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Association between lipids and apolipoproteins on type 2 diabetes risk; moderating effects of gender and polymorphisms; the ATTICA study

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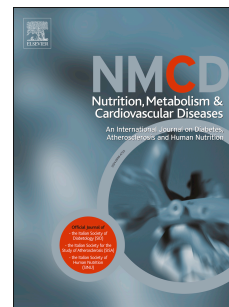
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1     **Association between lipids and apolipoproteins on type 2 diabetes risk; moderating**  
2                   **effects of gender and polymorphisms; the ATTICA study.**

3  
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30 **Highlights**

- 31 • ApoA1 levels, ApoB:LDL and TG:apoA1 ratios are associated with 10-year risk of
- 32 T2DM in males only
- 33 • In males only a unit change in apoB: LDL cholesterol increased risk of type 2
- 34 diabetes by 303%
- 35 • In males only a unit change in triglycerides: apoA1 increased risk of type 2 diabetes
- 36 by 85%
- 37 • HOMA-IR predicted the 10-year incidence of T2DM only in apoA1 75 GG carriers
- 38 • Physical activity may moderate the influence of HOMA-IR on T2DM incidence only
- 39 in carriers of apoA1 75 GG.

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**Abstract**

**Background and Aims:** Type 2 diabetes mellitus (T2DM) is a condition defined by hyperglycaemia, but also often presents with dyslipidaemia and suppressed HDL cholesterol. Mendelian randomization studies have suggested a causal link between low HDL cholesterol and T2DM. However, influences of gender, polymorphisms and lifestyle, all known to influence HDL cholesterol, have not been fully explored in a prospective cohort.

**Methods and Results:** In 2001-2002, a random sample of 1514 males (18-87 years old) and 1528 females (18-89 years old) were recruited in the ATTICA study. The 10-year follow-up (2011-2012) included 1485 participants. Lipids and lipoproteins levels, glucose and insulin levels were measured together with apolipoprotein A1 (apoA1) 75 G/A genotype, which is known to influence HDL-cholesterol. In total, 12.9% of the study sample developed T2DM within the 10-year follow-up period. In multivariable models, for each mg/dL increase in apoA1 levels in males, 10-year T2DM risk decreased 1.02%; while every unit increase in apoB/LDL-cholesterol ratio increased risk 4-fold. Finally, for every unit increase in triglycerides/apoA1 ratio, the risk increased 85%. HOMA-IR independently predicted T2DM 10-year incidence only for carriers of GG polymorphism (all,  $p < 0.05$ ), but not in carriers of the GA polymorphism (all,  $p > 0.05$ ).

**Conclusion:** ApoA1 was associated with decreased T2DM risk and TG/ApoA1 and apoB/LDL were associated with increased risk of T2DM, only in males. ApoA1 polymorphism, which is associated with lower HDL cholesterol, influenced the predictive effects of HOMA-IR on T2DM incidence, which appeared to be moderated by physical activity, suggesting potential scope for more targeted preventative strategies.

**Keywords:** Lipids; HDL cholesterol; Apolipoprotein A-1; Type 2 Diabetes Risk; Prospective cohort.

70

71 **Abbreviations:**

72 apoA1: Apolipoprotein A-1

73 apoB: Apolipoprotein B

74 BMI: Body mass index

75 CVD: cardiovascular disease

76 HDLc: High-Density Lipoprotein cholesterol

77 IPAQ: International Physical Activity Questionnaire

78 LDLc: Low-Density Lipoprotein cholesterol

79 TG: Triglycerides

80 T2DM: Type 2 Diabetes Mellitus

81

82

**83 Introduction**

84           Type 2 diabetes mellitus (T2DM) is a rapidly growing global health challenge [1-3].  
85           Traditionally, management and prevention of T2DM have focused mainly on glycaemia [4,  
86           5], despite it often presenting with dyslipidaemia. It is plausible that after hyperinsulinaemia,  
87           dyslipidaemia typified by suppressed HDL cholesterol is the second most dominant feature of  
88           Metabolic Syndrome in T2DM [6]. Recently, a Mendelian randomisation study suggested a  
89           potential causal link between suppressed HDLc and T2DM risk [7]. Despite this emerging  
90           evidence, the use of lipid and lipoprotein biomarkers still mainly focuses upon predicting  
91           cardiovascular disease (CVD) risk [8, 9] including in people with T2DM [10], with little  
92           consideration of these biomarkers when assessing T2DM risk [11].

