The gut microbiota metabolism of pomegranate or walnut ellagitannins yields two urolithin-metabotypes that correlate with cardiometabolic risk biomarkers: Comparison between normoweight, overweight-obesity and metabolic syndrome

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Abbreviations: ApoA-I, apolipoprotein A-I; ApoB, apolipoprotein B; CVD, cardiovascular diseases; EA, ellagic acid; ETs, ellagitannins; IDL, intermediate-density lipoprotein;

LC-MS, liquid chromatography-mass spectrometry; MetS, metabolic syndrome; ODMA, O-desmethylangolesin; PCA, principal component analysis; SCFAs, short chain fatty

acids; TMAO, trimethylamine-N-oxide; UM-A, Urolithin metabotype A; UPLC-ESI-qToF-MS, ultra performance liquid chromatographyeelectro spray ionizationequadrupole time of flight- mass spectrometry.

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30 **Keywords:**

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37

38 **SUMMARY**

39 *Background & aims:* Urolithins are microbial metabolites produced after consumption of
40 ellagitannincontaining foods such as pomegranates and walnuts. Parallel to isoflavone-metabolizing
41 phenotypes, ellagitannin-metabolizing phenotypes (urolithin metabotypes A, B and 0; UM-A, UM-
42 B and UM-0, respectively) can vary among individuals depending on their body mass index (BMI),
43 but correlations between urolithin metabotypes (UMs) and cardiometabolic risk (CMR) factors are
44 unexplored. We investigated the association between UMs and CMR factors in individuals with
45 different BMI and health status. Methods: UM was identified using UPLC-ESI-qToF-MS in
46 individuals consuming pomegranate or nuts. The associations between basal CMR factors and the
47 urine urolithin metabolomic signature were explored in 20 healthy normoweight individuals
48 consuming walnuts (30 g/d), 49 healthy overweight obese individuals ingesting pomegranate extract
49 (450 mg/d) and 25 metabolic syndrome (MetS) patients consuming nuts (15 g-walnuts, 7.5 g-
50 hazelnuts and 7.5 g-almonds/d). Results: Correlations between CMR factors and urolithins were
51 found in overweight-obese individuals. Urolithin-A (mostly present in UM-A) was positively
52 correlated with apolipoprotein A-I ($P < 0.05$) and intermediate-HDL-cholesterol ($P < 0.05$) while
53 urolithin-B and isourolithin-A (characteristic from UM-B) were positively correlated with total-
54 cholesterol, LDL-cholesterol ($P < 0.001$), apolipoprotein B ($P < 0.01$), VLDL-cholesterol, IDL-
55 cholesterol, oxidized-LDL and apolipoprotein B:apolipoprotein A-I ratio ($P < 0.05$). In MetS patients,
56 urolithin-A only correlated inversely with glucose ($P < 0.05$). Statin-treated MetS patients with UM-
57 A showed a lipid profile similar to that of healthy normoweight individuals while a poor response to
58 lipid-lowering therapy was observed in MB patients. Conclusions: UMsare
59 potentialCMRbiomarkers. Overweight-obese individuals withUM-B are at increased risk of
60 cardiometabolic disease, whereas urolithin-A production could protect against CMR factors. Further
61 research is warranted to explore these associations in larger cohorts and whether the effect of
62 lipidlowering drugs or ellagitannin-consumption on CMR biomarkers depends on individuals' UM.

63 **1. Introduction**

64 The gut microbiota is increasingly regarded as a key mediating factor in the development of obesity
65 and cardiometabolic disorders [1]. Emerging evidence also indicates a potential relation between gut
66 microbiota and certain chronic disease risk factors [1]. Previous reports correlate some gut microbial-
67 derived metabolites such as trimethylamine-N-oxide (TMAO), short-chain fatty acids (SCFAs) and
68 hippurate with cardiovascular disease (CVD) risk in animal models [2,3]. Whether the real factor that
69 correlates with CVD risk is the specific microbial metabolite and/or the gut microbial environment
70 capable of producing such metabolite is not entirely understood. In this regard, the potential role of
71 gut microbial metabolism of isoflavones in cardiometabolic risk has been previously suggested [4e6].
72 Indeed, the cardiometabolic risk seems to be different depending on individuals' isoflavone-
73 metabolizing phenotype, e.g. specific gut microbial environments capable of daidzein
74 biotransformation to yield the metabolites O-desmethylangolesin (ODMA) and/or equol [5,7].

75 Somehow parallel to the characteristic biotransformation of isoflavones, ellagitannins (ETs) and
76 ellagic acid (EA) are also metabolized by the gut bacteria to give up some specific metabolites so-
77 known as urolithins [8]. This metabolism has been reported in humans and different mammals after
78 consumption of ETs and EA rich foods such as some berries (strawberries, raspberries and others),
79 pomegranates, walnuts and oak-aged wines, among others [8,9]. These bioavailable metabolites,
80 mainly urolithin-A, exert antiinflammatory activities in vitro [10] and in vivo [8,11,12]. Urolithins
81 were proposed as biomarkers of dietary ETs consumption [9] and had been identified as potential
82 contributors to the favorable effects against CVD attributed to the consumption of pomegranates and
83 other ET-containing foods [13]. As in the case of isoflavones, there is a considerable interindividual
84 variability in the bioconversion of ETs to urolithins, which depends on the intestinal microbiota
85 composition leading to three ETs-metabolizing metabotypes: 'urolithin metabotype A' (UM-A; only
86 urolithin-A conjugates are produced), 'urolithin metabotype B' (UM-B; in addition to urolithin-A,
87 isourolithin-A and/or urolithin-B are produced), and 'urolithin metabotype 0' (UM-0; urolithins are
88 not produced) [14]. Although the three urolithin metabotypes (UMs) have been confirmed in different
89 studies, weight gain and diseases linked to gut microbial imbalance (dysbiosis) favor the growth of

