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# Habitual dietary methyl donor's intake and metabolic profile in obese individuals: a cross-sectional study

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**Considering the role of dietary methyl donor (DMD) in numerous biochemical processes, we hypothesized that DMD could play an important role in metabolic syndrome such as hyperlipidemia, hypertension, insulin resistance, and appetite in obese individuals. This cross-sectional study was conducted on 335 obese people. We collected dietary data using a valid and reliable 147-question Food Frequency Questionnaire (FFQ). Multivariate multinomial logistic regression was used to estimate the odds ratio (OR) and 95% confidence interval (CI) for the association between dietary methyl intake and cardio-metabolic risk factors. After adjusting for confounding variables, individuals at the fourth and third quartile of DMD, were more likely to have lower low-density lipoprotein cholesterol (LDL-C) (OR = 0.968, CI = 0.943–0.994, P = 0.015 and OR = 0.978, CI = 0.957–0.998, P = 0.03 respectively) versus first quartile. Also, total cholesterol (TC) showed a significant decrease in forth quartile of DMD in model III (OR = 0.974, CI = 0.951–0.997, P = 0.029). Current results suggested that, high DMDs' consumption, significantly associated with decreased risk of cardiometabolic risk factors.**

**Keywords** Diet, Methyl donor, Metabolic syndrome, Obesity

**Abbreviations**

| | |
|---|---|
| T2DM | Type 2 diabetes mellitus |
| CVD | Cardiovascular disease |
| MetS | Metabolic syndrome |
| DMD | Dietary methyl donors |
| SAM | S-adenosylmethionine |
| Hcy | Homocysteine |
| NASH | Nonalcoholic steatohepatitis |
| SES | socio-economic status |
| BIA | Bioelectrical impedance analysis |
| WC | Waist circumference |
| HC | Hip circumference |
| BMI | Body mass index |
| WHR | Waist-to-hip ratio |
| IPAQ | International Physical Activity Questionnaire |
| VAS | Visual analogue scale |
| FFQ | Food frequency questionnaire |
| USDA | US Department of Agriculture's |
| FBS | Fasting blood glucose |
| TG | Triglyceride |
| TC | Total cholesterol |
| HDL-C | High-density lipoprotein cholesterol |
| LDL-C | Low-density lipoprotein cholesterol |
| ELISA | Enzyme-linked immunosorbent assay |
| HOMA-IR | Homeostatic model assessment for insulin resistance |

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| | |
|---|---|
| QUICKI | Quantitative insulin sensitivity check index |
| ANOVA | One-way analysis of variance |
| ANCOVA | Analysis of covariance |
| OR | Odds ratio |
| CI | Confidence interval |

The prevalence of obesity as a chronic disease is increasing worldwide[1–3]. It has been shown that the combined number of overweight and obesity is more than 1.9 billion adults worldwide (r). Also, a study in Iran indicated that the prevalence of obesity among adults is 25%[4]. Obesity is linked to serious health problems like type 2 diabetes mellitus (T2DM)[5], cardiovascular disease (CVD)[6], metabolic syndrome (MetS)[7] and many types of cancer[8]. Also shown that the obesity can play an important role in increased risk of dyslipidemia, hypertension and insulin resistance[9]. Owing to the high prevalence of obesity and its related health outcomes, effective weight loss and maintenance strategies are necessary[2]. In addition to a wide range of unmodifiable factors such as genetics, environmental and gender, modifiable factors like diet and physical activity can play a remarkable role in the incidence of obesity[10,11]. The literature also suggests that micronutrients can participate in etiology of obesity[12,13]. Dietary methyl donors (DMD) are some nutrients such as vitamin B6 and B12, folate, betaine, choline and methionine, taking part in methylation cycle that produces S-adenosylmethionine (SAM) that can methylate different molecules and regulate multiple processes[14]. The one-carbon methylation cycle converts homocysteine (Hcy) to methionine[15]. Disturbance in this process can lead to a condition known as hyperhomocysteinemia (Serum Hcy levels > 15 µmol/L) and its related disease such as MetS, CVD and all-cause mortality, particularly in populations with abdominal obesity[16,17]. Epidemiological studies indicated that DMD are inversely associated with MetS, fatty liver and different types of cancer[3,18,19]. An inverse relationship between intake of folate and vitamin B12, but not B6, and blood pressure has been demonstrated in Japanese children[20]. Le Marchand et al.. suggested that higher intakes of folate and vitamin B6 are associated with a lower risk of colon cancer[21]. Also, a recent study indicated a negative relationship between vitamin B12 and folate levels and the severity of nonalcoholic steatohepatitis (NASH)[22]. Numerous experimental studies have also revealed the anti-adipogenic effects of methyl donors[23,24]. Similarly, various human studies have been conducted to investigate the relationship between DMD and the risk of obesity[2,25]. Based on available studies, both dietary and plasma methyl donors improve body composition[2,26]. A study among lean young males found that betaine supplementation with a 6-week concurrent progressive resistance training program was associated with improved body composition by both increased lean body mass and decreased fat mass[27]. In contrast, another study involving 34 young men did not find any change in body composition after betaine supplementation[28]. To the best of our knowledge, there are limited studies that evaluates the isolate effects of DMD in obesity, and the combined effects of all DMD in obese adults in Iran have not been investigated yet. Therefore, the current cross-sectional study was conducted to investigate the association between a combination of all of DMD on obesity and its related metabolic risk factors.