93           The mechanisms of how apolipoproteins and HDLc influence insulin action, a key  
94           aspect of T2DM pathogenesis has recently been explored [12]. The relationship between low  
95           HDLc and development of T2DM has been previously described [13, 14], with causality  
96           partially assessed [15] as plasma insulin increased and plasma glucose decreased following  
97           an infusion of HDLc (including apoA1) in individuals with T2DM. The role of HDLc at  
98           physiological levels is less clear and dependent on experimental conditions. However, it  
99           appears that HDLc is potentially protective of  $\beta$ -cells against stressors, including glucose and  
100           oxidised LDLc [16, 17], whereas triglyceride-rich particles may be detrimental [18]. The  
101           protective effects of HDLc may be attributable to apoA1 [17]; whereas low levels of HDLc  
102           are a known risk factor [12].

103           There are several known modifiers of cardiovascular risk including gender, genetic  
104           factors and lifestyle which appear to act via altering an individual's lipid profile [19], which  
105           may also influence the risk of developing T2DM. Males typically have lower HDLc  
106           compared to pre-menopausal females, leading to suggestions that CVD risk reduction  
107           strategies should be tailored accordingly [20]. Beyond gender, a number of polymorphisms

108 have been identified in apolipoprotein genes. A key single nucleotide polymorphism in the  
109 apoA1 gene is apoA1-75 G/A, which is associated with a lower apoA1 and HDLc  
110 concentration for the GG genotype compared to the AA and a lesser extent GA [21]. The G  
111 allele has also been associated with increased myocardial infarction risk [22]. It is, therefore,  
112 logical to explore the potential moderating effects of this polymorphism in cohorts at risk of  
113 developing T2DM.

114 Although HDLc has been proposed to be linked causally with T2DM; the link with  
115 and apoA1 and T2DM is less well defined, especially with respect to modifying effects of  
116 gender, polymorphisms or lifestyle. Therefore, this study aimed to explore potential  
117 associations of lipids, apolipoproteins, gender and apoA1 polymorphisms, and risk of  
118 developing T2DM in a cohort of Greek healthy adults.

## 119

## 120 **Materials and methods**

## 121

### 122 *Baseline sampling procedure (2001-2002)*

123 The ATTICA study is a large-scale, health and nutrition, prospective survey, which was  
124 carried out during 2001-2002, in the province of Attica, where Athens is a major metropolis.  
125 People with a history of CVD or other atherosclerotic diseases or having chronic viral  
126 infections or living in institutions were excluded from participation. Of the initially invited  
127 4056 individuals and after excluding those with CVD (i.e., n=117) or those having chronic  
128 viral infections (n=107), 3042 finally agreed to participate (75% participation rate); 1514 of  
129 the participants were male (aged  $46\pm 13$  y; range 18-87 y), and 1528 were female (aged  $45\pm 13$   
130 y; range: 18–89y). Trained personnel (i.e., cardiologists, general practitioners, dietitians and  
131 nurses) interviewed the participants, using a standard questionnaire.

132 More details about the aims, design and methods used in the ATTICA Study may be found  
133 elsewhere in the literature [23].

134

### 135 ***Baseline measurements***

136 Baseline assessment included information about socio-demographic characteristics (age and  
137 gender), history of diabetes, family history of diabetes, smoking status and physical activity.

138 Smokers were defined as those who smoked at least one cigarette per day or had quit  
139 within the previous year; the rest were defined as non-smokers. The International Physical  
140 Activity Questionnaire (IPAQ) was used to evaluate the level of physical activity [24].

141 Participants were classified into two groups; either sedentary lifestyle or at least moderately  
142 active during a substantial part of the day. Weight (kg), height (m), as well as clinical  
143 characteristics, were measured using standardized procedures. Body mass index (BMI) was  
144 calculated as weight (kg) divided by standing height (in square meters).

145

146 Biochemical measurements were carried out in the same laboratory that followed the criteria  
147 of the World Health Organization Lipid Reference Laboratories. Blood samples were  
148 collected from the antecubital vein between 8, and 10 am, in a sitting position after 12 hours  
149 of fasting and alcohol abstinence. Blood glucose levels (mg/dl) were measured with a  
150 Beckman Glucose Analyzer (Beckman Instruments, Fullerton, CA, USA). Serum insulin  
151 concentrations were assayed by radioimmunoassay (RIA100, Pharmacia Co., Erlangen,  
152 Germany). Insulin resistance was assessed by the calculation of the homeostasis model  
153 assessment (HOMA-R) approach (glucose in mg/dl x insulin in  $\mu$ U/ml / 22.5) [25]. Diagnosis  
154 of T2DM was based on the criteria of the American Diabetes Association (ADA) [26], i.e.,  
155 participants who had fasting blood glucose  $>125$  mg/dl during the examination or who  
156 reported the use of antidiabetic medication were defined as having diabetes. Serum total