90 bacteria able to produce isourolithin-A and/or urolithin-B rather than urolithin-A-producing bacteria
91 [15]. Gordonibacter species (spp.) have the ability to transform EA into different urolithins in pure
92 culture [16,17] and are positively correlated with urolithin-A in feces and urine, whereas occurrence
93 of isourolithin-A and/or urolithin B are inversely correlated with fecal concentration of Gordonibacter
94 spp. [18]. Higher plasmatic levels of urolithin-A recently have been described in individuals with less
95 severe metabolic syndrome (MetS) traits because inverse correlations with both abdominal adiposity
96 and impaired glycemic control were observed [19]. However, other critical urolithins and their
97 association with relevant cardiometabolic risk blood lipid biomarkers have not yet been analyzed. To
98 pursue the hypothesis supported by previous studies [14,15,18,19], we aimed to the study of UMs as
99 metabolomic signatures involved in cardiometabolic risk. In this regard, we evaluated the association
100 between UMs, BMI, glycemia, and serum lipoprotein-lipid profiles. Both healthy normoweight and
101 overweight-obese groups of subjects were compared with MetS patients.

102 **2. Materials and methods**

103 *2.1. Intervention studies and study products*

104 Human nutritional intervention studies were conducted in line with the Helsinki Declaration. The
105 design of the trials including eligibility criteria for participants was previously reported in detail
106 [18,20,21] (Fig. 1). In the first trial, healthy normoweight group (n = 20, 9 women and 11 men; BMI
107 < 25 kg/m2) consumed 30 g per day of unpeeled walnuts for 3 days [18] (Fig. 1A). In the second trial
108 (NCT01916239), overweight-obese healthy individuals (n = 49, 17 women and 32 men; BMI > 27
109 kg/m2) without drug treatment, ingested 1 daily capsule of pomegranate extract (450 mg/day) for 3
110 days. Capsules were kindly provided by Laboratorios Admira S.L. (Spain) [18] (Fig. 1B). In the third
111 intervention study (ISRCTN36468613), 50 volunteers (28 men and 22 women; 35 with statins and
112 15 without statins) with at least three MetS risk factors as defined by the Adult Treatment Panel III
113 [20,21] were recruited in a prospective, randomized, controlled, parallel-designed and 12- week
114 intervention feeding trial. Nut diet group (n = 25, 15 men and 10 women) consumed 30 g/d of raw

115 unpeeled mixed nuts (15 g of walnuts, 7.5 g of hazelnuts and 7.5 g of almonds) (Fig. 1C). The nuts
116 used in the study were donated by Borges S.A., Reus, Spain.

117 As our aim was to correlate blood cardiometabolic risk biomarkers with UMs, blood samples were
118 collected before (baseline) the consumption of ET-containing foods, whereas both 24 h-urine and
119 feces were collected after consuming the products (in order to stratify the volunteers according to
120 their UMs). Total volume of urine was measured and the samples (urine and feces) were stored at -
121 80 ºC until they were analyzed. Blood samples were taken and rapidly centrifuged at 2,000g for 10
122 min at 4 ºC. Serum and plasma EDTA aliquots were separated and stored at -80ºC until they were
123 analyzed.

124 *2.2. Biochemical measurements*

125 Basal serobiochemical parameters were determined using automated biochemical auto-analyzers
126 [21,22]. A quantitative determination of insulin was carried out using an IMMULITE 2000 analyzer
127 (DPC, Los Angeles, California, USA). Fasting glucose and insulin values were used to calculate
128 insulin resistance using the HOMA-IR method [22]. Non-HDL-cholesterol was calculated as total-
129 cholesterol minus HDL-cholesterol. Apolipoprotein A-I and B (ApoA-I and ApoB) were analyzed
130 by immunonephelometry (Dade Behring, BN II, Marburg, Germany). HDL-cholesterol and
131 LDLcholesterol subfractions were measured with a Lipoprint System (Quantimetrix, Inc., Redondo
132 Beach, CA, USA). Densitometric reading and conversion to concentrations of lipoprotein classes and
133 subclasses were carried out using the Lipoware software. We grouped HDL particles into ‘large’,
134 ‘intermediate’ and ‘small’ species and LDL into ‘large’ and ‘small’ particles. Cholesterol
135 concentrations were determined [23]. VLDL-cholesterol and intermediate density lipoprotein (IDL)
136 cholesterol concentrations were also obtained with the same assay system. The manufacturer's
137 supplied reagents were used in each analysis.

138 *2.3. Chemicals*

139 Urolithins with a purity higher than 95% were obtained as described before [24].

140 *2.4. Urolithin extraction and liquid chromatography-mass spectrometry (LC-MS) analysis*

141 Urine and feces were extracted as described before [24]. Briefly, urine samples (1 mL) were vortexed,
142 centrifuged at 14,000g for 10 min at 4 ºC and the supernatant was filtered by using 0.45 mm PVDF
143 filter. Fecal samples (1 g) were homogenized in 10 mL of methanol/DMSO/water (40/40/20) + 0.1%
144 HCl, centrifuged at 3500g for 10 min and finally the supernatantwas filtered by using 0.45 mm PVDF
145 filter. Ultra performance liquid chromatography-electro spray ionization-quadrupole time of flight-
146 mass spectrometry (UPLC-ESI-qToF-MS) was used for samples analysis as previously reported [24].

