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# Dietary acid load adopts the effect of *ApoB ins/del* genetic variant (rs11279109) on obesity trait, cardiovascular markers, lipid profile, and serum leptin level among patients with diabetes: a cross-sectional study

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***ApoB insertion/deletion (ins/del)*** **genetic variant (rs11279109) is thought to be related to cardio-metabolic markers and obesity. This association has the potential to be modified by dietary patterns. Since the majority of studies concerned the role of dietary acid load (DAL) or ApoB in type 2 diabetes mellitus (T2DM) and its complications independently, and due to the insufficient data regarding the possible interactions between *ApoB* genetic variants and DAL on anthropometric and metabolic markers, we aimed to study the interaction between this genetic variant and dietary acid load (DAL) on cardio-metabolic markers, along with leptin among Iranian individuals with T2DM. 700 T2DM patients were randomly recruited. A validated semi-quantitative food frequency questionnaire was used for DAL calculation including potential renal acid load (PRAL) and net-endogenous acid production (NEAP). The polymerase chain reaction was used for genotyping the *ApoB ins/del* (rs11279109). The general linear model was applied to find the interactions in the crude and adjusted models. Patients with *del/del* genotype (rs11279109) with high PRAL intake have lower low-density lipoprotein cholesterol (LDL-C) ($P_{interaction} = 0.004$), LDL/HDL ratio ($P_{interaction} = 0.02$), total cholesterol (TC) ($P_{interaction} = 0.04$), triglyceride (TG) ($P_{interaction} = 0.04$), leptin ($P_{interaction} = 0.04$) and interleukin-18 (IL-18) ($P_{interaction} = 0.04$). Moreover, the interaction of gene and DAL in the PRAL method on TG concentration ($P = 0.04$), waist circumference (WC) ($P = 0.04$), and LDL/HDL ratio ($P = 0.04$) were significant. Eventually, a positive relationship was observed between the presence of the *del/del* genotype (rs11279109) and higher levels of TG, TC, LDL-C, IL-18, and LDL/HDL, in individuals with lower adherence to DAL, after adjusting for various covariates. Further studies are needed to investigate and confirm these findings.**

**Keywords** ApoB, Genetic variant, Dietary acid load, Inflammation, Obesity, Lipid profile

**Abbreviations**

| | |
|---|---|
| *Ins/del* | *Insertion/deletion* |
| DAL | Dietary acid load |

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| | |
|---|---|
| T2DM | Type 2 diabetes mellitus |
| PRAL | Potential renal acid load |
| NEAP | Net-endogenous acid production |
| LDL-C | Low density lipoprotein cholesterol |
| TC | Total cholesterol |
| TG | Triglyceride |
| IL-18 | Interleukin-18 |
| WC | Waist circumference |
| CVDs | Cardiovascular diseases |
| GWAS | Genomewide association studies |
| VLDL | Very low-density lipoprotein |
| CHD | Coronary heart disease |
| MUFA | Monounsaturated fatty acid |
| PUFA | Polyunsaturated fatty acid |
| SFA | Saturated fatty acid |
| FFQ | Food frequency questionnaire |
| SOD | Superoxide dismutase |
| TAC | Total antioxidant capacity |
| PTX3 | Pentraxin-3 |
| PGF2α | 8-Isoprostane F2α |
| hs-CRP | High-sensitivity C-reactive protein |
| PCR | Polymerase chain reaction |
| ANCOVA | Analysis of covariance |
| DII | Dietary inflammatory indexes |
| TLC | Therapeutic lifestyle change |

In recent decades, the prevalence and incidence of type 2 diabetes mellitus (T2DM) have steadily risen[1] and appear to be associated with a 1.3 to 2 times higher risk of mortality, mostly resulting from cardiovascular diseases (CVDs)[2]. Since there is a clear link between CVDs and T2DM, treatment goals must focus on CVD risk reduction[3–5]. The lipoprotein abnormalities, which commonly occur in T2DM, may be considered a result of lipoprotein lipase enzyme deficiency and modulation of lipid metabolism-related genes in hyperglycemia[6–8]. Also, the inflammatory state contributes significantly to the progression of atherosclerosis and is involved in various metabolic disorders related to diabetes[9]. T2DM is the result of both environmental and genetic risk factors as well as gene-environment interactions[10]. Genome-wide association studies suggested the Apolipoprotein B (*ApoB*) gene as one of the main candidate genes predisposing individuals to dyslipidemia[11,12]. *ApoB* is a structural protein and acts as an integral part of chylomicrons during lipoprotein metabolism. It is required for the synthesis of triglyceride-rich (TG-rich) lipoproteins such as very low-density lipoprotein (VLDL) in the liver[13]. It has been shown that serum *ApoB* concentration directly reflects the amount of low-density lipoprotein (LDL) particles in the plasma[14]. Elevated levels of these atherogenic lipoproteins trigger arterial inflammation by enhancing the migration of monocytes and T lymphocytes to the endothelial surface and the transformation of monocytes/macrophages into foam cells, which secrete several plasma inflammatory markers[15]. There are multiple genetic variants at the *ApoB* gene locus[13,16]. The insertion/deletion of 3 amino acid residues (leucine-alanine-leucine) in the *ApoB* signal peptide forms the *insertion/deletion* (*ins/del*) genetic variant (rs11279109) which is a common genetic variant and located in the first exon of the *ApoB* gene[7,17]. The hydrophobicity and translocation of the synthesized *ApoB* from the endoplasmic network to the cytoplasm were decreased in the *del* allele through LDL clearance reduction and increasing TG accumulation in the liver, causing a greater uptake of TG by adipose tissues and obesity[18–22]. It is also proposed that the *del* allele is related to a decrease and an increase in the secretion and degradation of *ApoB*, respectively[23–28]. It is additionally reported that *ApoB ins/del* genetic variant (rs11279109) is likely to be determined as a significant factor causing variety in the TG and insulin levels, especially in response to dietary intake[29]. A meta-analysis reported an elevated level of LDL, *ApoB*, and risk of CHD among the *ins/del* genotype (rs11279109)[30], however, a few studies showed no association between the *ApoB ins/del* genetic variant (rs11279109) and serum lipid levels[31,32]. These controversial results reveal the necessity of more gene-diet interaction studies. Based on evidence from patients with T2DM, the levels of cardio-metabolic markers may benefit from appropriate diet and exercise due to improvement of glycemic control and insulin sensitivity[33]. Previous studies showed that the most common barriers in diabetes management are related to the lack of adherence to nutrition recommendations and exercise[33–39]. Moreover, several epidemiological studies reported a significant relationship between dietary intake and lipid profile or inflammation[36,37,40]. The dietary composition also has a major effect on the acid-base balance[41,42]. Dietary acid load (DAL), as a nutritional indicator is estimated according to the potential renal acid load (PRAL) and net endogenous acid production (NEAP) and reflects the acid-forming potential of a diet. Following a Western dietary pattern, containing a high amount of animal protein, processed meat, excessive intake of artificially sweetened beverages, and low consumption of fruit and vegetables can initiate inflammation by promoting metabolic acidosis condition[43]. Several studies have suggested a linkage between metabolic risk factors and DAL. They found higher levels of LDL-C and TC among the highest DAL categories[44,45]. It is also suggested that a pro-inflammatory status can be followed by a high DAL[43,46,47]. To our knowledge, only a few studies investigated the interaction effect of *ApoB ins/del* genetic variant (rs11279109) and diet on metabolic markers. According to a study among Iranian patients with diabetes, high levels of TG and LDL-C has been reported in *del*-allele carriers who had a higher intake of monounsaturated fatty acid (MUFA) and carbohydrate, and there was an elevated level of serum leptin among *del*-allele carriers with high protein, polyunsaturated fatty acid (PUFA), MUFA,

