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Original article

Healthy dietary pattern and their corresponding gut microbiota profile are linked to a lower risk of type 2 diabetes, independent of the presence of obesity

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SUMMARY

Background: Prediabetes and old age are both high risk factors for developing Type 2 Diabetes (T2D), while obesity is one of the most important factors triggering the disease. Nutritional interventions are the most effective tool for preventing T2D, as they improve different biochemical and anthropometric outcomes and growth-promoting/inhibiting gut microbiota populations. However, to date there are no specific dietary recommendations to stop the development of T2D in elderly groups, for whom hypocaloric diets and other commonly used weight-loss programs could be considered dangerous. The objective of our study, thus, was to understand the impact of dietary patterns on T2D risk as related to gut microbiota profile in obese and non-obese elderly prediabetic subjects.

Methods: A cross-sectional study was performed in 182 subjects ≥65 years old with prediabetes, divided into obese (OB) or non-obese (NOB) subgroups, and their risk of developing T2D was measured according to FINDRISK score and biochemical parameters. Also, clusters into different dietary patterns in each group by PCA analysis was related with gut microbiota, which was analyzed from stool samples by qPCR. The creation of clusters was used to re-evaluate T2D risk.

Results: OB was at higher risk of developing T2D and showed worse metabolic outcomes. Unhealthier and healthier dietary pattern clusters were observed for both OB (OB-6 and OB-5 respectively) and NOB (NOB-2 and NOB-3 respectively) groups. Results obtained from the gut microbiota showed that only Prevotella was higher in NOB, but when comparisons were made between clusters, a clear relation with dietary pattern was observed; showing in healthier dietary clusters a decrease in Prevotella, an increase of Faecalibacterium prausnitzii and an increase in lactic acid bacteria. T2D risk was greater in the obese group between unhealthier dietary clusters. No difference between healthier dietary clusters was observed.

Conclusion: A healthy dietary pattern and the growth-promoting beneficial and growth-inhibiting disadvantageous gut microbiota populations linked to it provide protection against the development of T2D in an obese population with advanced age and preDM.

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1. Introduction

Prediabetes (preDM) is defined as a state that precedes type 2 diabetes (T2D), which, together with its complications, is a major cause of early death in most countries [1].

Moreover, preDM, characterized by fasting blood glucose levels of 100–125 mg/dL [2], has been recognized as clinically relevant due to its association with metabolic syndrome and cardiovascular disease (CVD) [3]. Alongside T2D and obesity, preDM has become prevalent worldwide [1]. The natural history of preDM predicts that up to 70% of individuals with this condition will develop T2D, with the annual conversion ratio in prediabetic population around 10% [4,5]. However, the number of new-onset T2D in individuals ≥65 years old particularly stands out, increasing 4.5-fold as compared to 3-fold in total population [6].

The prevalence of T2D increases with age but also with the presence of obesity [7], as the risk of developing T2D at 65 years old increases >25% with a BMI ≥30 kg/m^2 [8].

Clinical guidelines recommend weight loss for the prevention of T2D through lifestyle modifications but fail to include specifications for old age [2]. The effects of weight loss in the elderly have been under debate, as some epidemiological studies have shown that it leads to increased mortality [9,10]. This is probably due to the unintentional weight loss of concretely fat-free or lean mass loss that also occurs [11], which is associated with various health problems. Among these is increased insulin resistance [12] and, with it, an increased risk of developing T2D.

Generally, nutritional interventions used for weight loss are based on hypocaloric diets that can lead to malnutrition in the elderly [13]. A more interesting intervention in this age group would be one based on changing dietary patterns instead of focusing on caloric restrictions, but first it is essential to know which dietary patterns are present, information that is currently missing for this population.

Meanwhile, in addition to the known effects that a specific diet can produce on different clinical parameters such as glycaemia, impact on gut microbiota has also been established. Diets provide a range of growth-promoting and growth-inhibiting factors for specific bacterial phylotypes that ferment different substrates [14]. Unhealthy dietary habits have been found to produce dysbiosis, which, in turn, can affect metabolism and increase susceptibility to different diseases. Gut microbiota has been suggested to play an important role in the development of T2D, in conjunction with or independently of obesity [15].

We hypothesize that elderly obese-prediabetic subjects are at higher risk of developing T2D than non-obese ones but that a healthy diet will be linked to a better gut microbiota profile, therefore, obese subjects who show beneficial dietary and gut microbiota pattern will have a lower risk of developing T2D.

2. Methods

This study was a cross-sectional study developed in three primary care centers in Barcelona (CAP Les Corts, Borrell and Casanova) from 2014 to 2016.

2.1. Participants

Subjects included, from whom informed consent was obtained for experimentation and study was performed in accordance with The Code of Ethics of the World Medical Association, were males and females ≥65 years old with glucose levels between 100 and 125 mg/dL and without a previous diagnosis of diabetes. Subjects were excluded from the study if they were taking antidiabetic drugs or had previously received any nutritional education to improve their blood glucose levels or were taking antibiotics.