147 *2.5. Bacterial DNA extraction and real-time qPCR*

148 Powerfecal® DNA isolation kit (Mo-Bio Laboratories, Carlsbad, CA USA) was used according to
149 the manufacturer's instructions for bacterial genomic DNA extraction. For Gordonibacter spp.
150 quantification by real-time qPCR, specific primers were used as previously described [15,18].

151 *2.6. Statistical analysis*

152 SPSS v. 19.0 (SPSS Inc, Chicago, USA) was used for statistical analyses. Comparisons among groups
153 and UMs were carried out by a variance model (ANOVA) with the Bonferroni post hoc test. The
154 association between two variables was measured with the Spearman correlation. Kendall correlation
155 was used for the pairs, where isourolithin-A + urolithin-B concentration was one of the two variables.
156 Statistical significance was recognized when $P < 0.05$. Discriminant principal component analysis
157 (PCA) and plotted of groups were done using R with the github package.

158 **3. Results**

159 *3.1. Serum lipids, glycemia and UMs*

160 Lipid and lipoprotein profile, plasma glycemia and urinary and fecal excretion of urolithin-A and
161 isourolithin-A + urolithin-B after dietary ETs consumption were quantified in healthy normoweight,
162 healthy overweight-obese and MetS groups (Table 1). Healthy overweight-obese individuals were
163 considered in the same group because they showed a similar lipid profile (Fig. 2A). Totalcholesterol

164 values were similar among healthy normoweight, healthy overweight-obese and MetS groups (Table
165 1). Similar levels of triglycerides, total-cholesterol, LDL-cholesterol, non-HDLcholesterol and LDL-
166 cholesterol:HDL-cholesterol ratio were also found in both healthy overweight-obese and MetS
167 groups. Only in MetS individuals using statins as lipid-lowering therapy (n = 14), LDL-cholesterol
168 levels (3.24 mmol/L) were significantly lower (P = 0.045) than those in the overweight-obese group
169 and similar to those of the normoweight group (data not shown). HDL-cholesterol concentrations
170 were also lower in MetS than in overweight-obese individuals. Only plasma glucose, triglycerides:
171 HDL-cholesterol and the total-cholesterol: HDL-cholesterol ratios were significantly higher in MetS
172 than in overweight-obese individuals (Table 1). PCA of the current results identified two components
173 accounting for 75% of the total variance. The first principal component (PC1) explained 49% of the
174 variance and was determined by total-cholesterol, non-HDL-cholesterol and LDL-cholesterol. The
175 second component (PC2) explained 26% of the variance and was represented mostly by HDL-
176 cholesterol, Gordonibacter spp. and glucose. The PCA plot illustrated that PC2, but not PC1, clearly
177 differentiated between overweight-obese and MetS groups (P < 0.001) (Fig. 2A). Furthermore, fecal
178 Gordonibacter spp. concentration positively correlated with HDL-cholesterol (r = 0.221; P = 0.032)
179 and negatively with both plasma glucose (r =-0.320; P = 0.003) and VLDL-cholesterol levels (r =
180 0.238; P = 0.027). On the other hand, both PC1 (P = 0.006) and PC2 (P = 0.027) discriminated among
181 UMs (Fig. 2B). The differences in some serum markers were accentuated when normoweight and
182 MetS individuals were compared. In this case, plasma glucose, triglycerides, HDL-cholesterol, non-
183 HDLcholesterol, as well as total-cholesterol:HDL-cholesterol, triglycerides:HDL-cholesterol, and
184 LDL-cholesterol:HDLcholesterol ratios were statistically different between both groups (Table 1).
185 Some differences between healthy normoweight and overweight/obese groups were also observed in
186 serum markers, mainly in LDL-cholesterol, HDL-cholesterol concentrations and LDL-
187 cholesterol:HDL-cholesterol ratio (Table 1). The percentage of individuals with UM-B was higher in
188 MetS (41%) than in overweight-obese (31%) and normoweight groups (20%) (Table 1). In contrast,
189 the percentage of individuals with UMA was lower in those individuals with MetS (50%) than in
190 overweight-obese (57%) and normoweight (70%) groups.

191 *3.2. Correlation between baseline serum markers and urolithins*

192 Healthy overweight-obese subjects with dyslipidemia but not subjected to any medical treatment
193 were further investigated. Oxidized-LDL, ApoA-I, ApoB and cholesterol concentrations in various
194 lipid subfractions from LDL and HDL were analyzed (Table 2). The main urolithins excreted in urine
195 of overweightobese individuals after pomegranate extract consumption correlated with serum lipid
196 markers (Table 2). Likewise, differences in serum lipid markers among UMs were also significant
197 (higher levels of total-cholesterol, LDL-cholesterol and non-HDLcholesterol were observed in UM-
198 B than in UM-A in overweightobese individuals) (Fig. 3). In MetS individuals, the same tendency
199 was observed, but the differences were only significant in those individuals under statin treatment
200 (Fig. 3). Isourolithin-A + urolithin-B concentration in urine was positively correlated with total-
201 cholesterol ($P < 0.001$), LDL-cholesterol ($P < 0.001$), oxidized-LDL ($P < 0.05$), small LDL-
202 cholesterol ($P < 0.05$), large LDL-cholesterol ($P < 0.05$), ApoB ($P < 0.01$), VLDL-cholesterol ($P <$
203 0.05), IDL ($P < 0.05$) and ApoB:ApoA-I ratio ($P < 0.05$), while urinary urolithin-A was positively
204 correlated with ApoA-I and intermediate HDL ($P < 0.05$) in healthy overweight-obese individuals
205 (Table 2). In MetS patients, urine urolithin-A levels only correlated inversely with plasma glucose
206 ($r=-0.537$, $P < 0.05$) and glucose with total-cholesterol ($r = 0.313$, $P < 0.05$). No correlation was found
207 between urine isourolithin- A + urolithin-B and plasma glucose or serum markers (data not shown).
208 Fecal Gordonibacter concentration was positively correlated with urinary excretion of urolithin-A
209 and negatively correlated with isourolithin-A + urolithin-B. However, serum lipid markers were not
210 significantly correlated with fecal Gordonibacter concentration when healthy overweight-obese
211 individuals were separately analyzed (Fig. 4). Correlation among serum lipids was also analyzed (Fig.
212 4). In general, a positive correlation was demonstrated between total-cholesterol and LDL-
213 cholesterol, oxidized- LDL, LDL-cholesterol subfractions, non-HDL-cholesterol, small HDL-
214 cholesterol, IDL and ApoB. In contrast, HDL-cholesterol positively correlated with large HDL-
215 cholesterol and ApoA-I ($P < 0.001$) and negatively correlated with HOMA-IR ($P < 0.05$) and insulin
216 ($P < 0.05$) (Fig. 4).