and saturated fatty acid (SFA) intake[7,48]. Another study on healthy individuals revealed an increased level of TC among *del*-allele carriers with higher PUFA and SFA intake[7,30]. Since the majority of studies concerned the role of DAL or *ApoB* in T2DM and its complications independently, and due to the insufficient data regarding the possible interactions between *ApoB* genetic variants and DAL on anthropometric and metabolic markers, we intended to investigate the interaction between *ApoB ins/del* genetic variant (rs11279109) and DAL including PRAL/NEAP on anthropometric measurements, and metabolic markers among patients with T2DM.

## Materials and methods

### Study design and subjects

This study is part of a larger cross-sectional investigation on patients with T2DM from the Iranian Diabetes centers conducted in Tehran[49]. 3100 patients with type 2 diabetes mellitus attended Iranian Diabetes centers, where 1100 individuals were ultimately excluded due to exclusion criteria and 2000 patients remained for the genetic assessment. Accordingly, 700 patients (276 men, 424 women) were included based on both genotyping and inclusion criteria using a simple random sampling method (Fig. 1). More details on the required sample size calculation are provided in the "Statistical analysis" section. Participants aged 35–65 with fasting blood

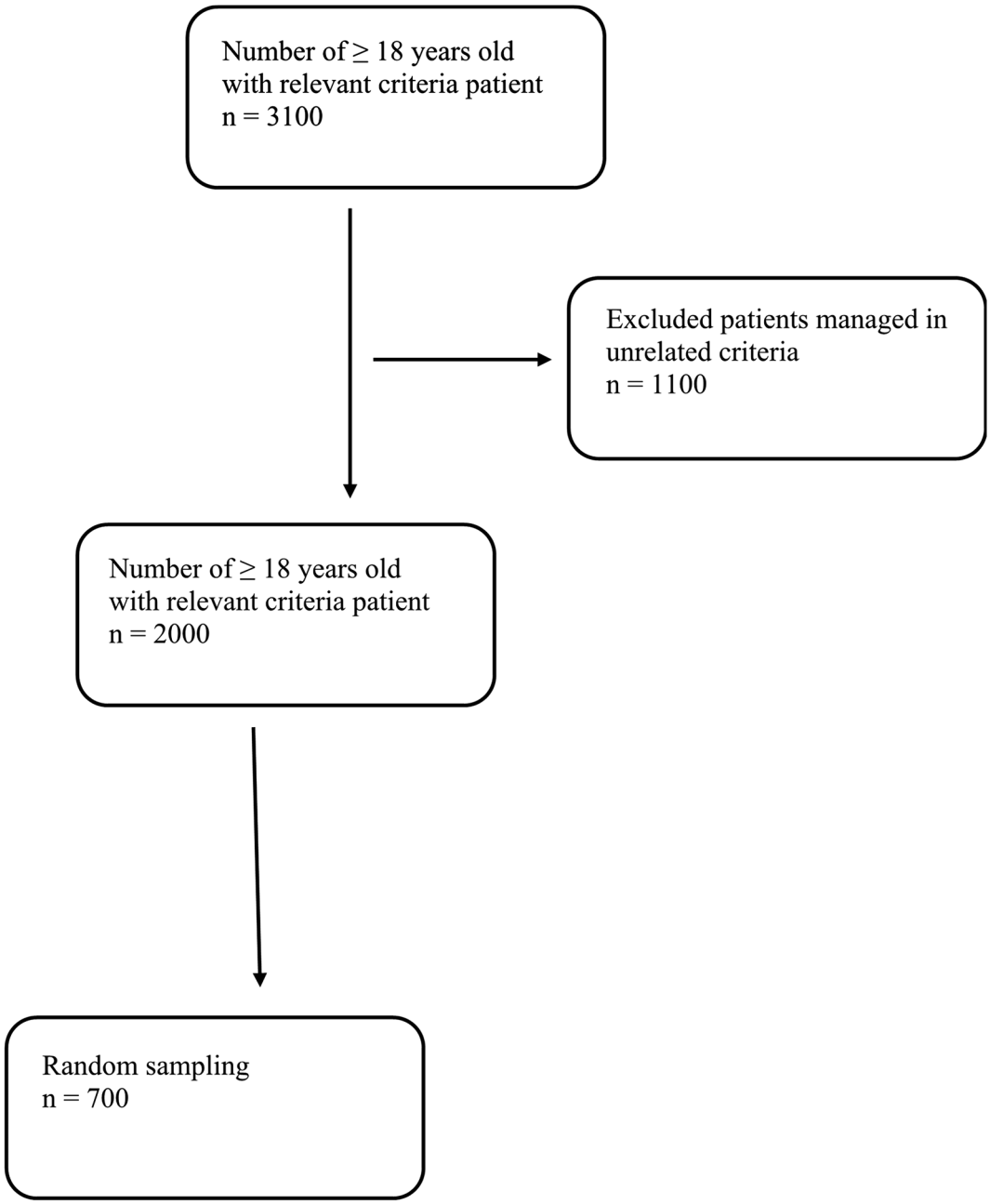


**Fig. 1**. The study sample enrollment.

glucose ≥ 126 mg/dl, consuming drugs for diabetes treatment, or both were included after taking written informed consent by explaining the purpose of the study. Patients aged under 35 and over 65 years, having any other chronic conditions, insulin use, pregnancy, lactation, and alcohol consumption 24 h before collecting blood samples were excluded. This study was approved by the Ethics Committee of Tehran University of Medical Sciences with a protocol number of IR.TUMS.VCR.REC.1395.15060 and was conducted based on the Declaration of Helsinki.

## Assessment of anthropometric measures and physical activity

The estimation of weight and height was based on minimal clothing by Seca falcon scales, with an accuracy of 100 gr and 0.5 cm, respectively. The measurement of a midpoint between the lower edge of the chest and the upper edge of the iliac crest and the last rib formed waist circumference (WC). The division of weight (kg) by the square of height ($m^2$) determined body mass index (BMI). Subjects were considered underweight, normal, and overweight/obese if their BMI values were < 18·5, 18.5–24.9. and 25 ≤ kg/m2, respectively[50]. Metabolic equivalent task (MET h/day) was used for physical activity calculation[8] via a validated physical activity questionnaire[51].