Based on the clinical records of the primary care centers, a list of 929 patients who met the inclusion criteria was created. 403 were contacted at random, of which 195 were cited for a screening appointment. A final total of 182 subjects were selected for inclusion in the study.

All participants provided written informed consent, and the study protocol was approved by the Ethics Committee of Hospital Clinic de Barcelona.

2.2. Measures

- Anthropometrics: Subjects were weighed and measured without clothing and shoes. BMI was calculated as weight (kg)/height (m)^2, and waist circumference was measured at the midpoint between the last rib and the iliac crest and hip circumference at the widest point of the gluteus.
- Body composition was measured using Lunar iDXA body composition (GE Healthcare) according to the standard operating protocol.
- Blood pressure was taken after individuals sat quietly for 5 min in a clinical examination room. The mean of three different measurements, obtained every 3 min using an OMRON M6 AC sphygmomanometer, was recorded.
- Fasting blood samples were collected by a nurse from each primary care center and were sent, refrigerated at 4 °C, to the central laboratory of Hospital Clinic i Provincial de Barcelona, where they were analyzed by the Biomedical Diagnosis Centre (CDB).
- T2D risk: The Finnish Diabetes Risk Score (FINDRISK) questionnaire was completed according of the creation groups of “low”, “medium”, “high” and “very high” risk of developing T2D.
- Lifestyle habits: Physical activity was estimated using VREM, a short questionnaire adapted from the Minnesota Leisure Time Physical Activity Questionnaire for individuals of advanced age. Nutritional patterns were measured using a 3-day dietary record revised corrected by a nutritionist and analyzed by the DIAL nutritional calculation program.
- Gut microbiota: Genomic DNA was extracted from fecal samples using the PSP® Spin Stool DNA Plus kit (Stratec, Berlin, Germany). Fecal samples were collected by the volunteers, immediately frozen and bring to the laboratory in less than 4 hours and were kept at −80°C until analysis. Gut microbiota composition was determined by quantifying the abundance of target bacterial indicators by quantitative PCR (qPCR), namely *Firmicutes, Bacteroidetes, Faecalibacterium prausnitzii (F. prausnitzii), Escherichia coli (E. coli), Prevotella,* and lactic acid bacteria. These indicators were chosen based on previous association with T2D or preDM. Total bacteria quantification was also performed in order to calculate relative abundances of the indicators and to normalize data. 16S rRNA targeted primers, probes, standards for quantification (16S rRNA gene inserted in a pCR®4-TOPO® cloning plasmid and linearized with NotI), and PCR conditions are detailed in Supplementary Table S2. Standard curves, inhibition controls and non-template controls were included in each run, and samples were run in duplicate. A 7500 Real Time PCR system was used for all quantifications. Data is given as the Log10 of the ratio between 16S rRNA gene copies of the target microorganism and total bacterial 16S rRNA genes detected in the same sample.
- Statistical analysis: Descriptive data are presented as the mean and standard deviation (SD) or median and interquartile ranges (IQRs) for continuous outcomes, or number and percentage (%) for categorical outcomes. Non-parametric Mann-

Whitney U test was used for the comparison of continuous outcomes when normality and equality of variance could not be assumed. Student's t test was used for the rest of continuous outcomes and Chi-square test for categorical outcomes. Nutrient pattern analysis was performed using Principal Component Analysis (PCA) based on the intake of 169 nutrients divided into monounsaturated, polyunsaturated, saturated fatty acids, cholesterol and carbohydrates (starches and sugars). In order to capture variability of nutrient intake independently, two separate PCA were performed for obese and non-obese subjects. False discovery rate (FDR) correction was used for multiple comparisons. All significance tests were 2-tailed, and values of $P < 0.05$ were considered significant. All analyses were conducted using the R V.3.0.3 for Windows statistical software package.

3. Results

Our recruited subjects were 49.7% female and 50.3% male. Their mean age was 71.5 ± 5.4 years, while the mean duration of PreDM was 4.7 ± 3.4 years.

T2D risk was calculated, with 86.18% of subjects showing a "high" or "very high" risk of developing the disease, according to FINDRISK test. Since one of the most important risk factors was body weight, participants were divided into two groups according to whether they were obese (OB) or not (NOB), with 45.6% of them presenting a BMI $\geq$30 kg/m^2.

Clinical characteristics of both groups were similar, with the exception of antithrombotic drug intake, which was more frequent in the OB group, along with systolic (SBP) and diastolic (DBP) blood pressure, which were also higher in the OB group. (Table 1).

Table 1
Characteristics from each group.