217 **4. Discussion**

218 Measurements of lipoprotein-lipid profiles together with blood pressure and glucose levels have been
219 traditionally used to identify individuals at high risk of CVD [25]. However, the emerging role of the
220 gut microbiota in CVD risk is giving rise to the development of new predicting CVD tools [26]. In
221 this regard, we report here for the first time that ellagitannin-metabolizing phenotypes (e.g. UM-A or
222 UM-B from gut microbiota) are useful as potential cardiometabolic risk biomarkers. There is
223 increasing evidence that links UMs with gut dysbiosis and health status [15,18,19,27]. However, the
224 possible association between urine urolithin metabolites and cardiometabolic risk had not yet been
225 explored. In the present study, the percentage of individuals with UM-B was higher in overweight-
226 obesity and even more in those individuals with MetS. Notably, overweight-obese individuals with
227 UM-B were at increased CVD risk, whereas UM-A seemed to be a protective metabotype against
228 cardiovascular risk factors. Fecal Gordonibacter spp. levels were previously identified to be higher
229 in UM-A individuals than in those with UM-B [15], which could be implicated in the protective effect
230 of the UM-A. Our results on UMs and cardiometabolic risk are somehow parallel to those reported
231 for isoflavones-metabolizing metabotypes. Similarly, Frankenfeld et al. [28] theorized that daidzein
232 metabotypes could be useful as new biomarkers for evaluating disease risk. They found that the so-
233 called ODMA-producer metabotype, but not the equol-producer metabotype, was related with obesity
234 in adults [5]. Recently, Hazim et al. [7] showed that the vascular benefits associated to the equol-
235 producer metabotype were related to the specific gut microbial environment rather than to the direct
236 effect exerted by the metabolite equol. In the present study, we examined the correlation between
237 UMs and a panel of serum lipids that provide an additional CVD risk criterion beyond classical target
238 LDL levels. For example, it is known that persons treated with statins and target LDL levels, but with
239 high either non-HDL or ApoB levels, remain at increased CVDrisk [25].We observed that differences
240 between UM-A and UM-B, and total cholesterol and LDL-cholesterol values were significant only
241 in overweight-obese individuals and MetS group under statin treatment but not in the MetS group
242 free of statins. This is interesting and suggests that lipid-lowering treatments could be more effective

243 in UM-A individuals. In overweight-obese individuals, additional serum markers such as oxidized-
244 LDL, ApoB and ApoA-I, which are increasingly used to provide optimal management of patients at
245 moderate to increased CVDrisk [25,29],were also measured. Positive associations between several
246 CVDrisk biomarkers (total-cholesterol, LDL-cholesterol, oxidized-LDL, small LDL-cholesterol,
247 large LDL cholesterol, ApoB, VLDL-cholesterol, IDL and ApoB:ApoA-I ratio) and the excretion of
248 isourolithin-A + urolithin-B were identified. ApoB is a more precise measure of the total atherogenic
249 particles concentration than LDL-cholesterol concentration, since ApoB molecules are not only
250 carried by LDL, but also by other atherogenic lipoproteins such as VLDL and IDL [25]. In the present
251 study, ApoB concentration was highly correlated with non-HDL-cholesterol levels and according to
252 previous research, it may help to identify coronary heart disease risk in a subpopulation of individuals
253 with normal cholesterol levels [30]. The ratio of proatherogenic (ApoB) to antiatherogenic (ApoA-I)
254 apolipoproteins (ApoB:ApoA-I) has been strongly associated with ischemic heart disease risk, even
255 among individuals with a low total-cholesterol:HDL-cholesterol ratio [30]. Decreased LDL particle
256 size is another CVD risk biomarker that is independent of LDL concentration [30]. Similarly, persons
257 with low levels of either HDL-cholesterol or ApoA-I (the principal apolipoprotein on HDL particles)
258 are also likely to experience CVD events, in spite of having normal LDL-cholesterol levels [25]. In
259 the present study, urolithin-A was positively correlated with ApoA-I ($P<0.05$) in healthy overweight-
260 obese individuals. Some reports attribute to ApoA-I rather than to the overall number of HDL
261 particles or their cholesterol contents, the anti-inflammatory and antioxidant properties of HDL
262 particles [25]. It is known that inflammation participates in the link between obesity and metabolic
263 diseases. In this regard, production of urolithin-A, a metabolite with strong in vivo anti-inflammatory
264 activity could counteract the low-grade pro-inflammatory state associated with obesity and MetS.
265 Therefore, as recently reported for equol [7], further research is warranted to elucidate the direct
266 and/or indirect benefit associated to urolithin-A production in preventing CVD. Overall, our results
267 suggest that healthy overweight-obese individuals with UM-B are at increased risk of
268 cardiometabolic disease. Metabolic syndrome patients with UM-A and under statin treatment had a
269 lipid profile like that of the healthy normoweight group while a poor response to lipid-lowering

