lipidómicos, pueden presentar variaciones notables en función de distintos contextos clínicos y ambientales [259]. Consecuentemente, se debe seleccionar cuidadosamente la población a estudio o, por lo menos, controlar por el mayor número de variables que puedan estar implicadas, para reducir la probabilidad de encontrar resultados dispares entre unos estudios y otros. En tercer lugar, quizá el suero o el plasma no sean el medio más adecuado para analizar el lipidoma y se deba analizar en muestras celulares o tisulares [545–547].

A pesar de las limitaciones metodológicas de nuestro quinto y sexto trabajos, con muestras relativamente pequeñas y diseños transversales, los resultados invitan a seguir explorando el potencial de estas técnicas avanzadas mediante diferentes abordajes [510,525].

5. El valor de la inflamación en el riesgo cardiovascular

En nuestro séptimo trabajo, objetivamos que en una gran muestra de adultos con DTI, dos sencillos parámetros y ampliamente disponibles (leucocitos circulantes y RNL) se asociaron, directa y progresivamente, con el número de placas carotídeas [548]. Sin embargo, el RNL fue el único que se mantuvo asociado significativamente en los modelos multivariantes [548].

El RNL es un biomarcador que refleja el equilibrio entre dos aspectos del sistema inmunitario: la inflamación aguda y la crónica (indicada por los niveles de neutrófilos) y la inmunidad adaptativa (linfocitos circulantes). En población general se ha asociado consistentemente con múltiples condiciones, como los eventos cardiovasculares mayores [345,349], mortalidad por cualquier causa y cardiovascular [346,347], ECV coronaria [348], hemorragia cerebral [549,550], ictus isquémico [551,552], sepsis y enfermedades infecciosas [553] y a un peor pronóstico en personas con cáncer [554], entre otros. Datos del *National Health and Nutrition Examination Survey* (NHANES) indican un valor medio del RNL de 2,15 en población general y de 2,49 en personas con diabetes de cualquier tipo [346,555]. Se ha sugerido un valor normal de RNL entre 1 - 2, límite entre 2 - 3 y patológico a partir de 3 [556]. En la cohorte de personas con diabetes del NHANES, se estableció el punto de corte en 3,48, por ser el que se asociaba de forma más significativa con la supervivencia global [346]. En nuestro estudio, el valor medio fue RNL inferior a los reportados (1,93) y aquellos con más carga aterosclerótica (≥ 3 placas), mostraron valores medios de 2,27, por lo que hipotetizamos que valores más bajos podrían ser clínicamente relevantes en esta población [548].

Este marcador ha sido poco estudiado en la población con DTI. Se dispone de unos pocos estudios pediátricos donde se ha relacionado con las complicaciones microvasculares [352,353] y la cetoacidosis diabética [557,558]. Fuera del contexto pediátrico, un estudio en 226 participantes asiáticos con DTI, objetivó niveles de RNL progresivamente mayores en función de la albuminuria en modelos univariantes [559]. Otro estudio, realizado en una pequeña muestra, halló una relación independiente entre el RNL y diversos parámetros de rigidez arterial en personas con DTI [354]. Es necesaria más evidencia en esta población antes de poder extrapolar el uso de esta razón a la práctica clínica. No obstante, la sólida evidencia de la relación del RNL con la ECV en población sin DTI, conjuntamente con nuestros resultados positivos en una gran muestra representativa de la población adulta con DTI de nuestro entorno, indican que podría ser un biomarcador útil y fácilmente implementable. Además, el tratamiento antiinflamatorio con canakinumab (inhibidor de la interleucina-1 β) reduce el RNL de forma dosis-dependiente, por lo que también podría ser de ayuda en el seguimiento y monitorización terapéutica [345].

En el octavo y último artículo de la presente tesis, mostramos como algunos parámetros inflamatorios avanzados (la razón altura/anchura de GlycA y GlycB), en contraposición a parámetros clásicos (leucocitos circulantes y PCRus), se asociaron con la presencia y número de placas carotídeas en aquellos que no tomaban estatinas [560].