	NOB (n=99)	OB (n=83)	P-value
Sex , % female	43.4	56.6	0.104
Age, year	71.15 (4.59)	71.67 (6.04)	0.33
Personal history			
Evolution IFG, years	4.77 (3.36)	4.78 (3.46)	0.976
Family history DM, % cases	36.4	43.4	0.417
Hypertension, % cases	56.6	71.1	0.062
CVD, % cases	12.1	22.9	0.084
Dyslipidemia, % cases	63.6	51.8	0.144
Drugs			
Antihypertension, % consumers	58.6	72.3	0.076
Antithrombotic, % consumers	21.2	38.6	**0.016**
Cholesterol-lowering drugs, % consumers	49.5	37.3	0.135
Gastric protector, % consumers	24.2	24.1	1
Thyroid hormone therapy, % consumers	8.1	15.7	0.173
Antidepressant, % consumers	16.2	24.1	0.249
Bronchodilators, % consumers	4	8.4	0.354
Opioid analgesics, % consumers	10.1	14.5	0.503
Other, % consumers	62.6	74.7	0.114
Toxic habits			
Smoking, % cases	6.1	8.4	0.366
Previous smoking, % cases	52.5	42.2	
Occasional drinking, % cases	58.8	53	0.177
Daily drinking, % cases	24.7	19.3	
Blood pressure			
Systolic blood pressure, mmHg	131.76 (16.32)	140.69 (19.74)	**0.002**
Dyastolic blood pressure, mmHg	77.98 (9.53)	81.84 (9.52)	**0.009**
Blood chemistry			
HbA1c, %	6.02 (0.43)	6.21 (0.47)	**0.006**
Glucose, mg/dL	104.04 (12.76)	110.21 (13.8)	**0.002**
Insuline, mU/L	13.97 (5.92)	19.39 (8.49)	**<0.001**
Total cholesterol, mg/dL	197.23 (34.11)	202.45 (31.79)	0.29
Cholesterol- HDL, mg/dL	49.94 (11.25)	52.39 (14.51)	0.212
Cholesterol-LDL, mg/dL	125.69 (29.4)	127.95 (26.55)	0.594
Tryglicerides, mg/dL	113.4 (60.12)	116.45 (47.21)	0.703
AdipoQ, μg/mL	10.89 (5.45)	11.15 (5.72)	0.762
Anthropometrics			
Weight, kg	73.06 (9.92)	86.74 (11.83)	**<0.001**
BMI, kg/m^2	26.62 (2.06)	33.54 (3.65)	**<0.001**
Waist, cm	95.2 (8.91)	107.51 (10.17)	**<0.001**
Hips, cm	102.42 (5.78)	114.75 (8.76)	**<0.001**
Percentage approximation ideal weight, %	120.63 (9)	151.81 (16.75)	**<0.001**
Body composition	**NOB (n=51)**	**OB (n=37)**	**P-value**
Centile fat mass, index	85.25 (18.5)	94.38 (7.64)	**0.002**
Fat mass total, %	37.8 (5.05)	44.44 (6.17)	**<0.001**
Fat mass total, gr	26263.12 (4691.9)	36422.38 (5538.48)	**<0.001**
Fat mass, torso %	42.31 (4.87)	49.69 (5.83)	**<0.001**
Fat mass in abdomen, %	47.68 (5.63)	54.58 (6.05)	**<0.001**
Fat mass in hips, %	40.42 (7.93)	47.61 (9.57)	**<0.001**
Lean mass, total %	56.69 (6.12)	53.40 (5.98)	**<0.001**

Values are expressed as mean (SD) for quantitative data, and mean % of subjects for frequency variables. Statistically significant differences are highlighted in boldface.
No differences were observed in the general characteristics of the group in terms of age, sex, personal history or toxic habits. Regarding the consumption of drugs, only antithrombotics showed to be higher in the OB group. OB also demonstrated greater blood pressure, anthropometrics measures, fat mass and fat distribution in abdomen, torso and hips. Worse blood chemistry values were observed in OB, with higher HbA1c, glucose, insulin. NOB= non-obese, OB=obese, IFG= impaired fasting glycemia, DM= diabetes mellitus, HbA1c=standardized glycosylated hemoglobin NGS, AdipoQ= adiponectine, BMI= body mass index.

As expected, OB showed higher weight, body mass index (BMI) and waist and hip circumference. Ideal weight was calculated by Lorentz formula, and the percentage of approximation to it was more distant for OB (Table 1).

Total fat amount and the accumulation of fat in different parts of the body, as well as the centile in which they were classified, were higher in the OB than in NOB group. On the other hand, OB showed a lower percentage of lean mass (Table 1).

The mean of the percentage risk of developing diabetes according to FINDRISK showed a significant difference ($p < 0.001$) between groups: 40.17 ± 8.45 in the OB group and 32.40 ± 12.20 in the NOB group. Specifically, the percentage of individuals classified as having a "high" or "very high" risk of developing T2D was different ($p < 0.001$): 100% of OB participants and 77.78% of NOB participants.

OB was associated with significantly higher glucose, HbA1c and insulin (Table 1). When homeostatic model assessment for insulin resistance (HOMA-IR) was calculated, OB showed a higher index than NOB (5.37 ± 2.73 and 3.68 ± 1.85 respectively, $p < 0.001$), and for beta-cell function (HOMA-β), OB also showed a stronger compensatory effect of pancreatic function than NOB (155.06 ± 70.59 and 127.5 ± 45.81 respectively, $p = 0.003$).

3.1. Lifestyle habits

The percentage of sedentariness, classified according FAO/WHO/UNU [16], in the OB group was higher than in the NOB group, at 61.2% and 40.65%, respectively ($p = 0.01$). Moreover, OB expended a lower energy expenditure practicing physical activity than the NOB group, as calculated by METS (272.1 ± 184.7 and 330.2 ± 192.4 respectively, $p = 0.05$) and by Kcal (721.7 ± 554.9 and 537.4 ± 386 respectively, $p = 0.02$).