## Methods and materials

### Participants

In this cross-sectional study, the participants were those participated in two previous projects including 335 obese people from Tabriz and Tehran, Iran (57% males and 41% females)[29]. The study subjects were selected through public announcement using the convenience method and were enrolled if they met inclusion criteria (aged range 20–50 years old, BMI ≥ 30 kg/m2). The exclusion criteria were having recent bariatric surgery, CVD, cancer, renal and hepatic diseases, T2DM, thyroid diseases, history of weight changes ≥ 5 kg in the last six months, being pregnant, lactating, menopausal and taking any weight affecting medications. Trough one-to-one interview, all phases of recruitment and data collection were performed by a certified nutritionist. All participants provided written, fully informed satisfaction prior to participating in the study and the Ethics Committee of Tabriz University of Medical Sciences, Tabriz, Iran approved this study. (Registration number: IR.TBZMED.REC.1403.370).

### General characteristics and anthropometric assessments

We collected Socio-demographic information, including sex, age, smoking status, education attainment, marital status, occupation, medical history, and family size using a demographic questionnaire. Socio-economic status (SES) score was assessed by considering factors such as education level, family size, occupational status and home ownership[29]. Education was graded on a 0–5 Likert scale (illiterate: 0; less than diploma: 1; diploma and associate degree: 2; bachelor's degree: 3; master's degree: 4; and higher: 5). Participants were assigned 1, 2 and 3 points based on family size (family size of 3: 1, 4–5: 2 and more than 6: 3). Female and male participant's occupational status was categorized as follows: housewife: 1, employee: 2, student: 3, self-employed: 4, and others: 5 and unemployed: 1; laborer, farmer, and rancher: 2; others: 3; employee: 4; and self-employed: 5, respectively. In addition, if they were a landlord or a tenant, they would get a score of 2 or 1. Finally, a total SES score ranging from 0 to 15 was given to each participant. An expert researcher administered all surveys related to anthropometric indicators in one day for each participant. Body composition was evaluated by bioelectrical impedance analysis (BIA) method (Tanita, BC-418 MA, Tokyo, Japan). The height and weight of the participants were assessed using a wall-mounted stadiometer and a Seca scale (Seca co., Hamburg, Germany) and the results were rounded to the nearest 0.5 cm and 0.1 kg, respectively. A fixed tension tape was used to measure each participant's waist circumference (WC) and hip circumference (HC) at the midpoint between the lower costal edge and the iliac crest and the widest part of the hip, respectively. Furthermore, the body mass index (BMI) and waist-to-hip ratio (WHR) were calculated. The concise form of the International Physical Activity Questionnaire (IPAQ) was used to evaluate the level of physical activity[30,31]. Visual analogue scale (VAS) was used to evaluate the state of the appetite in fasting state in the morning. We calculated VAS by marking a 100-mm line at each end

of the line with the opposing words "I'm not at all hungry" and "I have not been so hungry." This questionnaire asked about hunger, satiety, fullness, and future food intake as well as cravings for sweet, salty, and fatty foods[32]. The appetite state was the distance between the left side of the line and the mark. Blood pressure was measured twice in the same arm using a mercury sphygmomanometer after at least 15 min of rest, and the average of two measurements was used for analysis.