## Dietary intake and dietary acid load assessment

The regular dietary intake of participants was evaluated throughout the last year for 147 food items by a trained dietitian via interviews and using a semi-quantitative food frequency questionnaire (FFQ) which was validated by Mirmiran et al.[52]. The amounts listed for each food were converted to grams per day using household measures[53]. Two common scores including PRAL and NEAP, were used and categorized into tertiles for evaluation of DAL. The NEAP score includes total protein and potassium intake as the major acid-producing foods and the PRAL estimates the intestinal absorption rates for protein, potassium, calcium, magnesium, and phosphate. We used both scores for the DAL assessment.

The NEAP and PRAL scores were assessed by the following previously established algorithms[54,55]:

$$\begin{aligned}
\mathrm{NEAP\ (mEq/day)} &= (54.5 \times \mathrm{protein\ (g/day)}/\mathrm{potassium\ (mEq/day)}) - 10.2\\
\mathrm{PRAL\ (mEq/day)} &= (0.49 \times \mathrm{protein\ (g/day)})\\
&\quad + (0.037 \times \mathrm{phosphorus\ (mg/day)})\\
&\quad - (0.021 \times \mathrm{potassium\ (mg/day)})\\
&\quad - (0.026 \times \mathrm{magnesium\ (mg/day)})\\
&\quad - (0.013 \times \mathrm{calcium\ (mg/day)})
\end{aligned}$$

## Biochemical assessment and genotyping

Whole blood samples were obtained after 12-h fasting. The enzymatic method was utilized for measuring serum TC and TG (using kits from Pars Azmoon, Iran). Turbidimetry on a Roche Hitachi analyzer (Roche, Germany) was carried out for HDL-C and LDL-C levels measurement. Also, the evaluation of leptin level was performed by the ELISA method (Bioassay Technology Co, China, and Mediagnost, Germany, respectively). Interleukin-18 (IL-18) was assessed by means of the ELISA method (Shanghai Crystal Day Biotech Co., Ltd). The sensitivity of the IL-18 ELISA kit was 28 ng/ml. Whole blood was used for isolating genomic DNA through the salting-out extraction method[56]. Polymerase chain reaction (PCR) was used to detect the *ApoB ins/del* (rs11279109) in a 25 µL mixture, containing 50 ng of DNA, 0.2 mM of each primer (Forward: 5'-CAGCTGGCGATGGACCCGCCG A-3' Reverse: 5'-ACCGGCCCTGGCGCCCGCCAGCA-3'), and 75 ng genomic DNA. PCR was done at 94 °C for 50 s and 64 °C for 90 s in 35 cycles. The genotypes were ascertained by 8% polyacrylamide gel electrophoresis[7].