270 therapy was observed in UM-B patients. Therefore, in the new era of 'personalized nutrition', 271 stratification of subjects in relation to their UMs could supply an additional tool for CVD risk 272 assessment that must be replicated in large cohorts. In addition, further research is warranted to 273 explore whether the impact of lipid-lowering therapy or dietary interventions with ellagitannin-274 containing foods such as pomegranate or walnuts on cardiometabolic risk factors differs according to 275 individuals' UMs.

276 **Contribution authors**

277 JCE and MVS: designed the study; AGS, JSS and CAL recruited the volunteers and conducted the 278 trials; AGS and FATB: performed UPLC-qToF analyses; CA, AO and JSS: performed serum lipids, 279 insulin, glucose and HOMAIR determinations; MVS: performed RTqPCR assays and contributed to 280 the statistical analysis of data; MVS and JCE: wrote the manuscript; and AGS, JSS, CAL, CA, AO 281 and FATB: critically reviewed the manuscript for important intellectual content. All authors read and 282 approved the final manuscript.

283 **Conflict of interest**

284 None of the authors had a conflict of interest.

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370 FIGURES

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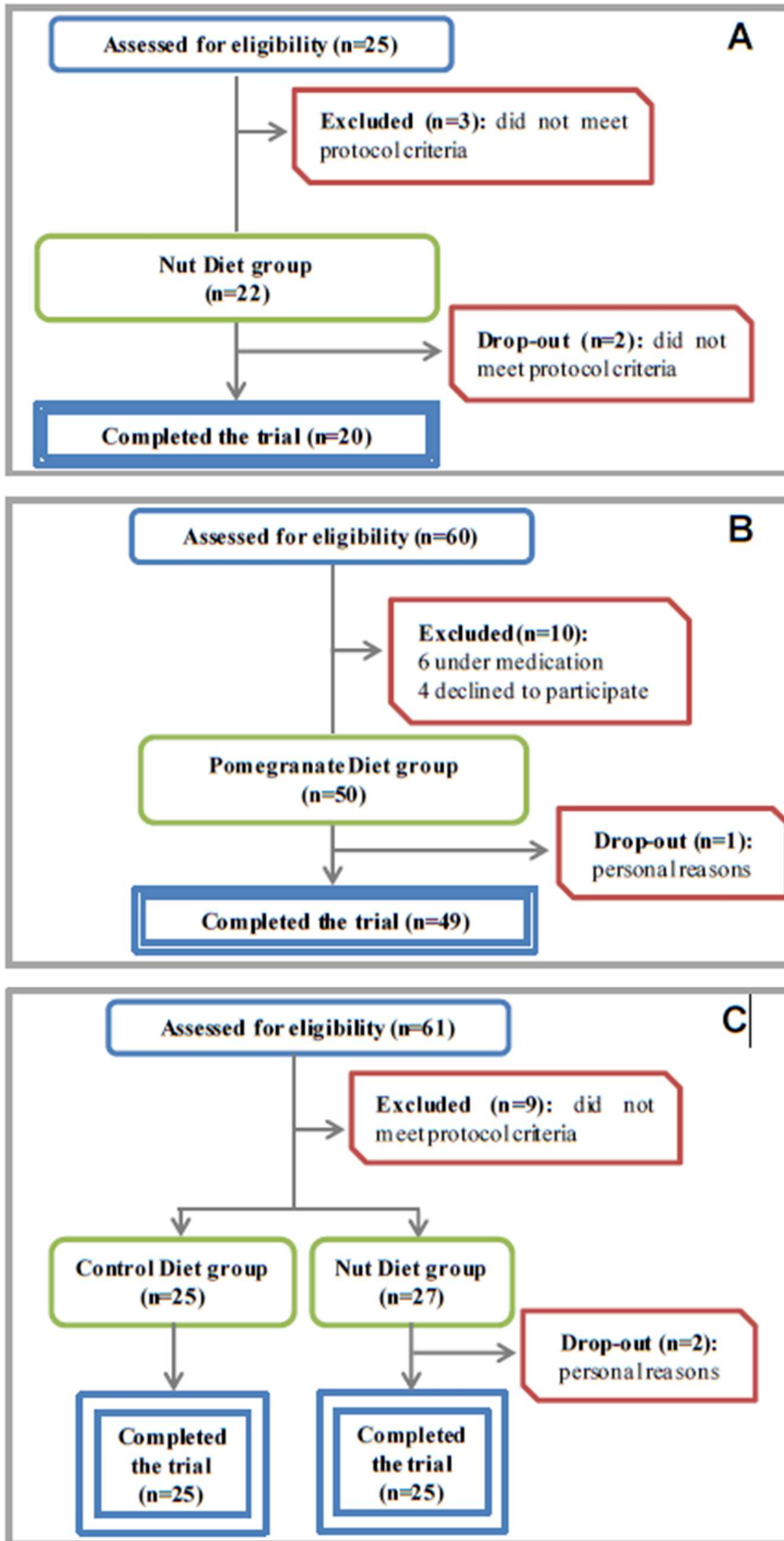

Fig. 1. Study design. Flow of participants through the trial of healthy normoweight (A) healthy overweight-obese (B) and MetS groups (C).