Harris-Bennedict formula was used to evaluate daily energy expenditure with adjustments made for elderly and overweight or obese conditions, as described previously [17]. Total daily energy expenditure was lower in OB as compared to NOB (1167.71 ± 201.62 and 1402.11 ± 187.50 respectively, $p < 0.001$).

Analyses of 3-day dietary records revealed that the energy intake was similar in NOB and OB groups (1992.9 ± 564.6 and 1958.44 ± 503.9 Kcal/day, respectively), however, although both groups ingested more Kcal than they expended per day, OB showed a greater excess because their daily energy expenditure was lower (Fig. 1).

As for total calories consumed (TCC), both groups followed a diet rich in lipids (>35% of TCC) and poor in carbohydrates (<45% of TCC). Although both groups consumed a low percentage of carbohydrates, they exceeded for the recommendation for simple sugars (>10% of TCC) [18,19]. Furthermore, in terms of lipid consumption, both groups consumed an excess of saturated fatty acids (SFA) (>7% of TCC) but a correct proportion of unsaturated fatty acids (UFA) to polyunsaturated fatty acids (PUFA) (<10% of TCC), and monounsaturated fatty acids (MUFA) (10–20% of TCC) [2].

Differences between groups also appear in nutrient consumption (Table 2). NOB showed a lower intake of cholesterol and a higher intake of PUFA, especially omega 3 fatty acids (PUFA ω3); in particular, NOB consumed more linolenic acid but also more linoleic acid, an omega 6 fatty acid (PUFA ω6). For their part, OB showed less protein consumption (gr/Kg weight).

Other nutrients were considered in the analyses (data not shown), revealing a better quality diet in NOB due to a higher consumption of micronutrients with antioxidant properties, such as phytosterols ($p = 0.038$), available organic acid ($p = 0.011$), luteine ($p = 0.034$), citric acid ($p = 0.015$) and tocopherols ($p < 0.005$).

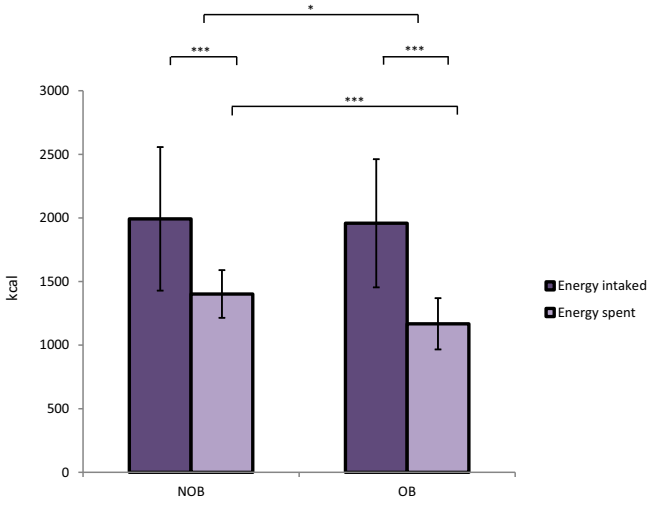

Fig. 1. Energy balance from each group comparing energy intake recorded from dietary records and energy expended ,calculated taking into consideration basal energy expenditure and physical activity. While no difference in energy intake was observed, the NOB group expended more energy than OB. The difference between energy expended and energy ingested was different in each group and also between groups, being higher in OB. NOB= on-obese, OB=obese.

3.1.1. Clusters

Nutrient pattern analysis by means of PCA showed that participants were distributed among six clusters (Supplementary Figure S1).

From them, four different dietary patterns were clearly identified on the first principal planes (Fig. 2), with two different subpatterns observed in each group. NOB showed, on the one hand, subpattern NOB-2, based on amino acids (A) and SFA and MUFA (B), and, on the other, subpattern NOB-3 based on PUFA, in particular PUFA ω3 (C), antioxidant nutrients (D) and based on minerals and purines (E). On the contrary, the OB group showed subpattern OB-5 based on foods rich in PUFA (F), vitamin B and some minerals (G), antioxidant nutrients (H) and based on other minerals and purines (I). The remaining subpattern of this group, OB-6, was characterized by amino acids (J), other antioxidant nutrients (K), B-group

Table 2
Nutrient intake differences between groups.