## Statistical analysis

The sample size was computed through the following formula by considering the type I error of α = 0.05 and type II error of β = 80%:

$$\begin{aligned}
\mathrm{Sp2} &= [(\mathrm{n1}-1)\times \mathrm{SD} + (\mathrm{n2}-1)\times \mathrm{SD}]/[(\mathrm{n1}-1)+(\mathrm{n2}-1)-2]\\
&= [(30-1)\times(0.25)+(6-1)\times(0.53)]/[(71-1)+(6-1)-2]\\
&= 0.042;\ \mathrm{Sp} = 0.208\mathrm{d} = (\mu 1-\mu 2)/\left(\sqrt{}^{2}\times \mathrm{Sp}\right) = 0.5/(\sqrt{2}\times 0.208)\\
&= 1.7\\
\mathrm{N} &= (\mathrm{Z1}-\alpha/2 + \mathrm{Z1}-\beta)\,2/\mathrm{d};\\
&\quad \alpha = 0.05,\ 1-\beta = 0.05 = (1.96+0.84)\,2/1.7 = 5
\end{aligned}$$

The frequency of the *del* allele is unclear in the Iranian population. Provided that the frequency of the minor allele was 1–16% in a different population[57], here, 1% was chosen as the frequency of the *del* allele, hence, the minimum sample size for the present study was 500 subjects (5 / 0.01 = 500). Regarding the necessity of a large sample size for determining the gene-diet interaction, the final sample size was increased to 700 by reason of the indefinite distribution of the genetic variant and further increase of statistical power. The normality was checked by the Kolmogorov-Smirnov test. We analyzed variables among three genotype groups (*ins/ins*, *ins/del*, and *del/del*) and tertiles of indexes using Analysis of variance (ANOVA). The interaction effect of *ApoB ins/del* (rs11279109) and PRAL/NEAP scores on BMI, WC, TG, TC, LDL-C, HDL-C, LDL/HDL, IL-18, and leptin were tested using the generalized linear models (GLMs) test in three multivariate interaction models: (crude) model, P1 = P value with adjustments for potential confounding factors including age, sex, smoking, alcohol, lipid-

lowering medications, and P2 = P value with adjustments for variables in model 1 plus for job, education, family history of diabetes, glucose-lowering medications, physical activity, and energy intake. The data were analyzed by IBM SPSS software version 25 and P-value < 0.05 was considered statistically significant.

## Results

### Association of DAL with metabolic markers

700 patients with T2DM (Fig. 1) were studied in this cross-sectional study. Genotype distribution of *ApoB ins/del* (rs11279109) in the type 2 diabetes population was 67.1%, 29.4%, and 3.4% for *ins/ins*, *ins/del*, and *del/del* genotypes (rs11279109), respectively. 67.8%, 29.4%, and 2.7% of participants in *ins/ins*, *ins/del*, and *del/del* genotype groups were men, respectively. The genotype frequency had no deviation from Hardy–Weinberg equilibrium ($P=0.97$). Details of the baseline characteristic and biochemical variables between *ApoB ins/del* (rs11279109) genotypes are presented in Table 1. There were no significant differences according to *ApoB ins/del* genotypes, however, legume intake for patients with *ins/ins* and *ins/del* genotype was 19.33 gr/d. Also, those with *del/del* genotype consumed 26.12 gr/d which was more than other groups (Supp. 1).

Table 2 shows the general, biochemical, and anthropometric parameters of study participants in the tertiles of DAL. In particular, subjects in the highest tertile of NEAP and PRAL presented higher consumption of energy ($P_{NEAP}$ <0.001, $P_{PRAL}$ <0.001), protein ($P_{NEAP}$ <0.001, $P_{PRAL}$ <0.001), total fat ($P_{NEAP}$ = 0.005, $P_{PRAL}$ = 0.03) and carbohydrate ($P_{NEAP}$ <0.001, $P_{PRAL}$ = 0.01)). For dietary intake differences, a significant result was observed between tertile 1 and 3. In addition, participants with higher PRAL had lower HDL ($P_{PRAL}$ = 0.04) and physical activity ($P_{PRAL}$ = 0.03). Finally, men with the higher DAL (PRAL and NEAP) had higher estimated energy requirements (EER) ($P_{NEAP}$=0 0.03, $P_{PRAL}$ = 0.03). In terms of HDL, we did not observe a significant difference in a post-hoc analysis, but in EER for men, a significant result was observed between tertiles 2 and 3 of both NEAP and PRAL.

### The interaction between *ins/del* (rs11279109) and DAL (NEAP and PRAL) on metabolic factors

Figures 2 and 3 show the interaction between *ins/del* (rs11279109) and DAL (PRAL and NEAP) on several biochemical markers. Only in the PRAL method, the interactions of *ins/del* genetic variant (rs11279109) and DAL on the serum level of TC ($P_{interaction}$ = 0.04, P1 = 0,04, P2 = 0.04), LDL-C ($P_{interaction}$ = 0.004, P1 = 0,006, P2 = 0.003), LDL/HDL ($P_{interaction}$ = 0.02, P1 = 0,02, P2 = 0.03) and IL-18 ($P_{interaction}$ = 0.04, P1 = 0,04, P2 = 0.03) were statistically significant in both crude and adjusted models. *Del* allele carriers who were placed in the higher tertile of PRAL showed lower TC, LDL, LDL/HDL, and IL-18.

Furthermore, in the PRAL method, the interaction of gene and DAL on TG concentration was significant in crude ($P=0.04$) and adjusted model 1 (P1 = 0.04), but this interaction was not significant in model 2 (P2 = 0.06). Finally, a significant interaction was found between PRAL and *ins/del* (rs11279109) on serum levels of leptin just in the crude (P1 = 0.04) model, but in adjusted models (model 1, P1 = 0.23 and model 2, P2 = 0.28), this significant interaction was lost. In particular, patients with the *del/del* genotype (rs11279109) with high PRAL have lower TG, TC, LDL-C, LDL/HDL ratio, IL-18, and leptin.

Besides, *del* homozygotes with higher NEAP, had lower WC ($P=0.04$), LDL/HDL($P=0.04$), and TG ($P=0.04$) in the crude model but in adjusted models, this significant interaction was lost ($P>0.05$). No gene-diet interaction was found between DAL (NEAP and PRAL) and *ins/del* (rs11279109) in associations with other metabolic markers.