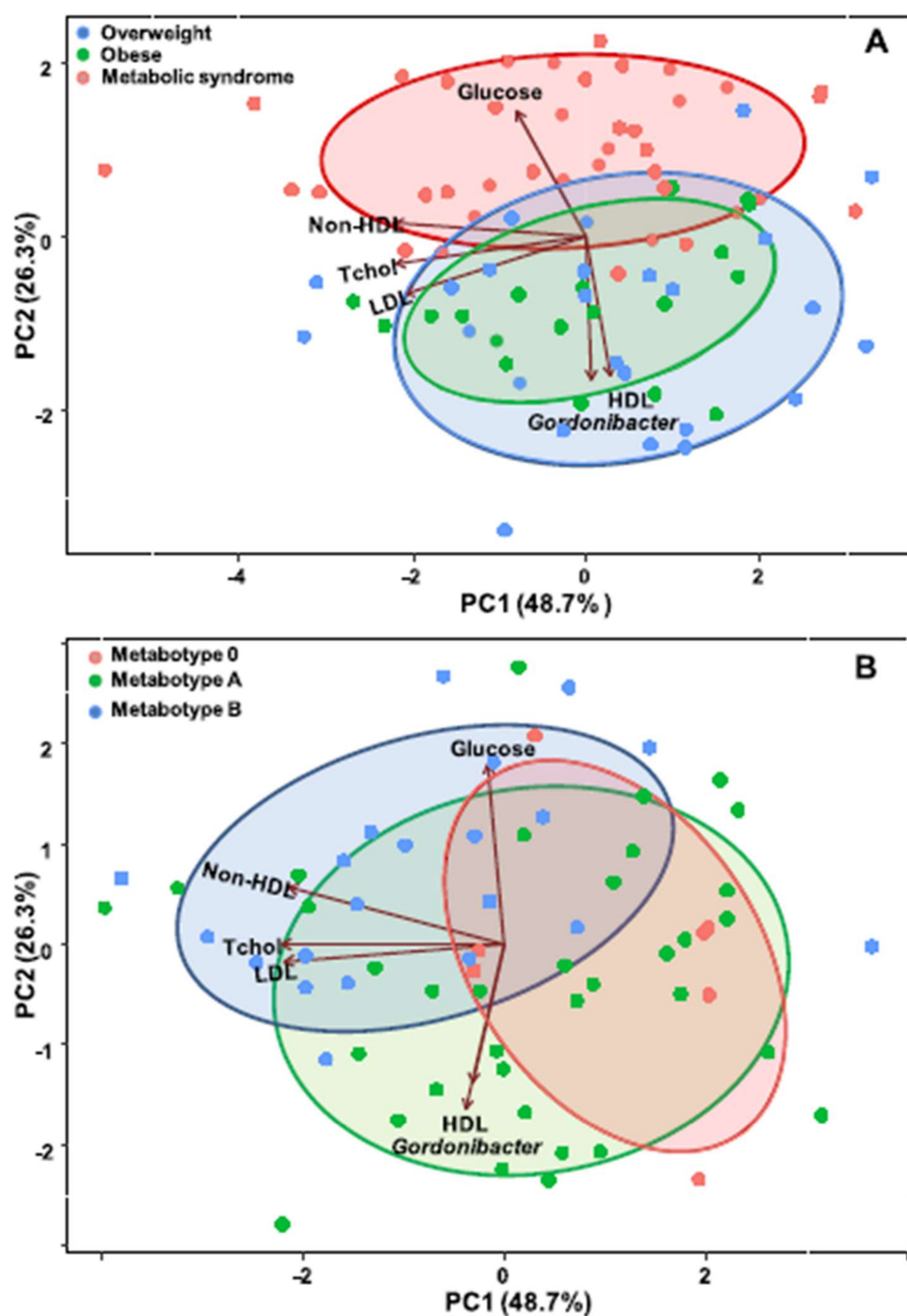

Fig. 2. Variations in serum markers and fecal concentrations of *Gordonibacter* spp. in the healthy overweight-obese and MetS groups represented by PCA. Plot analysis of overweight-obese versus MetS groups (A). Plot analysis of overweight-obese and MetS groups separated by UMs (B). HDL, HDL-cholesterol; LDL, LDL-cholesterol, Non-HDL, non-HDL-cholesterol; Tchol, total cholesterol.