	NOB (n=86)	OB (n=64)	P-value
Carbohydrates, kcal % diet	39.42 (6.61)	38.8 (6.42)	0.553
Simple sugar, gr	78.26 (27.67)	80.04 (29.65)	0.963
Starch, gr	86.53 (33)	87.11 (34.61)	0.967
Soluble fiber, gr	4.96 (1.92)	4.69 (1.98)	0.341
Insoluble fiber, gr	9.52 (4.15)	8.85 (3.77)	0.301
Lipids, kcal % diet	40.88 (5.99)	40.12 (6.02)	0.599
Cholesterol, gr	278.13 (148.15)	308.1 (115.51)	**0.03**
SFA, %	11.82 (2.49)	11.5 (2.6)	0.367
MUFA, %	19.3 (4.02)	19.43 (4.14)	0.527
PUFA, %	6.28 (1.79)	5.61 (1.42)	**0.008**
PUFA ω6, gr	11.01 (5.04)	9.71 (4.06)	0.115
Linoleic acid C18:2, %	4.82 (1.49)	4.35 (1.3)	**0.031**
PUFA ω3, gr	2.8 (1.61)	2.23 (1.26)	**0.012**
Linolenic acid C18:3, %	0.82 (0.43)	0.67 (0.28)	**0.003**
Proteins, kcal % diet	17.04 (2.97)	17.86 (3.24)	0.22
Proteins, gr/Kg weight	1.07	0.97	**0.004**

Values are expresses as mean (SD). Statistically significant differences are highlighted in boldface.
No differences were observed in the consumption of carbohydrates and total lipids. Only for one of three macronutrients, OB showed a lower consumption of proteins considering gr of protein/Kg of weight. The OB group also demonstrated a higher intake of cholesterol and less PUFA, linoleic acid, PUFA w3 and linolenic acid. NOB= non-obese, OB=obese, SFA= saturated fatty acids, MUFA= monounsaturated fatty acids, PUFA=polyunsaturated fatty acids, ω6= omega 6, ω3=omega.

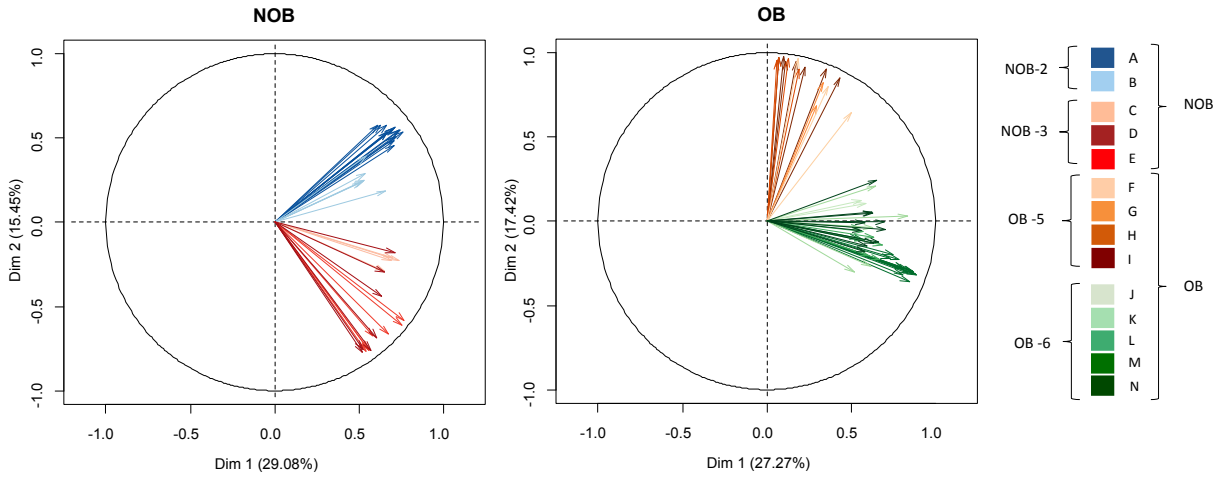

Fig. 2. PCA analysis of different nutrients found in each dietary pattern explaining the 30% of NOB and the 51% of OB in dimensions 1 and 2. NOB= on-obese, OB=obese. Groups of nutrients: A: Alanine, Serine, , Arginine, Glycine, Proline, Tyrosine, Histidine, Isoleucine, Lysine, Methionine, Threonine, Tryptophane, Phenylalanine, Leucine, Valine. B: Palmitoleic C16:1, Palmitic C16:0, Oleic C18:1 cis 9, Lipids, MUFA, SFA. C: PUFA, PUFA ω6, PUFA ω3, Linoleic C18:2. D: Vegetable fiber, Vit. E, Vit. K, TRAP, Oxalic acid, Tocopherol total, α - Tocopherol. E: Nickel, Chrome, Fluor, Magnesium, Potassium, Adenine, Guanine. F: Linolenic C18:3, PUFA ω3. G: Calcium, Vit. B2, Iron. H: Vit K, TRAP, Oxalic acid, Vegetable fiber. I: Nickel, Chrome, Fluor, Magnesium, Potassium, Adenine, Guanine. J:Alanine, Serine, , Arginine, Glycine, Proline, Tyrosine, Histidine, Isoleucine, Lysine, Methionine, Threonine, Tryptophane, Phenylalanine, Leucine, Valine. K: Fiber, Insoluble fiber, Soluble fiber, Vit. E, Tocopherol total, α-Tocopherol, Folates. L: Vit. B1, Vit. B9, Vit. B7, Vit. B3, Coline. M: PUFA, PUFA ω6, Linoleic C18:2, SFA, Stearic C18:0, FA cis, Palmitoleic C16:1, Palmitic C16:0, Oleic C18:1 cis 9, Lipids, MUFA, SFA. N: Carbohidrates, Simple sugar.

vitamins (L), and PUFA, specifically omega 6, but also SFA and MUFA (M) and carbohydrates, especially simple sugars (N). The two remaining clusters (NOB-1 and OB-4), did not show any clear dietary pattern when focusing on 6 dimensions. Clusters NOB-3 and OB-5 were similar in nutrient composition, while NOB-2 and OB-6 also showed very similar patterns.