## Discussion

In the present study, the interaction effect of *ApoB ins/del* genetic variant (rs11279109) with DAL index on inflammation, leptin, lipid profile, and obesity was evaluated in type 2 patients with diabetes. Here, participants with greater adherence to DAL had significantly higher energy intake as well as consuming more protein, carbohydrate, total fat, and dietary cholesterol along with increased WC and decreased levels of HDL-C. Regarding the *ApoB ins/del* genetic variant (rs11279109), it may modulate the effect of DAL on cardio-metabolic biomarkers. In particular, the serum levels of TG, TC, LDL-C, IL-18, and LDL/HDL increased unexpectedly in *del/del* homozygotes (rs11279109) by less adherence to DAL. Considering the role of *ApoB ins/del* (rs11279109) in the modification of lipid profile and BMI, it is proposed as one of the main potential etiology for dyslipidemia and obesity[7,58–60]. By considering high levels of serum lipid profiles in *del/del* homozygotes (rs11279109), they are more vulnerable to dyslipidemia and CVDs, however, TG, LDL-C, and TC levels raised by the observation of Vimaleswaran et al. with regard to *ins/ins* homozygotes after dietary fat intake in healthy adults[61]. This controversy might be explained by the health status of subjects who participated in different studies.

It is suggested that the production and assemblage of VLDL are impaired in *del* allele carriers which leads to the storage of fat in hepatocytes and suppressing LDL-receptor secretion, resulting in promoting the synthesis of hepatic TG and *ApoB − 100* as well as lowering their clearance[62,63]. This condition provokes more TG uptake by adipocytes which has a direct relationship with their hypertrophy and hyperplasia[64,65]. Additionally, leptin production could be affected by the downregulation of *ApoB* synthesis which causes leptin resistance and obesity at the end[66], however, weight and BMI stability in the present study might be the rational explanation for the insignificant interaction between DAL, genotypes, and leptin given that the consumption of a low-calorie diet and the subsequent weight loss was proposed as one of the main mechanisms for decreasing leptin in patients with diabetes[67]. The significance of the interaction between the genetic variant and DAL on WC was also lost in the second step of confounders adjustment in the present study. The possible direct relationship between energy intake and WC has been reported previously[68–70] which controlling the effect of energy intake in the aforementioned interaction might be a clarification for such insignificance.

| ApoB ins/del (rs11279109) | | | | |
|---|---|---|---|---|
| | ins/ins | ins/del | del/del | P[a] |
| Sex (Male/ Female) | 173(67.8)/259(65.2) | 75(29.4)/123(31) | 7(2.7)/15(3.8) | 0.67 |
| Age (year) | 54.42 ± 6.56 | 53.32 ± 6.69 | 52.32 ± 5.68 | 0.07 |
| BMI (kg/m2 ) | 29.42 ± 4.86 | 29.21 ± 4.41 | 28.92 ± 3.35 | 0.79 |
| Energy intake (kcal/day) | 2514.39 ± 859.20 | 2576.85 ± 1051.54 | 2424.60 ± 803.45 | 0.63 |
| NEAP | -9.02 ± 0.25 | -9.01 ± 0.25 | -9.02 ± 0.28 | 0.76 |
| PRAL | -11.34 ± 20.49 | -8.72 ± 20.62 | -13.15 ± 27.16 | 0.28 |
| HDL-c(mg/dl) | 52.63 ± 11.63 | 53.80 ± 11.94 | 49.77 ± 9.7 | 0.22 |
| LDL-c(mg/dl) | 107.02 ± 33.91 | 112.81 ± 33.67 | 114.86 ± 56.07 | 0.11 |
| CH(mg/dl) | 190.73 ± 61.13 | 199.89 ± 66.70 | 190.86 ± 97.12 | 0.24 |
| LDL/HDL | 2.47 ± 8.23 | 3.08 ± 13.08 | 2.30 ± 0.89 | 0.76 |
| TG(mg/dl) | 174.30 ± 99.82 | 173.87 ± 90.59 | 193.18 ± 137.82 | 0.67 |
| Leptin(ng/ml) | 24.69 ± 13.97 | 26.96 ± 16.09 | 27.77 ± 15.98 | 0.53 |
| IL-18(pg/ml) | 248.07 ± 29.01 | 251.18 ± 27.60 | 256.31 ± 37.07 | 0.71 |

**Table 1**. Baseline characteristic and metabolic markers according to ApoB ins/del (rs11279109) genotype in T2DM patients. Data are presented as mean ± standard deviation (SD) or percent. Abbreviation: potential renal acid load (PRAL), net-endogenous acid production (NEAP), BMI: body mass index, HDL-c high-density lipoprotein cholesterol, LDL-c = low-density lipoprotein cholesterol, CH = cholesterol, TG =triglyceride, IL-18 = interleukin 18, a Obtained from ANOVA or Chi-square test, where appropriate.