### Dietary assessments

A semi-quantitative food frequency questionnaire (FFQ) of 147 food items adapted for the Iranian population was used to collect dietary information[33]. The FFQ was a list of food items that frequently consumed in specified portion sizes in Iran. An expert nutritionists asked participants to report frequency and amount of each food item they consumed on a daily, weekly, monthly, or yearly basis during the prior year. Then, by using household measures, the reported frequency of consumed foods and portion sizes for each food items were converted to grams. The US Department of Agriculture's (USDA) national nutrient databank used to analyze daily energy and nutrient contents.

### Calculation of methyl donor micronutrients intake

Total DMD intake was calculated from 6 dietary micronutrients including vitamin B6 and B12, folate, betaine, choline and methionine[14]. First, we used USDA national nutrient databank to extract the amount of these micronutrient in each food items. Then, total intake of DMD was calculated for each participant. Participants were divided into quartiles based on the total amount of DMD. Based on the quartile of participants methyl donor intake, the score 1 to 4 has been considered in ascending order.

### Biochemical assessment

After 12 h of fasting, each subject gave a 10 ml venous blood sample, which was centrifuged at 4500 rpm for 10 min at 4 °C to separate serum and plasma. The samples were kept at -70 °C until analysis. A commercially available kit was used to assess fasting blood glucose (FBS), triglyceride (TG), serum total cholesterol (TC) and high-density lipoprotein cholesterol (HDL-C) (Pars Azmoun, Tehran, Iran). Additionally, low-density lipoprotein cholesterol (LDL-C) levels were determined by the Friedewald Eq. (34). Serum insulin concentrations were evaluated with Enzyme-linked immunosorbent assay (ELISA) kits (Bioassay Technology Laboratory, Shanghai Korean Biotech, Shanghai City, China). We calculated homeostatic model assessment for insulin resistance (HOMA-IR) using the formula: fasting insulin (μIU/ml) ×fasting glucose (mmol/L) /22.5 and quantitative insulin sensitivity check index (QUICKI) as 1/ [log fasting insulin (μU/mL) + log glucose (mmol/L)]. The cutoff value for these variables was defined according to the guidelines of the National Cholesterol Education Program Adult Treatment Panel III (NCEP-ATP III)[35,36]: LDL-C ≥ 100 mg/dl; HDL-C < 50 mg/dl in women and < 40 in men; TC ≥ 200 mg/dl; TG ≥ 150 mg/dl; FBS ≥ 100 mg/dl; SBP ≥ 130 mmHg or DBP ≥ 85 mmHg.

### Statistical analyses

Statistical Package for Social Sciences (version 21.0; SPSS Inc.,) was used to carry out statistical analysis with a statistical significance level of $< 0.05$. In order to compare the difference between discrete and continuous variables among different DMD quartiles, chi-square and one-way analysis of variance (ANOVA) were used. We compared biochemical variables after adjustment for confounding factors such as age, gender, BMI, PA and energy intake using analysis of covariance (ANCOVA). Multivariate multinomial logistic regression was used to estimate the odds ratio (OR) and 95% confidence interval (CI) for the association between DMD intake and cardio metabolic risk factors. This risk was reported in the three different models including: Model I: crude, Model II: adjusted for age and sex, Model III: adjusted for age, BMI, sex, physical activity, SES and energy intake.