En la línea del séptimo estudio [548], nuestra hipótesis fue que los parámetros capaces de aunar de manera más amplia la inflamación sistémica, como el RNL o el perfil avanzado mediante ¹H-RMN, podrían ser mejores herramientas que los que tan solo identifican una parte del proceso inflamatorio (e.g. PCRus o leucocitos circulantes). Por un lado, GlycA computa la señal de diversos de los principales reactantes de fase aguda, como la α 1-glicoproteína ácida, haptoglobina, α 1-antitripsina, α 1-antiquimotripsina y la transferrina [356]. Además, muestra una menor variabilidad intraindividual en comparación a los niveles de PCRus y se ha sugerido ser un mejor predictor del estado global de las citoquinas inflamatorias [356,561,562]. Por el otro lado, GlycB se propuso como

marcador hace relativamente pocos años, por lo que dispone de una menor cantidad de estudios clínicos. De la misma manera, las razones entre sus alturas y anchuras también han sido poco reportadas. La señal de GlycB proviene de los grupos acetilo el ácido N-acetilneuramínico (ácido siálico) de las glicoproteínas, aunque está peor caracterizada que GlycA [356,368,370,563]. La razón altura/anchura de GlycA y GlycB proporciona información relacionada no sólo con la concentración de glicoproteínas (altura), sino también con sus características termodinámicas y cinéticas, como la agregación y la flexibilidad estructural de las moléculas (anchura) [370,564]. Un cambio en la flexibilidad conformacional de las proteínas puede, por ejemplo, modificar su función, aumentando la vida media y dificultando su degradación proteolítica [565].

Si bien varios estudios, en personas sin DTI, han relacionado los niveles de GlycA con la ECV [358,359,566], tan solo uno ha comparado su capacidad predictiva [358]. En base a la cohorte del estudio MESA (n = 6523), *Duprez D et al*, compararon la capacidad predictora de ECV de varios parámetros inflamatorios [358]. Así pues, al añadir GlycA al resto de covariables clínicas y demográficas, se reclasificó el riesgo de ECV, hacia arriba o a hacia abajo, hasta un tercio de la muestra, sin mejoras sustanciales al adicionar también los niveles de PCRus, IL-6 y dímero-D [358]. Además, también mejoró la predicción de la mortalidad, cáncer y hospitalización o muerte por enfermedad inflamatoria crónica [358]. En cuanto a la aterosclerosis, un estudio llevado a cabo en Brasil en una gran muestra en prevención primaria, objetivó que GlycA se relacionó de forma independiente con la presencia de placas carotídeas, mientras que los niveles de PCRus no lo hicieron [567]. Con respecto a GlycB, *Cediel G et al*, mostraron una asociación entre sus niveles y la probabilidad de reingreso por insuficiencia cardíaca [568].

En personas con DTI, *Puig-Jové C et al*, objetivó un área y razón altura/anchura de GlycA significativamente mayor en los sujetos con disfunción miocárdica de una muestra danesa de 304 adultos [371]. Asimismo, estos dos parámetros fueron de los que más contribuyeron a explicar la presencia de disfunción miocárdica de entre una serie de covariables [371]. Otro estudio, realizado en nuestro ámbito en 84 participantes adultos con DTI sin ECV previa,

demostró una relación directa e independiente de las concentraciones de GlycA y las razones altura/anchura de GlycA y GlycB, con la velocidad de la onda del pulso aórtica [373]. Por otro lado, el área de GlycF, un marcador aún más novedoso, el cual surge de la concentración de los grupos acetilo en N-acetilglucosamina, N-acetilgalactosamina y N-acetilneuramínico que no están unidos a proteínas [569], se asoció linealmente con la presencia de placas carotídeas en mujeres con preeclampsia previa, independientemente de si asociaban DTI [374]. Además, al igual que en nuestro estudio, los marcadores clásicos (PCRus y leucocitos circulantes), no se asociaron. Sin embargo, las relaciones de los diversos parámetros inflamatorios con el GIM carotídeo en el grupo con DTI (n = 56), fueron inconsistentes [374]. Por último, un estudio llevado a cabo en cerca de 600 personas con diabetes (54% con DTI), no encontró que la adición del perfil avanzado de glicoproteínas (¹H-RMN) mejorase la predicción de la retinopatía diabética por encima de las variables clínicas y de laboratorio convencionales [372].