157 cholesterol, HDL-cholesterol and triglycerides were measured using chromatographic  
158 enzymic method in a Technicon automatic analyser RA-1000 (Dade Behring, Marburg,  
159 Germany). HDL-cholesterol was determined after precipitation of the Apolipoprotein B  
160 (apoB) containing lipoproteins with dextran-magnesium-chloride. Non-HDL cholesterol was  
161 calculated by the formula: total cholesterol minus HDL cholesterol. Lipoprotein (a) was  
162 measured by a latex-enhanced turbidimetric immunoassay. LDL cholesterol calculated using  
163 the Friedewald formulae:  $(8) - [13] - 1/5$  (triglycerides) (only for participants with  
164 triglycerides < 400 mg/dL). apoB and apoAI were measured by rate immunonephelometry.  
165 Internal quality control was in place for assessing the validity of cholesterol, triglyceride and  
166 HDL methods. The intra and inter-assay coefficients of variation of cholesterol levels did not  
167 exceed 9 %, triglycerides 4 % and HDL 4 %. Serum for the measurement of blood lipids was  
168 harvested immediately after admission.

169 The combination of ratios used in the analysis was based on that published previously in  
170 association with cardiovascular risk [27, 28]. Comparing the cholesterol marker with its  
171 corresponding apolipoprotein was selected as it has been shown to be a surrogate of function  
172 of the apolipoprotein, which would not be the case for a triglyceride to apolipoprotein ratio.

173

#### 174 ***DNA extraction and genotyping***

175 Genomic DNA was extracted from 2-5 mL of fresh or frozen whole blood using standard  
176 methods (Qiam-DNA extraction kit, QIAGEN, Hilden, Germany), as described previously  
177 (29). The coding sequence variant was a G to T substitution in exon 7 in codon 298, which  
178 alters the amino acid at this residue from Glu to Asp. Genotyping of apoA1 gene  
179 polymorphisms were performed by polymerase chain reaction (PCR) restriction length  
180 polymorphism assay previously described [29]. The location of the MspI restriction site was  
181 used to identify polymorphisms, with presence of the restriction site at -75 bp (G allele) and

182 at +83 bp (C allele) in the 433 bp product resulted in four fragments of 45, 66, 113 and 209  
183 bp. The absence of the restriction site at -75 bp (A allele) resulted in three fragments of 45,  
184 179 and 209 bp. The absence of the restriction site at +83 bp (T allele) created a larger  
185 fragment of 254 bp instead of two fragments of 45 and 209 bp [21].

186

### 187 *10-year follow-up evaluation (2011-2012)*

188 During 2011-2012, the 10-year follow-up was performed. Of the n=3042 participants,  
189 n=2583 were allocated during the follow-up (85% participation rate). A detailed evaluation of  
190 the participants' medical status was performed. Among various endpoints, development of  
191 T2DM was recorded based on diagnosis by a physician; n=210 patients diagnosed with  
192 diabetes at baseline and n=1347 participants with no data regarding diabetes status at the 10-  
193 year follow up were not included in the present analyses, yielding a working sample of  
194 n=1485 participants without diabetes at baseline, as presented elsewhere [30]. Further details  
195 about the baseline procedures and the 10-year follow up of the study have been presented  
196 elsewhere [31].

197

### 198 *Statistical analysis*

199 Incidence of diabetes was calculated as the ratio of new cases (n=191) to the total number  
200 (n=1485) of participants in the follow-up. Normality for continuous variables was tested  
201 through histograms and P-P plots. Normally distributed continuous variables are presented as  
202 mean values±standard deviation, not normally distributed variables are presented as median  
203 (1<sup>st</sup>, 3<sup>rd</sup> quartile) and categorical variables as frequencies (relative frequencies). Associations  
204 between categorical variables were tested using chi-squared test. Comparisons of mean  
205 values of normally distributed variables between those groups were performed using  
206 Student's t-test, after ensuring equality of variances using Levene's test. For non-normally