| Tertile of PRAL | | | | | Tertile of NEAP | | | |
|---|---|---|---|---|---|---|---|---|
| | T1 | T2 | T3 | | T1 | T2 | T3 | |
| Variables | (-97.83 - -16.35) | (-16.34 - -1.83) | (-1.82–62.75) | $P^a$ | (-9.76 - -9.15) | (-9.14 - -8.94) | (-8.93 - -8.09) | $P^a$ |
| Age (year) | 54.38 ± 6.12 | 53.44 ± 6.78 | 54.24 ± 6.83 | 0.27 | 54.25 ± 6.39 | 53.69 ± 6.47 | 54.07 ± 6.92 | 0.66 |
| BMI (kg/m2 ) | 29.39 ± 4.74 | 29.13 ± 4.58 | 29.51 ± 4.73 | 0.68 | 29.32 ± 4.88 | 29.13 ± 4.27 | 29.59 ± 4.88 | 0.60 |
| WC (cm) | 91.35 ± 10.75 | 91.49 ± 10.53 | 93.53 ± 10.76 | 0.06 | 91.34 ± 11.44 | 91.46 ± 9.59 | 93.56 ± 10.95 | 0.05 |
| Physical activity (MET min/week) | 38.99 ± 5.77 | 37.11 ± 4.64 | 37.18 ± 5.88 | **< 0.001** | 38.27 ± 5.12 | 37.90 ± 5.46 | 37.09 ± 5.92 | 0.07 |
| Energy intake (kcal/day) | 2589.30 ± 906.66 | 2308.30 ± 699.40 | 2693.66 ± 1073.96 | **< 0.001** | 2377.62 ± 825.28 | 2477.41 ± 756.36 | 2735.76 ± 1104.97 | **< 0.001** |
| Protein (g/d) | 86.49 ± 29.16 | 81.11 ± 27.29 | 100.33 ± 41.64 | **< 0.001** | 77.62 ± 25.13 | 87.76 ± 26.31 | 102.46 ± 43.42 | < 0.001 |
| Carbohydrate (g/d) | 352.42 ± 135.53 | 306.44 ± 97.04 | 357.10 ± 175.10 | **< 0.001** | 325.87 ± 125.29 | 328.97 ± 109.94 | 361.01 ± 177.47 | **0.01** |
| Total Fat (g/d) | 103.20 ± 47.42 | 91.36 ± 38.03 | 103.26 ± 43.98 | **0.005** | 94.59 ± 42.57 | 97.85 ± 41.28 | 105.35 ± 46.34 | **0.03** |
| Cholesterol (g/d) | 205.17 ± 86.10 | 221.38 ± 293.27 | 240.43 ± 114.42 | 0.15 | 189.05 ± 76.71 | 232.43 ± 291.51 | 245.30 ± 120.43 | **0.005** |
| EER.(Men) | 2564.45 ± 284.64 | 2549.62 ± 257.21 | 2651.21 ± 296.91 | **0.03** | 2540.61 ± 290.02 | 2570.60 ± 251.01 | 2650.54 ± 300.22 | **0.03** |
| EER.(Women) | 2002.60 ± 175.97 | 2018.67 ± 170.66 | 2037.69 ± 206.77 | 0.31 | 2004.3 ± 186.09 | 2011.24 ± 157.48 | 2045.43 ± 206.06 | 0.17 |
| HDL-c(mg/dl) | 54.46 ± 12.58 | 51.90 ± 11.25 | 52.30 ± 11.04 | **0.04** | 54.41 ± 12.59 | 52.23 ± 11.34 | 51.96 ± 10.94 | **0.05** |
| LDL-c(mg/dl) | 112.11 ± 39.50 | 106.76 ± 31.88 | 108.24 ± 32.56 | 0.25 | 109.83 ± 37.86 | 109.84 ± 34.36 | 106.90 ± 31.20 | 0.59 |
| CH(mg/dl) | 191.07 ± 58.87 | 198.66 ± 72.11 | 190.81 ± 61.29 | 0.35 | 189.12 ± 57.04 | 201.85 ± 73.06 | 189.09 ± 61.11 | 0.05 |
| LDL/HDL | 2.11 ± 0.71 | 2.89 ± 11.59 | 2.96 ± 12.49 | 0.60 | 2.85 ± 11.62 | 2.16 ± 0.69 | 2.94 ± 12.49 | 0.66 |
| TG(mg/dl) | 177.28 ± 98.40 | 170.81 ± 92.84 | 176.33 ± 104.36 | 0.76 | 172.23 ± 95.12 | 176.27 ± 96.64 | 175.71 ± 104.14 | 0.90 |
| Leptin(ng/ml) | 25.85 ± 13.93 | 25.55 ± 15.55 | 24.83 ± 14.68 | 0.91 | 26.55 ± 13.84 | 24.49 ± 14.62 | 25.40 ± 15.85 | 0.67 |
| IL-18 (pg/ml) | 251.98 ± 32.58 | 246.58 ± 27.20 | 249.91 ± 26.43 | 0.62 | 254.03 ± 32.70 | 243.81 ± 25.48 | 250.17 ± 26.66 | 0.18 |

**Table 2**. The association between metabolic markers with NEAP and PRAL in T2DM patients. Abbreviation: potential renal acid load (PRAL), net-endogenous acid production (NEAP), BMI: body mass index, HDL-c high-density lipoprotein cholesterol, LDL-c = low-density lipoprotein cholesterol, CH = cholesterol, TG =triglyceride, IL-18 = interleukin 18. Significant values are in [bold].

According to previous studies, the Western Dietary Pattern, which consists of high amounts of acid-forming foods like refined grains, animal products, and dietary fats, was associated with DAL and might contribute to the acid-base imbalance[71,72] which is in line with findings of the present study. Besides, further odds of obesity were obtained over the median and quartiles of NEAP[73–75]. A marginally significant association was also suggested between DAL and WC by Murakami et al.[76].

A number of studies with inconsistent findings have considered the interaction effect of *ApoB ins/del* genetic variant (rs11279109) with dietary status on cardio-metabolic biomarkers. *Del* allele carriers had a higher level of TG and LDL-C together with lower BMI and WC by consuming more MUFA, carbohydrate, and n-3 PUFA, respectively[7,49]. An insignificant interaction was obtained between the *ApoB ins/del* genetic variant (rs11279109) and dietary inflammatory indexes (DII) on obesity by Mokhtary et al.[77], however, a significant change was proposed on LDL-C by considering the interaction of *ApoB ins/del* genetic variant (rs11279109) and DII in which the LDL level was elevated in *del* carriers by increasing the DII score[78].