Un hallazgo que merece mención es la asociación de los parámetros inflamatorios avanzados únicamente en los que no estaban en tratamiento con estatinas [560]. Algunos tratamientos hipolipemiantes, como los inhibidores de PCSK9, pueden disminuir los niveles de varios de estos parámetros de forma modesta [570]. No obstante, el tratamiento con estatinas, por el momento, no ha demostrado modificarlos [571]. De hecho, nuestros datos no muestran diferencias en ningún parámetro inflamatorio acorde al tratamiento con estatinas, aunque tampoco para los niveles de PCRus, los cuales si suelen reducirse por el tratamiento [571,572]. Además, aquellos tratados con estatinas fueron de mayor edad y prevalencia de HTA, por lo que una explicación plausible sería que el inicio previo del tratamiento haya podido difuminar las asociaciones del estudio [560].

En resumen, la inflamación juega un papel central en la fisiopatología de la ECV y puede ser aún más trascendente en las personas con enfermedades autoinmunes, como la DTI. El RNL, fácilmente calculable a partir del hemograma, dispone de poca evidencia clínica, pero toda a favor de su utilidad. Además, dada su universal disponibilidad en clínicas y hospitales, no resultaría metodológicamente complicado analizar su capacidad predictiva a gran escala. Por

otro lado, el estudio avanzado de glicoproteínas inflamatorias mediante ^1H -RMN se encuentra unos pasos atrás de cara a su posible implementación en práctica habitual. Creemos que la principal ventaja de esta técnica es poder analizar conjuntamente múltiples parámetros (e.g. metabolómicos, inflamatorios y lipoproteicos), en muestras seleccionadas y suficientemente grandes. El análisis de la enorme cantidad de datos que de ello se pueda derivar, podría apoyarse en el uso de la inteligencia artificial para poder dictaminar qué parámetros puedan ser clínicamente relevantes.

6. Limitaciones y fortalezas

La principal limitación de los trabajos de la presente tesis es el diseño transversal el cual impide establecer causalidad y es proclive a múltiples sesgos. Sin embargo, la finalidad de esta tesis no es otra que la de abrir nuevas puertas y proponer una dirección en la que seguir investigando. En este sentido, los estudios transversales suelen ser el primer paso antes de plantear diseños observacionales y prospectivos. En segundo lugar, las muestras de los estudios donde se evaluaron las técnicas mediante ^1H -RMN fueron relativamente pequeñas ($n < 200$) teniendo en cuenta que se analizó la asociación de múltiples parámetros con la variable resultado. De todos modos, en los cálculos *post hoc*, la potencia de los estudios fue correcta (superior al 80%). En tercer lugar, los criterios de inclusión (edad ≥ 40 años, ERD y/o ≥ 10 años de evolución con otro FRCV adicional) circunscriben la extrapolación de nuestros resultados al subgrupo de personas con DTI y alto RCV. Cabe destacar que la población adulta con DTI atendida habitualmente en el territorio nacional, aunque más joven y con menor duración de la diabetes, es parecida en cuanto a los niveles de IMC o la prevalencia de otros FRCV, como la HTA, el tabaquismo o las complicaciones microvasculares [573–575]. Por ello, creemos que nuestros resultados deberían poder reproducirse de manera similar en poblaciones menos seleccionadas.

Una de las fortalezas destacadas de este trabajo es la claridad con la que se establecen sus objetivos, lo que permite una estructura ordenada y dirigida. Esta

precisión en la definición del propósito de los estudios, facilita una ejecución metódica y coherente, asegurando que cada paso del proceso de investigación esté alineado con las metas planteadas. En segundo lugar, el uso de la presencia de placas carotídeas determinadas mediante ecografía como variable resultado, un contrastado *proxy* de ECV [576], aporta mayor solidez a los resultados. En tercer lugar, la novedad de los hallazgos permite explorar nuevas vías para poder aportar soluciones a esta vulnerable y poco estudiada población. Por último, la mayoría de los trabajos que componen esta tesis han contado con un número de participantes elevado ($n = 401 - 728$), incrementando la validez interna y el poder estadístico de los resultados.

Estamos convencidos de que los resultados de esta tesis resultan especialmente relevantes por estar focalizada en resolver un problema clínico imperante, detectar correctamente a las personas con diabetes tipo 1 de mayor riesgo cardiovascular y poder, en consecuencia, individualizar la estrategia preventiva. Este enfoque práctico y orientado a la traducción clínica es, sin duda, el objetivo principal de las investigaciones médicas.