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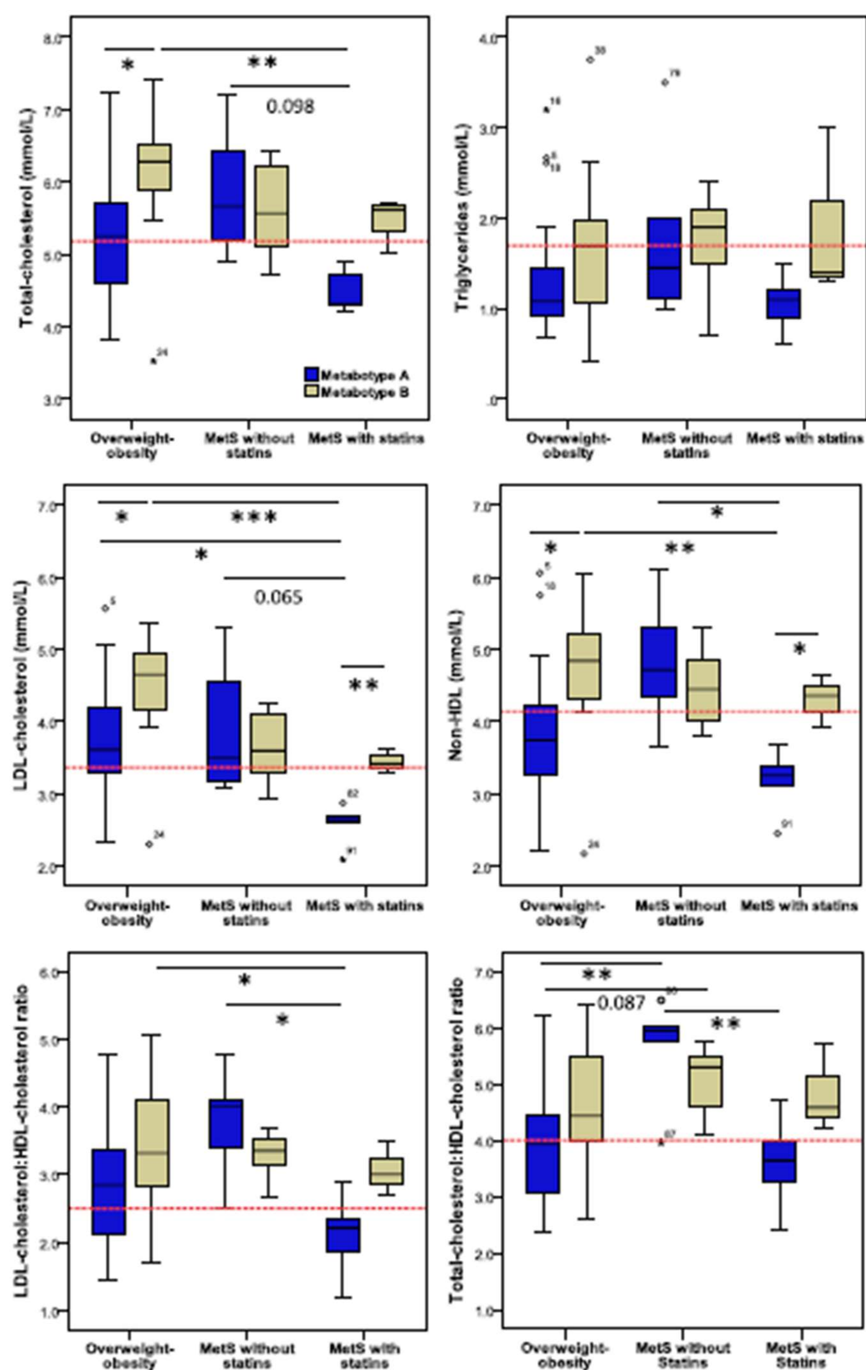

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Fig. 3. Differences in lipid profile depending on the UMs (UM-A, UM-B and UM-0) and health-disease status (healthy overweight-obese and MetS volunteers). Dotted lines indicate borderline values between normal and abnormal according to American Heart Association. $^{***}P \leq 0.001$, $^{**}P \leq 0.01$ and $^{*}P \leq 0.05$.

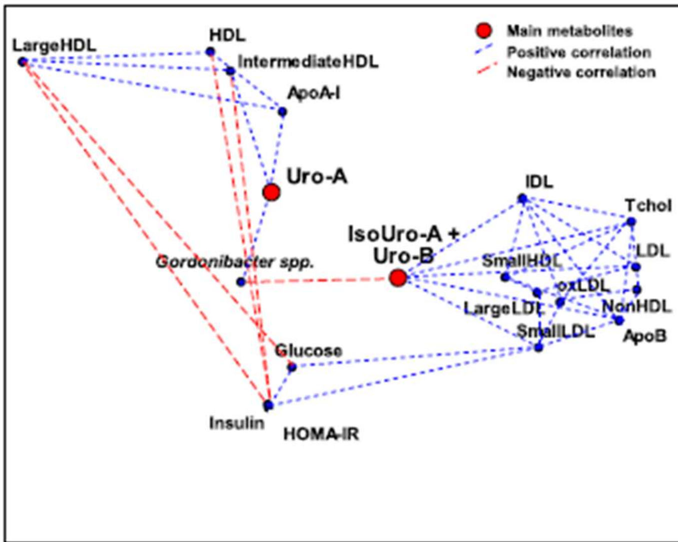

Fig. 4. PCA of the main microbial metabolites excreted in the urine of overweight-obese individuals after pomegranate extract consumption and correlation to fecal *Gordonibacter* spp. levels and serum markers. ApoA-I, apolipoprotein A-I; ApoB, apolipoprotein B; HDL, HDL-cholesterol; LDL, LDL-cholesterol; Non-HDL, non-HDL-cholesterol; oxLDL, oxidized-LDL; Tchol, total cholesterol; Uro-A, urolithin-A; IsoUro-A, isourolithin-A; Uro-B, urolithin-B.