In the current study, *del/del* homozygotes (rs11279109) consumed more protein foods significantly than other genotypes with an emphasis on legumes as a major part of protein consumption. Various studies proposed a protective role of legumes as opposed to the high levels of pro-inflammatory markers and serum lipid profile in accordance with ours. As indicated by Becerra et al. addition to others, a legume-rich diet tended to improve fasting serum TC, LDL-C, and TG[79–88], whereas a non-significant association was observed with serum lipid profile through inconsistent evidence[89]. Furthermore, an inverse linkage of legumes consumption and inflammatory markers e.g. hs-CRP, IL-6, and IL-18 were observed via multiple studies[80,82,90–93].

The precise underlying mechanisms of the aforementioned relationships are not clarified so far, yet, some attainable justifications might be available for the interaction between DAL and *ApoB ins/del* genetic variant (rs11279109) on the above-mentioned markers. On the one hand, a legume-rich diet might ameliorate serum lipid profile by means of high fiber content through fat absorption reduction and diminishes hepatic synthesis of cholesterol while binding to bile acid which assists in modifying lipid circulation[94,95]. On the other hand, decreased glycemic index and glycemic load of legumes, boost insulin sensitivity which hinders the mobilization of free fatty acids from adipose tissue in order to preserve low levels of LDL-C and TC[84]. Moreover, the level of blood cholesterol could be dropped by some specific phytochemicals like phytosterols which hydrolyzation of saponin to diosgenin might affect cholesterol absorption[96]. It was proposed that dietary fiber might reduce inflammation not only by downregulating lipid oxidation but also by normal bowel flora which could play a positive role in a healthy intestinal environment[82,97,98]. The Magnesium content of legumes may also contribute to inflammation suppression. Hypomagnesemia leads to inflammatory responses by several pathways which could contribute to the pathogenesis of various conditions such as diabetes, cardiovascular diseases, osteoporosis, and neurodegenerative diseases[99,100]. It could exacerbate inflammation by the activation of cellular oxidative stress, renin-angiotensin system, nuclear factor-κB signaling, phagocytic cells, and the transcription of cytokines and pro-inflammatory genes[101–114]. Accordingly, it seems that healthy eating habits and lifestyles might ameliorate the vulnerability of *del/del* homozygotes (rs11279109) for dyslipidemia. Even so, more investigations are

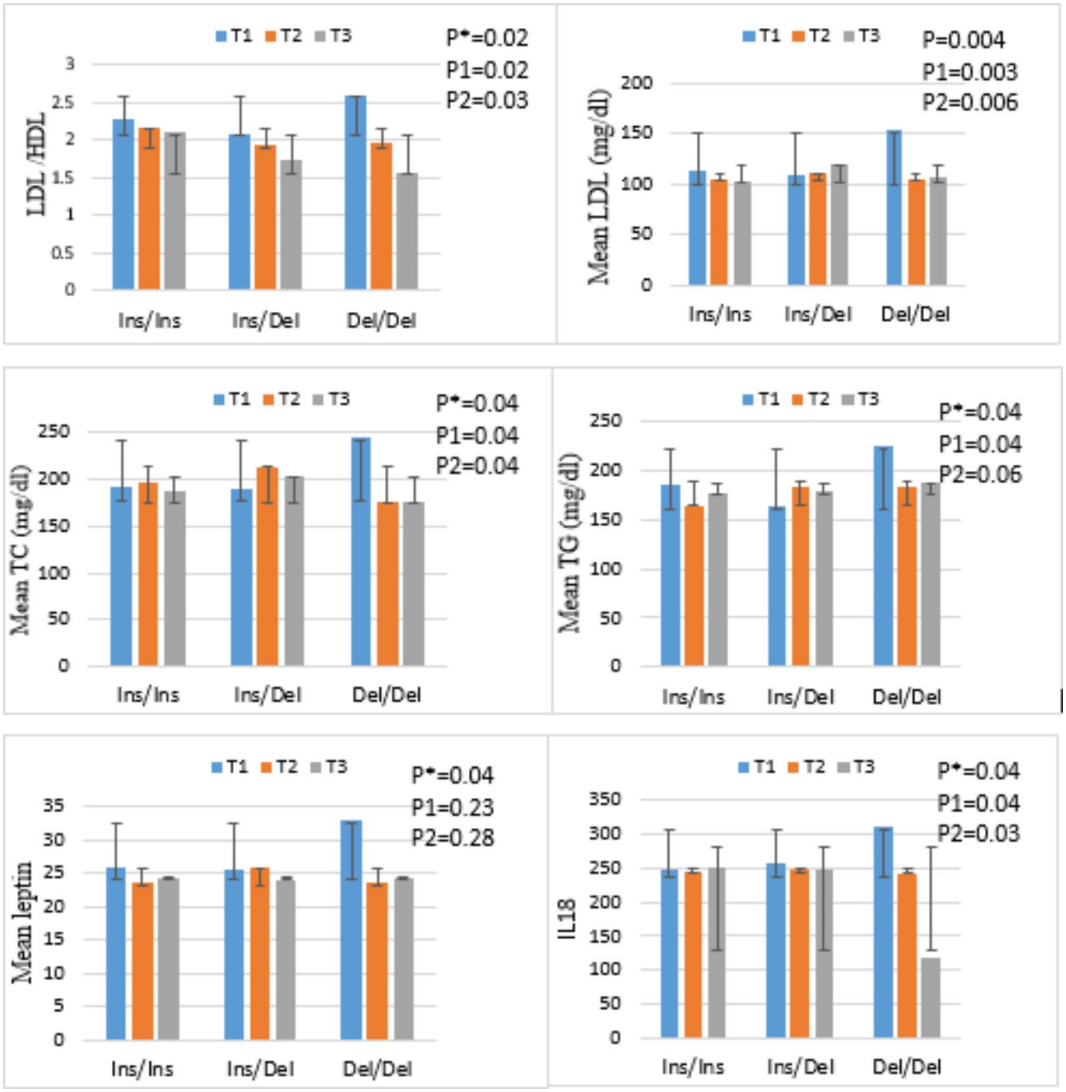


**Fig. 2**. Interaction effect between PRAL and *ApoB ins/del (rs11279109)* on LDL/HDL, LDL, TC, TG, Leptin, and IL-18. P* = P value with the unadjusted (crude) model, P1 = P value with adjustments for potential confounding factors including (age, sex, smoking, alcohol, lipid-lowering medications) P2 = P value with adjustments for variables in model 1 plus for (Job, education, family history of diabetes, glucose-lowering medications, physical activity, and energy intake).

required to shed light on the interaction of *ApoB ins/del* genetic variant (rs11279109) with DAL indexes on cardio-metabolic markers.