3.2. Microbiota

Prevotella relative abundance was three times higher in NOB than in OB subjects (p = 0.047). No statistically significant differences were found in the abundance of the other bacterial groups analyzed comparing obese and non-obese subjects (Supplementary Table S1).

3.2.1. Clusters

Differences for microbiota population were observed according to the four previously described clusters of dietary patterns (Table 3).

When comparing these four clusters, no significant differences were found. However, in NOB-2, *Prevotella* load tended to be higher than in any of the other clusters (p = 0.052), and also a trend towards differences in *F.prausnitzii* abundance were observed between clusters (p = 0.059) (Table 3 and Supplementary Figure S2). Within OB, those in the OB-5 cluster had a higher load of both lactic acid bacteria (p = 0.053) and *F. prausnitzii* (P = 0.042) than subjects in cluster OB-6. In contrast, within NOB, the NOB-2 cluster tended to have a higher load of *Prevotella* than cluster NOB-3, but without reaching statistical significance (P = 0.073).

More similarities were observed between clusters NOB-3 and OB-5 and between clusters NOB-2 and OB-6 than between clusters within the same group (NOB-2 and NOB-3, OB-5 and OB-6).

3.3. T2D risk

3.3.1. Clusters

Once we observed the differences among the 4 clusters in terms of dietary patterns and their effects on gut microbiota, we studied if these differences might play a role in the risk of developing T2D.

The risk percentage of developing T2D varied among the four clusters (p = 0.005), however, the differences did not come from comparisons between clusters within the same group (NOB-2 and

Table 3
Comparison of microbiota populations between clusters selected in dietary patterns described.

	NOB -2 (n=22)	NOB -3 (n=5)	OB -5 (n=8)	OB -6 (n=24)	P-value[a]	P-value[b]	P-value[c]
Firmicutes	206 [126.7–370.2]	199.1 [34.33–210.1]	193.1 [135.5–227.5]	146.1 [102.9–478.3]	0.960	0.625	0.902
Bacteroidetes	42.17 [15.09–63.51]	54.06 [34.29–67.62]	26.34 [20.06–35.53]	37.61 [17.06–66.31]	0.585	0.454	0.418
Prevotella	2.261 [0.181–10.91]	0.026 [0.025–0.055]	0.035 [0.022–0.703]	0.052 [0.014–1.927]	**0.052**	**0.073**	0.902
Lactic acid bacteria	0.077 [0.026–0.235]	0.366 [0.006–0.586]	0.31 [0.068–19.56]	0.056 [0.027–0.162]	0.292	0.819	**0.053**
Faecalobacterium prausnitzii	28.78 [11.78–61.96]	93.6 [23.39–108.2]	104.1 [54.44–189.9]	26.69 [11.36–79.25]	**0.059**	0.204	**0.042**
Escherichia coli	0.188 [0.06–2.323]	0.127 [0.072–1.306]	0.872 [0.328–10.32]	0.141 [0.033–1.862]	0.550	0.672	0.177
Firmicutes/Bacteroidetes ratio	7.73 [3–11.38]	3.11 [1.3–5.81]	7.48 [3.76–19.085]	6.25 [2.065–18.3]	0.406	0.111	0.539
Faecalibacterium prausnitzii/ Escherichia coli ratio	101.04 [26.68–619.1]	106.98 [71.64–184.43]	213.02 [34.715–264.74]	175.59 [11.28–1525.895]	0.922	0.536	0.825

Values are concentrations expressed as median [IQRs]. Statistically significant differences are highlighted in boldface.
No differences when comparing the four clusters were observed, with the exception of two differences found in the significance level limit for Prevotella and Faecalibacterium prausnitzii. Also, only one difference was observed in Prevotella when comparing clusters from the NOB group. A significant difference was seen in Faecalibacterium prausnitzii and a small one in lactic acid bacteria when comparing clusters from the OB group.
NOB= non-obese, OB=obese.
[a] Comparison among 4 clusters.
[b] Comparison between clusters 2 and 3.
[c] Comparison between clusters 5 and 6.

NOB-3 clusters with p = 0.916, OB-5 and OB-6 clusters with p = 1000).

We then looked at the individuals classified as having a "high" or "very high" risk of T2D and compared the clusters that were similar in dietary patterns. We observed that NOB-3 and OB-5 presented a similar T2D risk classification (p = 0.19), therefore, despite the difference in BMI, there was no greater risk of developing T2D in the obese group. In contrast, comparing OB-6 to NOB-2 showed more individuals in this "high" and "very high" T2D risk classification (p = 0.003), which means that obesity plays an important role in T2D risk when the quality of diet and intestinal microbiota are poorer.