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TABLES

Table 1

Differences in BMI, glycemia, serum lipid markers and percentage of the three UMs present in healthy normoweight, healthy overweight-obese and MetS volunteers.[1]

	Healthy normoweight	Healthy overweight-obese	MetS[2]	Optimal levels[3]
Subjects, *n*	20	49	50	
Age, y	33.6 ± 10.2	45.7 ± 6.7	51.8 ± 8.3	
Gender, male/female	11/9	32/17	28/22	
BMI, kg/m^2	22.8 ± 1.4^b	30.3 ± 3.5^a	31.4 ± 3.2^a	18.5–24.9
Glucose, mmol/L[4]	4.38 ± 0.72^b	5.21 ± 0.52^b	5.82 ± 0.54^a	3.3–6.1
Insulin, pmol/L[5]	ND[6]	68.90 ± 44.30^a	55.62 ± 24.93^a	<58.3
HOMA_IR[7]	ND[6]	2.21 ± 1.43^a	2.10 ± 1.01^a	<2.0
Triglycerides, mmol/L[8]	0.91 ± 0.29^b	1.40 ± 0.75^{ab}	1.61 ± 0.82^a	≤1.69
Cholesterol, mmol/L[9]				
Total	5.07 ± 0.89^a	5.41 ± 0.98^a	5.61 ± 1.09^a	≤5.2
HDL	1.86 ± 0.26^a	1.38 ± 0.29^b	1.14 ± 0.27^c	>1.55
LDL	2.86 ± 0.78^b	3.90 ± 0.82^a	3.61 ± 0.90^{ab}	<3.36
VLDL	ND	0.96 ± 0.35^a	0.84 ± 0.42^a	≤0.78
Non-HDL	3.13 ± 0.61^b	4.03 ± 0.94^{ab}	4.47 ± 1.14^a	<3.3
Ratios cholesterol				
Total: HDL	2.7 ± 0.5^b	4.0 ± 0.9^b	5.2 ± 1.8^a	≤3
Triglycerides: HDL	0.5 ± 0.1^b	1.1 ± 0.7^b	1.5 ± 1.0^a	<1.3
LDL:HDL	1.6 ± 0.6^b	2.9 ± 0.8^a	3.3 ± 1.4^a	<2
UM-A (%)	70^a	57^b	50^c	
UM-B (%)	20^a	31^b	41^c	
UM-0 (%)	10^a	12^a	9^a	

[1]Values are expressed as mean ± SD. Values within rows followed by different letters are significantly different ($P \leq 0.05$).
[2]MetS, metabolic syndrome.
[3]Optimal levels according to American Heart Association.
[4]To convert glucose to mg/dL, divide by 0.0555.
[5]To convert insulin to μIU/mL, divide by 6.945.
[6]ND, not determined.
[7]HOMA-IR, fasting glucose (mmol/L) × fasting insulin (μIU/mL)/22.5.
[8]To convert triglycerides to mg/dL, divide by 0.0113.
[9]To convert cholesterol to mg/dL, divide by 0.0259.

Table 2
Significant correlations between glucose, lipid profile and lipoprotein particles and the urinary excretion of main urolithins and metabotype factor in healthy overweight-obese individuals.[1]

	Mean ± SD[2]	Urolithin-A[3]	Isourolithin-A + urolithin-B[4]	Metabotype factor[5]
Glucose, mmol/L[6]	5.21 ± 0.52	NS	NS	NS
Insulin, pmol/L[7]	68.90 ± 44.3	NS	NS	NS
HOMA_IR[8]	2.21 ± 1.43	NS	NS	NS
Triglycerides, mmol/L[9]	1.40 ± 0.75	NS	NS	NS
Cholesterol, mmol/L[10]				
Total	5.41 ± 0.98	NS	0.358***	0.010
HDL	1.38 ± 0.29	NS	NS	NS
Large HDL	0.41 ± 0.19	NS	NS	NS
Intermediate HDL	0.67 ± 0.12	0.289*	NS	NS
Small HDL	0.30 ± 0.10	NS	NS	NS
IDL	1.50 ± 0.37	NS	0.266*	NS
LDL	3.90 ± 0.82	NS	0.371***	0.016
Large LDL	1.14 ± 0.31	NS	0.256*	0.082
Small LDL	0.38 ± 0.28	NS	0.270*	0.013
VLDL	0.96 ± 0.35	NS	0.291*	NS
Non-HDL	4.03 ± 0.94	NS	0.379***	0.009
Oxidized-LDL, U/L	75.6 ± 21.17	NS	0.237*	0.006
ApoA-I, g/L	1.48 ± 0.20	0.303*	NS	NS
ApoB, g/L	0.92 ± 0.23	NS	0.350**	0.008
Ratios cholesterol				
Total:HDL	4.0 ± 0.9	NS	NS	NS
Triglycerides:HDL	1.1 ± 0.7	NS	NS	NS
LDL:HDL	2.9 ± 0.8	NS	NS	0.080
Small HDL:Large HDL	0.9 ± 0.7	NS	NS	NS
Large LDL:Small LDL	5.0 ± 3.8	NS	NS	0.016
LDL:oxidized-LDL, mmol/U	0.05 ± 0.01	NS	NS	NS
LDL:ApoB, mmol/g	4.3 ± 0.5	NS	NS	NS
Non-HDL:ApoB, mmol/g	4.4 ± 0.4	NS	NS	NS
ApoB:ApoA-I	0.6 ± 0.2	NS	0.256*	0.037
Oxidized-LDL:ApoB, U/g	82.9 ± 16.4	NS	NS	0.068

[1] Significant at $^{***}P \leq 0.001$, $^{**}P \leq 0.01$ and $^{*}P \leq 0.05$.
[2] Standard deviation.
[3] Spearman correlation.
[4] Kendall correlation.
[5] ANOVA.
[6] To convert glucose to mg/dL, divide by 0.0555.
[7] To convert insulin to μIU/mL, divide by 6.945.
[8] HOMA-IR, fasting glucose (mmol/L) × fasting insulin (μIU/mL)/22.5.
[9] To convert triglycerides to mg/dL, divide by 0.0113.
[10] To convert cholesterol to mg/dL, divide by 0.0259.