207 distributed variables, the Kruskal-Wallis test was applied, and next the Mann-Whitney *test*  
208 was performed between groups. The relative risk of developing T2DM during the 10-year  
209 period according to the participants' baseline characteristics was estimated through the odds  
210 ratio (OR) and the 95% corresponding confidence interval, as derived from logistic  
211 regression models. This type of analysis was preferred since there were no accurate data  
212 about diabetes onset, but only diagnosis. Univariable logistic regression models were used to  
213 identify the variables that were possible independent predictors of T2DM onset but also  
214 known confounders (i.e., gender, age) were forced in the multivariable models and all of  
215 independent variables were tested for collinearity. Level of statistical significance was  
216 defined at  $\alpha=0.05$  and Bonferroni corrections were applied to all predictive models to  
217 counteract for multiple comparisons (in this case the eight models). An exploratory analysis  
218 of the influence of apoA1 polymorphism on relative risk of developing T2DM during the  
219 follow-up period was also undertaken; this included an analysis of interactions with both  
220 modifiable and non-modifiable risk factors. The SPSS version 23 (Statistical Package for  
221 Social Sciences, IBM Hellas SA, Greece) software was used for all statistical calculations.

222

## 223 **Results**

224 The study sample consisted of 1485 individuals (51% females) with a mean age of  $45\pm 13$   
225 years ( $p$  for gender difference  $>0.05$ ). Of these, 12.9% (191) developed T2DM within the 10-  
226 year follow-up period, but no difference was detected between genders ( $p=0.574$ ). Mean BMI  
227 at baseline of the total sample was  $26.3\pm 4.28\text{kg/m}^2$ , with males having significantly higher  
228 mean BMI than females ( $27.2\pm 3.6$  vs  $25.3\pm 4.7$  respectively,  $p<0.001$ ). Details of participant  
229 characteristics are in *Table 1*.

230

231 T2DM incidence was not associated with gender ( $p=0.574$ ), family history of T2DM  
232 ( $p=0.416$ ), sedentary lifestyle ( $p=0.191$ ) and age ( $p=0.458$ ). Smoking was more prevalent in  
233 males than females ( $p<0.001$ ). Concerning the biomarkers relating to glucose metabolism,  
234 fasting glucose, insulin and HOMA-IR were significantly lower in females than males (all  
235  $p<0.001$ ). Baseline lipoprotein and lipid biomarkers indicated that males had significantly  
236 higher total and LDLc, Triglycerides (TG) and apoB levels, but lower HDLc and apoA1  
237 compared to females (all  $p<0.001$ ), suggesting a profile highly related to gender.

238

239 Several logistic regression models were applied to investigate the net effects of different  
240 biomarkers on T2DM 10-year incidence (*Table 2*). All models were adjusted for the same set  
241 of potential confounders and stratified by gender due to significant biomarker profile  
242 differences reported at baseline ( $p$  for interaction  $<0.001$ ) (*Table 1*). No biomarkers were  
243 associated with T2DM 10-year risk in females, but there were significant associations for  
244 males. Specifically, for each mg/dL increase in apoA1 levels in males the 10-year T2DM risk  
245 decreased per 1.1%, independently of age, smoking, physical activity status, HOMA-IR,  
246 family history T2DM and BMI. Moreover, in males for every unit increase in apoB/LDL-  
247 cholesterol ratio, the 10-year T2DM risk was 4-fold increased independent confounding risk  
248 factors used in previous models. Finally, for males, every unit increase in triglycerides/apoA1  
249 ratio, the 10-year T2DM risk increased per 85%, independent of confounding risk factors. No  
250 other biomarker or ratios were associated with T2DM 10-year risk in males. Additionally, in  
251 this cohort, it was found that HOMA-IR was the most specific independent predictor of  
252 T2DM incidence in females, with no effect of lipids or lipoprotein as observed in males.

253

254 ApoA1-75G/A polymorphism data were available for 313 participants (GG: 215(68.7%) GA:  
255 89(28.4%) and AA: 9(2.9%)). The AA group was therefore excluded due to its small number,

256 although representative of the population; this prevalence would negate any meaning being  
257 able to be derived. Polymorphism distribution was not influenced by gender (GA prevalent at  
258 29.4% of males ( $n=45$ ) and 29.1% of females ( $n=44$ ),  $p=0.958$ ). No association was detected  
259 between polymorphism and T2DM 10-year incidence ( $p=0.931$ ). However, significant  
260 interactions were observed with apolipoprotein levels ( $p<0.001$ ) that led the analysis to  
261 stratification per polymorphism group (**Table 3**).