Las recomendaciones prácticas para el día a día que se pueden extraer del manuscrito son:

- 1) Debemos abandonar la perspectiva glucocéntrica imperante, así como los sistemas de clasificación del riesgo cardiovascular que no han sido diseñados para las personas con diabetes tipo 1.
- 2) Los biomarcadores y factores que se pueden recoger fácilmente (razón neutrófilos / linfocitos, antecedentes obstétricos, grado de adherencia a la dieta mediterránea) deben ser considerados para su implementación en la práctica diaria. Asimismo, conviene realizar esfuerzos para educar y reforzar la incorporación de hábitos dietéticos acordes al patrón mediterráneo.
- 3) Incorporar algún método de cribado de la aterosclerosis subclínica es de gran ayuda para reclasificar el riesgo cardiovascular, tanto hacia arriba, como hacia abajo, en la población adulta con DTI.

Esta tesis representa un punto de partida para el diseño de futuros estudios. Por ello, se proponen algunos pasos que creemos que se deben dar para proseguir la investigación en este campo. En primer lugar, es ya el momento de plantear estudios prospectivos con datos de progresión de la aterosclerosis y/o con variables cardiovasculares duras, para evaluar varios de los parámetros que se han ido proponiendo. Por el trasfondo científico previo y a la luz de nuestros hallazgos, los principales candidatos serían el antecedente de preeclampsia, la adherencia a la dieta mediterránea y la razón neutrófilos / linfocitos circulantes. Todos ellos ampliamente disponibles y de bajo coste tanto económico, como en el tiempo que consume recogerlos. En última instancia, como se ha comentado anteriormente, los estudios mediante técnicas más costosas, como la espectroscopia por resonancia magnética nuclear presentan una elevada variabilidad de muchos de los parámetros capaces de determinar y permiten extraer una gran cantidad de datos. En este sentido, las técnicas avanzadas deben estandarizarse para permitir la comparabilidad entre estudios y llevarse a cabo en muestras muy bien seleccionadas para facilitar la interpretabilidad de los datos, la cual se podría apoyar en el uso de herramientas de aprendizaje automatizado.

IX. Conclusiones

En la presente tesis se ha querido evidenciar la poca utilidad de las herramientas genéricas de predicción de eventos cardiovasculares en la población adulta con diabetes tipo 1. Tras ello, se han analizado una multitud de posibles marcadores y/o factores de riesgo cardiovascular y su relación con la presencia de aterosclerosis subclínica, determinada mediante un método confiable, contrastado y práctico, como la ecografía carotídea. De manera resumida, se concluye:

- 1) Entre las herramientas para detectar la aterosclerosis subclínica, la puntuación del calcio aorto-coronario es la que parece más útil para predecir eventos cardiovasculares en personas con diabetes tipo 1. No obstante, las lesiones más precoces pueden pasar desapercibidas con su uso. La ecografía carotídea es una técnica poco costosa, aplicable en los estadios iniciales de la aterosclerosis y que permite caracterizar dichas lesiones.
- 2) La escala de predicción cardiovascular genérica propuesta por las guías europeas de prevención cardiovascular de 2019 (ESC/EASD-2019) no identificaron correctamente a aquellos que albergaban placas carotídeas. En contraposición, la ecuación *Steno Type 1 Risk Engine*, específica de la población con diabetes tipo 1, demostró un buen rendimiento discriminativo.
- 3) Una mayor adherencia a la dieta mediterránea restringida en calorías se relaciona con una menor aterosclerosis carotídea. El consumo de frutas, nueces, legumbres y carne blanca en vez de roja parecen ser los hábitos más saludables en este sentido.
- 4) El antecedente de preeclampsia y la paridad se asocian a una mayor prevalencia y carga aterosclerótica en comparación a las mujeres nulíparas. A pesar de que su inclusión no mejore el rendimiento de la ecuación *Steno Type 1 Risk Engine*, los factores obstétricos, sobre todo la preeclampsia, deben ser tenidos en cuenta al establecer una estrategia preventiva.
- 5) El perfil avanzado de lipoproteínas, lipidómica y glicoproteínas inflamatorias mediante espectroscopia por resonancia magnética nuclear,

es una herramienta prometedora capaz de dar una perspectiva amplia de múltiples procesos patológicos. Algunos parámetros relacionados con el número de partículas aterogénicas e inflamatorios podrían ser especialmente útiles en esta población.

6) La razón entre los neutrófilos y los linfocitos circulantes se asocia sólidamente con la presencia y el número de placas carotídeas. La implementación de este sencillo parámetro podría ayudar en la fenotipación del riesgo cardiovascular de las personas adultas con diabetes tipo 1.

X. Bibliografia

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