In agreement with these results, we observed that insulinemia, as well as the compensation of β-cell function measured by HOMA-β, was not different between NOB-3 and OB-5 but was higher in OB-6 with respect to NOB-2 (p = 0.001, p = 0.005 respectively).

4. Discussion

Obesity is associated with an array of health problems and its prevalence clearly shows a gradual increase of impaired glucose metabolism, a finding that supports the important role of obesity in the development of T2D [11]. One particular study showed that an increase in BMI raised the incidence of T2D by 25% in the general population [12].

On the contrary, the relative risk of many diseases associated with a greater BMI declines with age [20]. Nevertheless, absolute mortality and health complications associated with obesity increase linearly with a corresponding increase in BMI until the age of 75 years [20]. Beyond the age of 80 years, the association between BMI and mortality becomes weakened, primarily because the elderly may have a low body weight due to unintentional weight loss, a common complication of many serious diseases, which could confound the interpretation of weight loss effects on mortality, and also due to sarcopenia, a decrease in lean mass which often accompanies functional limitations and poor health outcomes [21].

Obesity, even so, has a negative impact on older persons with an impaired health status [22]. In particular, we found that T2D risk also increases with obesity in the elderly, along with a worsening of many parameters such as levels of glucose, HbA1c and insulin, but also HOMA-IR and HOMA-β. With these results, we can also relate an increased risk of developing other diseases associated with T2D which are driven by the combination of insulin resistance and a pro-inflammatory state [23], such as CVD [24].

The relation between energy intake and expenditure is an important determinant of obesity. Therefore, the increase in total fat mass that occurs with aging must be attributable to an increase in energy intake, a decrease in energy expenditure, or both.

Physical activity reduces with age, and it has been estimated that it accounts for about one-half of the decrease in total energy expenditure that occurs with aging [25]. Therefore, it is likely that a decrease in energy expenditure is an important contributor to the gradual increase in body fat observed with age. Both groups in our study were characterized by lower energy expenditure than found in a group of people ≥60 years old described in a previous study [26]. Also, we found that the OB group performed less physical activity than the NOB group, in accordance with previous results in which BMI was found to be inversely related to measured physical performance in older persons [27].

In the last decade, sedentary behavior has emerged as a new risk factor for health [28].

Findings from a study in the UK reported that objectively measured sedentary time increased with age [29]. Indeed, our study OB group spent more time performing sedentary activities, which has been associated with higher plasma glucose [26]. In addition, excess body fat mass and a BMI of ≥30 in older individuals have been associated with physical dysfunction and are predictive of a decline in functional status and future disability [30].

The elderly prediabetic population in our study showed an elevated food intake in relation to daily energy expenditure. The calories they ingested were higher than in a previous study performed in the same region, Catalonia-Spain, in healthy individuals aged between 65 and 75 years [31]. In addition, according to the American Diabetes Association (ADA), healthy low-calorie eating patterns should be recommended for patients at a high risk of developing T2D [2].

The percentage of TCC from carbohydrates ingested by our population did not exceed recommendations [18,19]. Nevertheless, the percentage of TCC from simple sugars was above the recommendations [18,19], which is described that, independently the mechanisms by which the sugar intake might promote weight gain by increasing energy consumption to an extent that exceeds energy output and distorts energy balance [32], the increased consumption of simple sugar increase hyperinsulinaemia [33]. In addition, total fiber was less than previously described in same region population [30,34], and soluble fiber in particular was below the recommendations [35,36].

The percentage of TCC from lipids was higher than the recommendations [34–36], but recent evidence suggests that quality of fats is prior than quantity [37].In this regard, dietary patterns characterized by a low intake of SFA and a higher intake of UFA are protective and promote a beneficial effect on insulin sensitivity [38]. In our study, comparing to recommendations, for both groups ingested SFA was greater [36], but amounts of MUFA and PUFA were close to it [35]. Specifically, the intake of PUFA ω3, in particular, linolenic acid, which have been associated to an improvement to cardiovascular health [39,40], was higher in the NOB group. Meanwhile, the intake of cholesterol was greater in the OB group, exceeding the recommendations [35], which has been associated with an increase in the incidence of CVD.

The quantity and also the percentage of TCC from proteins were correct in both OB and NOB groups, but when taking body weight into consideration, the OB showed lower intake than NOB group. A modest increase in protein assists in achieving and maintaining weight reduction and also helps to minimize loss of lean mass [36].

As for micronutrients, different parameters related to antioxidant power consumed were lower for the OB group. Among them, less intake of tocopherol, which a cross-sectional study found to have an inverse correlation with fasting plasma insulin concentrations [41], luteine, which the same study found inversely correlated with insulin resistance [42], and others such as organic and citric acids, which are very present in plant-based foods. In fact, green leafy vegetables, which are the most rich in these nutrients, demonstrated a capacity to reduce the risk of developing T2D, as an increased consumption of them was associated with a decrease in the incidence of T2D [42].