262

263 As presented in *Table 3*, when the analysis was stratified per apoA175 G/A polymorphism  
264 status, none of the lipid or lipoprotein biomarkers were significantly related to T2DM 10-year  
265 risk (all  $p >0.05$ ) after adjusting for confounding risk factors. Although influencing T2DM  
266 risk in the cohort as a whole (crude (Odds Ratio (OR) =2.44, 95% Confidence Interval (CI):  
267 1.94-3.07), HOMA-IR was an independent predictor of 10-year incidence of T2DM, only for  
268 GG polymorphism carriers (all  $p<0.05$ ) in all presented models. Contrarily, HOMA-IR was  
269 not significantly associated with T2DM 10-year risk in any of the models for carriers of the  
270 GA polymorphism (all  $p>0.05$ ). Physical activity was found to be protective against T2DM  
271 only for GG carriers (Odds Ratio (OR) =0.206, 95% Confidence Interval (CI): 0.043-0.983)  
272 but not for GA carriers (OR=0.478, 95% CI: 0.033-6.84), after adjusting for confounding risk  
273 factors.

274

## 275 **Discussion**

276 This analysis investigated the influence of apolipoprotein and lipid biomarkers as predictive  
277 factors for developing T2DM during a 10-year follow-up, focusing on the potentially  
278 influencing effects of gender, apoA1 polymorphisms along with any interactions with insulin  
279 resistance (HOMA) and physical activity. This analysis has provided further evidence of how  
280 lipid profile and apolipoproteins influence the risk of developing T2DM in a Greek cohort

281 followed up for 10 years. Additionally, this is the first analysis to consider how gender and  
282 polymorphisms of apoA1-75 G/A may influence how lipoproteins and lipids influence  
283 ultimately risk of developing T2DM.

284

285 Males were found to have significantly higher total and LDLc, TG and apoB levels, but lower  
286 HDLc and apoA1 compared to females, suggesting an influence of gender upon lipid profile.  
287 However, there were no statistically significant differences in new cases of T2DM between  
288 genders. No lipid or apolipoprotein biomarkers were associated with T2DM 10-year risk in  
289 females, but associations were significant in males. Specifically, higher apoA1 levels were  
290 seen to be protective for males, while the increase in apoB/LDL-cholesterol ratio and increase  
291 in triglycerides/apoA1 ratio were aggravating factors independent of age, smoking, physical  
292 activity status, HOMA-IR, family history of diabetes and BMI.

293

294 When the analysis was stratified per apoA1-75G/A polymorphism status a known factor  
295 which influences both apoA1 and HDLc concentration, none of the biomarkers were  
296 significantly related to T2DM 10-year risk in the same multivariable models. An analysis to  
297 consider interactions between risk factors for T2DM found that HOMA-IR was an  
298 independent predictor of T2DM 10-year incidence for GG polymorphism carriers only [32].  
299 No significant associations with T2DM 10-year risk were found in any of the models for GA  
300 polymorphism carriers.

301

302 The potential differences between genders have been largely overlooked, in a previous study  
303 which followed up a Dutch cohort for a shorter timeframe [13]. It was noticeable that the  
304 ATTICA study sample where higher risk, having a stronger family history and greater  
305 prevalence of insulin resistance than the Dutch cohort, potentially highlighting the greater

306 suitability of the ATTICA cohort in studying diabetes prevention [33]. A population at higher  
307 risk of developing T2DM, by virtual of increased incidence of insulin resistance as seen in  
308 this study might explain the observation in this analysis that for apoA1 to reduce risk in  
309 males, but not in females. It might also be a reflection that the Dutch cohort data was only  
310 adjusted for glucose and not insulin, so unable to adjust for insulin resistance (HOMA-IR); a  
311 known predictive of risk of developing T2DM [34, 35]. The pattern which protective effects  
312 in males of apoA1/HDLc and increasing risk from apoB/LDLc ratios were consistent with  
313 Abbasi et al. [13] and data concerning apoB/LDLc ratio additionally concurs with the male-  
314 only cohort reported by Fizeleva et al. [36]. However, this is the first cohort to suggest an  
315 effect of apoA1/triglycerides as modifying risk of developing T2DM in a long-term  
316 prospective cohort. This data provides support to the logical theory; as raised triglycerides  
317 have been previously associated with increased T2DM risk [37] and insulin resistance.

318

319 The potential influencing effect of apoA1-75 G/A polymorphisms was explored in 304  
320 participants over 10-year follow up. This is believed to be the first analysis investigating any  
321 influencing effects of this polymorphism which is known to alter lipid profiles [21], with G/G  
322 carriers expressing less apoA1 and having lower levels of HDLc [32]. Although effects of the  
323 polymorphism were not seen with respect to the risk of developing T2DM, interactions were  
324 observed with HOMA-IR only being associated with increased risk of developing T2DM in  
325 those with GG polymorphism. This suggests that identifying insulin resistance and then  
326 treating it in carriers of the GG polymorphism may provide a potentially more focused and  
327 effective intervention. The potential for targeted interventions for the prevention of T2DM  
328 was further highlighted by modulating effects of physical activity which reduced the risk of  
329 developing T2DM in GG carriers by 79% (OR 0.206 95% CI 0.043-0.983) but not in GA  
330 carriers (OR 0.478 95% CI 0.033-6.84) after adjusting for other confounding risk factors.