This investigation was the first attempt to study the interaction effect of *ApoB ins/del* genetic variant (rs11279109) with DAL indexes on cardio-metabolic markers among type 2 patients with diabetes, still, it had some limitations. First and foremost, this cross-sectional study cannot verify any causality about the observed interactions, and the serum level of *ApoB* was not measured here. Furthermore, the bias in the study such as recall bias and over-or under-reporting of participants couldn't be neglected since we used the FFQ for dietary intake evaluation.

## Conclusion

In conclusion, carriers of the *del/del* genotype of the rs11279109 variant in the *ApoB* may have higher serum concentrations of LDL/HDL, TG, TC, LDL-C, and IL-18 due to lower adherence to DAL, possibly associated with reduced legume consumption. However, high-quality intervention studies are needed to confirm this relationship. This perspective opens new avenues for understanding gene-diet interactions and underscores the importance of considering genetic variability when addressing metabolic health in patients with type 2 diabetes.

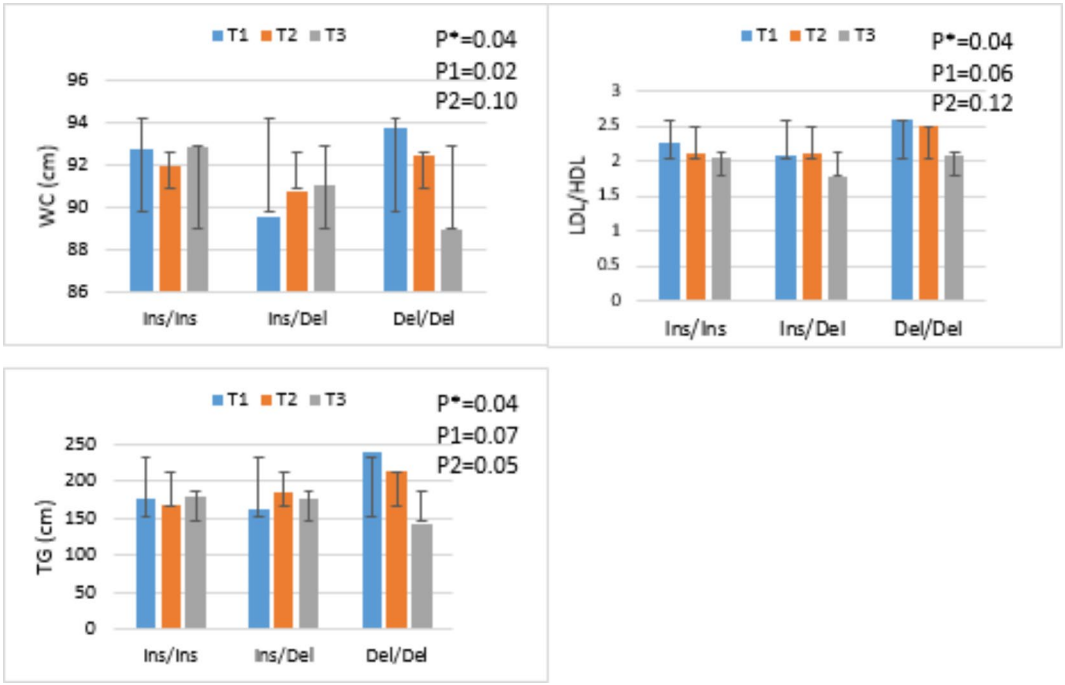


**Fig. 3**. Interaction effect between NEAP and *ApoB ins/del* (rs11279109) on WC, LDL/HDL, and TG. P* = P value with the unadjusted (crude) model, P1 = P value with adjustments for potential confounding factors including (age, sex, smoking, alcohol, lipid-lowering medications) P2 = P value with adjustments for variables in model 1 plus for (Job, education, family history of diabetes, glucose-lowering medications, physical activity, and energy intake).

## Data availability

Data described in the manuscript, code book, and analytic code will be made available upon request pending to Masoumeh Rafiee (masomeh.rafiei@gmail.com.)

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## Author contributions

Z. Esmaeily: Conceptualization, Methodology, Formal analysis, Investigation, Writing - Original Draft; F. Abaj: Conceptualization, Methodology, Formal analysis, Investigation, Writing - Original Draft; Z. Naeini: Conceptualization, Investigation, Writing - Original Draft; E. Alvandi: Performing the molecular experiments; M. Rafiee: Formal analysis, Writing – Editing, Interpretation of Data; F. Koohdnai: Conceptualization, Methodology, Supervision, Project administration.

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## Declarations

## Competing interests

The authors declare no competing interests.

## Ethics approval and consent to participate

This study was approved by the Ethics Committee of Tehran University of Medical Sciences with a protocol number of IR.TUMS.VCR.REC.1395.15060. Written consent was taken from participants by explaining the purpose of the study.

## Consent for publication

The consent of publication has been obtained from all participants.

## Additional information

**Supplementary Information** The online version contains supplementary material available at https://doi.org/10.1038/s41598-024-75352-x.

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