Subsequently, two different dietary patterns were observed in each group. Subgroups NOB-2 and OB-6 showed similar nutrient intake patterns rich in amino acids and B-group vitamins, SFA and MUFA, but pattern from the subgroup OB-6 was characterized to involved also the consumption of PUFA ω6, carbohydrates, and, especially, simple sugar. Another similar nutrient intake pattern was observed between subgroups NOB-3 and OB-5 characterized by the consumption of PUFA ω3, antioxidant nutrients, minerals and purines, but while the former was rich in PUFA ω6, the latter was rich in B-group vitamins and some minerals.

The effect of obesity on elderly prediabetic subjects seems to not have an impact on gut microbiota composition. Only *Prevotella* load, previously associated with Mediterranean diet and plant-based

diets [43] and with a polyphenols-rich diet [44], was higher in NOB. This is related to a higher consumption of PUFA and antioxidant nutrients such as polyphenols [45], which corresponded to the differences observed in nutrient intake between NOB and OB.

Previous studies have documented changes in the gut microbiota taken from subjects with obesity or certain dietary patterns and analysed for bacterial load indicators. Since no major shifts in gut microbiota have been observed in our cohort, our results could suggest that in these population groups nutrient intake has a stronger effect than obesity in modulating the composition of gut microbiota.

Interestingly, differences were observed between clusters of patients based on dietary patterns. *Prevotella* was found to be especially elevated in the NOB-2 cluster following a more unhealthy diet. These bacteria have been found in elevated concentrations in T2D patients [46] and in prediabetic populations following a diet rich in fat and protein [47]. But, on the other hand, this genus has been positively correlated with a higher consumption of oleic acid and inversely correlated with animal protein and animal fats [48]. Concretely, *Prevotella* is link with the Mediterranean diet probably due to consuming plant-foods rich in lipids of the MUFA type, such as olive oil and nuts, while the amino acid and SFA dietary patterns probably indicate a non-animal origin, thereby creating similarities with the dietary patterns found in the NOB-2 group.

Prevotella appeared even slightly higher in the OB-6 cluster, but the reason for this could also be due to a diet rich in carbohydrates and simple sugars [49], suggesting that high proportions of these bacteria can lead to obesity in high-caloric Western diets [50]. This could explain the lower load of lactic acid bacteria found in this cluster in comparison with OB-5, due to the dietary patterns of OB-6 should be linked with Western diets rich in animal protein and fat and low in fibre which have demonstrated a decrease in these bacteria load [51]. In contrast, the OB-5 cluster shows a very high load of lactic acid bacteria, which has been related with a fish-oil rich diet in mice [52] and can be linked to the dietary pattern of this cluster through PUFA ω3. A high load of this bacterial group has also been observed in a lactose-rich diet [53], which can be also linked to the presence of vitamin B2 and specific minerals such as calcium and iron, among others, in this cluster. Different lactic acid bacteria have been related with an improvement in glucose metabolism and a reduction in chronic inflammation [54] and have been found in reduced quantities in patients with T2D and obesity [55]. Finally, *F. prausnitzii* was higher in NOB-3 and OB-5. The dietary patterns of both subgroups were rich in PUFA ω3, antioxidant nutrients and were compounded for the same minerals and purines. These results can be linked with previously studies where an increase in these bacteria was found due to a diet rich in fruit, legumes and some vegetables [56–58]. Also, *F. prausnitzii* has been demonstrated to decrease with hydrolysed complex carbohydrates [59], which could translate into the carbohydrates appearing in the dietary pattern of OB-6 that come from simple carbohydrates found in ultra-processed foods [60]. A possible association between changes in this species of bacteria and insulin sensitivity has been previously suggested [61–63], and depletion of this species has been reported in patients with T2D [64–66]. Supporting the influence of *F. prausnitzii* on T2D, a study analysing *F. prausnitzii* profiles in obese subjects with and without T2D has suggested that differences in this species phylotypes may lead to differences in inflammatory status in the host, thus having an influence on disease development [65]. Furthermore, its anti-inflammatory properties have been consistently demonstrated [66].

We have shown in our study that individuals from a specific group of elderly people with preDM and obesity present a higher risk of developing T2D and worse metabolic outcomes. However, in those individuals who present a healthier dietary pattern and gut microbiota, the presence of obesity does not appear to modify insulinemia or the compensatory function of β cells, nor does it increase the risk of developing T2D.

Therefore, we can conclude that a healthy dietary pattern and the growth-promoting beneficial and growth-inhibiting disadvantageous gut microbiota populations linked to it provide protection against the development of T2D in an obese population with advanced age and preDM.

Clinical trial

This study was registered in clinicaltrials.gov with identifier number: NCT03557541.

Statement of authorship

DA Díaz-Rizzolo and R Gomis designed the study. DA Díaz Rizzolo and C Colungo performed clinical visits. DA Díaz Rizzolo and A Serra collected samples and codified data. M López-Siles and M Martinez–Medina were responsible for microbiota extraction and determination. B Kostov Formal analysis participated in all data analysis. DA Díaz-Rizzolo, A Sisó-Almirall and R Gomis were responsible for interpretation the results. All of the authors participated in the critical revision of the manuscript. All authors gave final approval of the version to be submitted.

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Appendix A. Supplementary data

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