331 This warrants further investigation as it suggests that there is a gene-lifestyle interaction  
332 where physical activity may be more effective in reducing T2DM risk in GG carriers. The  
333 variation in response to exercise has been reported with greater response with a more  
334 favourable HDLc particle size with physical training for GG compared to GA or AA carriers  
335 [38]. However, to date, this effect has not been linked to the development of clinical  
336 conditions such as T2DM. Further research is needed to understand these mechanisms  
337 relative to risk of developing T2DM and effects of physical activity on the apolipoprotein, or  
338 the nature of HDLc particle size or its concentration.

339

340 Longitudinal effects of lipid and apolipoprotein measures were previously investigated in a  
341 Dutch mixed gender cohort, where gender did not influence the association between lipids,  
342 apolipoprotein markers and T2DM risk. This was despite reporting a 30% reduction in risk  
343 for males for each standard deviation shift in HDLc compared to 26% for females (OR 0.70,  
344 95% CI 0.55-0.91 and OR 0.74 CI 0.57-0.96, respectively) [13]. A further Finish cohort of  
345 3686 male participants completing a mean follow-up of 5.9 years [36] found an association  
346 between worsening glycaemia and incidence of T2DM linked to the ratio of apolipoprotein  
347 and its associated lipoprotein. This further supports the theory that inadequate or altered  
348 capacity of lipids by apolipoproteins could be implicated in an increased risk of developing  
349 T2DM. As an individual's lipid profile is influenced by insulin and glycaemia as well as  
350 potentially vice versa, therefore caution should be taken when looking to assign potential  
351 causality. *In vitro* work has linked apoB positively, and apoA1 negatively, to increased risk  
352 of developing T2DM [16, 17], supportive of a theory that apoA1 and HDLc are protective  
353 against the development of T2DM. However, according to our data, the nature of this effect  
354 and potential inter-individual variation appears to be further influenced by gender and apoA1-  
355 75 polymorphisms.



356

357 Clinically, the prevention of T2DM has focused on the use of glycaemia and insulin-based  
358 markers. This has been most recently seen in the commissioning of clinical services,  
359 including NHS England National Diabetes Prevention Program, which focuses purely on  
360 changes in glycaemia and weight as outcome measures [4]. Such programs focus on a  
361 glucose centric perspective of T2DM prevention despite evidence from a clinical perspective  
362 that the pathology should be increasingly seen as a global metabolic abnormality. The twin-  
363 cycle hypothesis highlights this, identifying lipid metabolism and ectopic lipid accumulation  
364 in the pancreas and liver as key drivers of the pathology [39]. This, together with  
365 experimental models and epidemiological data suggest lipids, especially HDLc and  
366 potentially HDLc/apoA1 ratio, are predictive of developing T2DM. Additionally, in an age of  
367 personalised medicine, our data suggests that recognising differences in risk associated with  
368 gender and polymorphisms could be useful in targeting interventions.

369

### 370 *Limitations*

371 Despite the importance of this study highlighting differences in gender and polymorphism for  
372 T2DM risk, there are a number of limitations. Firstly, the effect of gender and apoA1-75  
373 polymorphism on lipid and apolipoprotein were only measured at baseline examination. The  
374 number of cases for the assessment of apoA1-75 polymorphism was relatively small within  
375 the whole cohort, which suggests potential bias cannot be ruled out and if the null findings  
376 are not certain although evident. The number of participants with an AA polymorphism was  
377 so small; thus, were excluded from the analysis. An alternative approach would be to  
378 combine this group with the GA. However, this did not affect the outcome. The change in  
379 lipid and apolipoprotein concentrations over the 10-year follow up was not measured, and  
380 variation could influence risk. However, this is the same methodology that has been used in

381 other prospective studies and is typical in this field, making results comparable. The decision  
382 to exclude individuals with a history of cardiovascular disease could potentially hinder  
383 external validity of this analysis, as it is plausible that the dyslipidaemia associated with  
384 cardiovascular disease might be protective role in type 2 diabetes incidence. However, as  
385 there is an association with type 2 diabetes and cardiovascular disease this is unlikely, and the  
386 impact of including individuals with pre-existing cardiovascular disease would, both due to  
387 the nature of the pathology and any therapeutic interventions might have would have only  
388 added additional confounding factors.

389

### 390 **Conclusions**

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392 Markers of lipids and apolipoproteins were associated with risk of developing T2DM only in  
393 males in this Greek cohort. Additional apoA1 polymorphisms appear to influence the  
394 predictive effect of HOMA-IR on T2DM incidence and the potential moderating role of  
395 physical activity; suggesting the potential for more targeted and individualized approaches  
396 for diabetes prevention strategies based on taking into account the influencing effects of  
397 genetic factors, lipid and apolipoprotein levels.

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408

**409 Author Contributions**

410

411 D.D.M., D.B.P., E.N.G., N.M.D., and N.N. conceptualised and wrote the paper. C.C., D.T.,  
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**Table 1.** Baseline lifestyle, biochemical variables and 10-year (2002-2012) incidence of diabetes, in the ATTICA study cohort ( $n=1485$ ).

	<b>Males (<math>n=726</math>)</b>	<b>Females (<math>n=759</math>)</b>	<b><i>p</i></b>
New diabetes cases, n (%)	97 (13.4)	94 (12.4)	0.574
Age, years	46 ± 13	45 ± 14	0.458
Ever Smoker, n (%)	456 (62.9)	346 (45.6)	<0.001
Body mass index, kg/m <sup>2</sup>	27.2 ± 3.6	25.3 ± 4.7	<0.001
Sedentary lifestyle, n (%)	408 (56.2)	452 (59.6)	0.191
Family history of diabetes, n (%)	<b>135 (18.6)</b>	<b>156 (20.5)</b>	0.416
Fasting glucose, mg/dl	91 ± 13	88 ± 12	<0.001
Fasting insulin, μU/ml	14.0 ± 3.5	11.8 ± 2.9	<0.001
HOMA-IR	3.1 ± 0.69	2.6 ± 0.59	<0.001
Total cholesterol, mg/dL	197 ± 41	191 ± 37	0.012
HDL-cholesterol, mg/dL	45 ± 15	53 ± 14	<0.001
LDL-cholesterol, mg/dL	126 ± 36	119 ± 38	0.001
Triglycerides, mg/dL	113 (79,160)	82 (59,117)	<0.001*
ApoA1, mg/dL	148 ± 23	163 ± 27	<0.001
ApoB, mg/dL	113 ± 29	100 ± 42	<0.001
<b>ApoB/ApoA1</b>	<b>0.781 ± 0.232</b>	<b>0.641 ± 0.357</b>	<b>&lt;0.001</b>
<b>ApoA1/HDL</b>	<b>3.47 ± 0.585</b>	<b>3.20 ± 0.762</b>	<b>&lt;0.001</b>
<b>ApoB/LDL</b>	<b>0.928 ± 0.235</b>	<b>0.862 ± 0.265</b>	<b>&lt;0.001</b>
<b>LDL/ApoA1</b>	<b>0.870 ± 0.283</b>	<b>0.755 ± 0.285</b>	<b>&lt;0.001</b>
<b>TG/ApoA1</b>	<b>0.932 ± 0.630</b>	<b>0.599 ± 0.391</b>	<b>&lt;0.001</b>
<b>TG/ApoB</b>	<b>1.18 ± 0.769</b>	<b>0.982 ± 0.850</b>	<b>&lt;0.001</b>

Data are presented as mean values and standard deviation for normally distributed variables and median (1<sup>st</sup>, 3<sup>rd</sup> quartile) for not normally distributed variables (\*). Categorical variables are presented as absolute and relative frequencies. *p*-values derived from independent samples test for the normally distributed variables and Mann-Whitney test for the non-normally distributed variables (\*) to test differences between genders and chi-square test was used for the categorical variables. HDL = high-density lipoprotein, LDL = low-density lipoprotein; HOMA-IR = homeostasis model assessment-insulin resistance; apoA1=apolipoprotein A-I; apoB=apolipoprotein B.

**Table 2.** Multivariable logistic regression models for various lipid biomarkers for the 10-year incidence of diabetes in the ATTICA study, stratified by gender ( $n=1485$ ).

Variable	Males ( $n=726$ )		Females ( $n=759$ )	
	OR	95% CI	OR	95% CI
<i>Model 1:</i> ApoB (per 1 mg/dL)	1.01	0.99-1.02	1.01	0.99-1.01
<i>Model 2:</i> ApoA1 (per 1 mg/dL)	0.98	0.97-1.00	1.00	0.98-1.01
<i>Model 3:</i> ApoB/ApoA1 (per 1 unit/per 1 SD)	3.72/1.36	0.97-14.2/0.99-1.85	1.51/1.16	0.74-3.05/0.90-1.49
<i>Model 4:</i> ApoA1/HDL (per 1 unit/per 1 SD)	0.99/0.99	0.57-1.72/0.72-1.37	0.78/0.83	0.48-1.28/0.57-1.21
<i>Model 5:</i> ApoB/LDL (per 1 unit/per 1 SD)	4.03/1.39*	1.05-15.5/1.01-1.90	1.68/1.15	0.53-5.21/0.85-1.55
<i>Model 6:</i> LDL/ApoA1 ((per 1 unit/per 1 SD)	1.40/1.10	0.45-4.29/0.80-1.51	1.13/1.04	0.38-3.34/0.76-1.41
<i>Model 7:</i> TG/ApoA1 (per 1 unit/per 1 SD)	1.85/1.47*	1.20-2.87/1.12-1.94	1.56/1.19	0.80-3.05/0.92-1.55
<i>Model 8:</i> TG/ApoB (per 1 unit/per 1 SD)	1.17/1.13	0.86-1.57/0.89-1.42	1.07/1.06	0.81-1.39/0.84-1.32

OR: Odds Ratio; CI: Confidence Interval; apoB: apolipoprotein B; apoA1: apolipoprotein A-I; HDL: High-Density Lipoprotein –cholesterol; LDL: Low-Density Lipoprotein –cholesterol; TG: triglycerides. OR and CIs derived from multivariable binary logistic regression models adjusted for age, smoking status, physical activity status, HOMA-IR, family history of type 2 diabetes, BMI. (\*) indicates Bonferroni corrected p-value significantly low.

**Table 3.** Multivariable logistic regression models for various lipid and lipoprotein biomarkers, for the 10-year incidence of diabetes in the ATTICA study, stratified by apoA1-75G/A polymorphism ( $n=304$ ).

Variable	GG ( $n=215$ )		GA ( $n=89$ )	
	OR	95% CI	OR	95% CI
<i>Model 1:</i> ApoB (per 1 mg/dL)	1.01	0.98-1.03	1.00	0.96-1.04
<i>Model 2:</i> ApoA1 (per 1 mg/dL)	0.98	0.96-1.01	0.99	0.96-1.03
<i>Model 3:</i> ApoB/ApoA1 (per 1 unit/per 1 SD)	1.50/1.13	0.25-8.87/0.65-1.98	0.80/0.93	0.01-60.1/0.24-3.59
<i>Model 4:</i> ApoA1/HDL (per 1 unit/per 1 SD)	0.75/0.82	0.29-1.94/0.42-1.59	0.76/0.83	0.14-4.06/0.26-2.65
<i>Model 5:</i> ApoB/LDL (per 1 unit/per 1 SD)	1.45/1.10	0.10-19.4/0.56-2.12	0.12/0.58	0.00-159/0-3.61
<i>Model 6:</i> LDL/ApoA1 (per 1 unit/per 1 SD)	2.10/1.24	0.11-38.4/0.53-2.87	2.11/1.24	0.02-162/0.32-4.35
<i>Model 7:</i> TG/ApoA1 (per 1 unit/per 1 SD)	2.11/1.50	0.74-5.96/0.85-2.64	1.02/1.01	0.25-4.03/0.47-2.13
<i>Model 8:</i> TG/ApoB (per 1 unit/per 1 SD)	1.86/1.66	0.75-4.62/0.79-3.50	2.57/2.16	0.43-15.4/0.50-9.36

OR: Odds Ratio; CI: Confidence Interval; apoB: apolipoprotein B; apoA1: apolipoprotein A-I; HDL: High-Density Lipoprotein –cholesterol; LDL: Low-Density Lipoprotein –cholesterol; TG: triglycerides. OR and CIs derived from various multivariable binary logistic regression models adjusted for gender, age, smoking status, physical activity status, HOMA-IR, family history of type 2 diabetes, BMI. (\*) indicates Bonferroni corrected p-value significantly low.

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## Highlights

- ApoA1 levels, ApoB:LDL and TG:apoA1 ratios are associated with 10-year risk of T2DM in males only
- In males only a unit change in apoB: LDL cholesterol increased risk of type 2 diabetes by 303%
- In males only a unit change in triglycerides: apoA1 increased risk of type 2 diabetes by 85%
- HOMA-IR predicted the 10-year incidence of T2DM only in apoA1 75 GG carriers
- Physical activity may moderate the influence of HOMA-IR on T2DM incidence only in carriers of apoA1 75 GG.