## Result

This cross-sectional study included 335 obese people with an average BMI of $32.6 \pm 9$ and an age range of $40.7 \pm 4$ years; 57% of whom were men. A comparison of general characteristics of study participants based on different DMD quartiles is shown in Table 1. Participants in the last quartile of DMD had higher WHR ($P = 0.03$), FFM ($P = 0.04$) and energy intake ($p < 0.01$). Also, men were more likely to be in at higher quartiles of DMD ($P = 0.01$). Other general characteristics were not significantly different between DMD categories. In addition, after multivariable adjustment, no significant difference was observed between cardiometabolic factors except of serum LDL-C level (0.04). Table 2 indicates dietary energy, macronutrient and some selected nutrients intake of study participants across quartiles of DMD intake after adjustment for some confounders such as age, gender, BMI, PA and energy intake. Except for fat ($P = 0.10$), the intake of total energy ($p < 0.01$), protein ($p < 0.01$), dietary fiber ($p < 0.01$), n-3 fatty acids ($p < 0.01$), thiamin ($p < 0.01$), iron ($p < 0.01$), red meat ($P = 0.03$), grains ($p < 0.01$), fruits ($p = 0.06$), vegetable ($p < 0.01$), beans ($p < 0.01$) and diary ($p < 0.01$) were significantly higher among subjects in the last quartile of DMD. Table 3 represents the odds of biochemical variables in highest versus lowest quartiles of DMD. Accordingly, those at the forth and third quartile of DMD, were more likely to have lower LDL-C (OR = 0.968, CI = 0.943–0.994, $P = 0.015$ and OR = 0.978, CI = 0.957–0.998, $P = 0.03$ respectively) versus first quartile in age, sex, PA, SES and energy intake-adjusted model. Also, TC showed a significant decrease in forth quartile of DMD in model III (OR = 0.974, CI = 0.951–0.997, $P = 0.029$). In addition, based on the crude and sex and age -adjusted model, participants in the higher DMD quartiles showed higher levels of appetite (OR = 1.062, CI = 1.013–1.113, $P = 0.013$ and OR = 1.055, CI = 1.006–1.107, $P = 0.027$ respectively) than those in the lower quartiles. There was no significant association between other biochemical variables and DMD quartiles.

| Variable | All participants $n=335$ | Quartile of DMD | | | | |
|---|---|---|---|---|---|---|
| | | $Q_1$ ≤2000.88 mg | $Q_2$ 2000.88–2516.28 mg | $Q_3$ 2516.28–3184.15 mg | $Q_4$ ≥3184.15 mg | *P** value |
| | Mean ± SD | Mean ± SD | Mean ± SD | Mean ± SD | Mean ± SD | |
| Age (y) | 40.78 ± 9.23 | 40.33 ± 8.94 | 41.55 ± 9.9 | 40.16 ± 8.02 | 39.75 ± 9.24 | 0.611* |
| Gender (% Male) | 191 (57%) | 43 (51.2%) | 41 (49.4%) | 51 (60.0%) | 56 (67.5%) | **0.014**** |
| Education (12 ≤) | 143 (76.4%) | 38 (80%) | 32 (76.1%) | 39 (78.0%) | 34 (70.8%) | 0.571** |
| Marital status (% Single) | 45 (13.4%) | 13 (15.5%) | 7 (8.4%) | 9 (10.6%) | 16 (19.3%) | 0.368** |
| Family size (%) (> 4) | 25 (13.2%) | 6 (12.8%) | 5 (11.9%) | 8 (16%) | 6 (12.2%) | 0.371** |
| BMI ($kg/m^2$) | 32.67 ± 4.80 | 32.32 ± 4.79 | 31.87 ± 4.11 | 33.84 ± 5.61 | 32.69 ± 4.54 | 0.186* |
| WC (cm) | 106.78 ± 9.62 | 105.72 ± 8.79 | 103.75 ± 8.56 | 109.54 ± 10.69 | 107.76 ± 9.42 | **0.001*** |
| WHR | 0.96 ± 0.07 | 0.92 ± 0.07 | 0.91 ± 0.08 | 0.93 ± 0.07 | 0.95 ± 0.05 | **0.039*** |
| FM (%) | 33.81 ± 9.13 | 33.45 ± 7.57 | 32.53 ± 7.57 | 37.26 ± 11.61 | 31.71 ± 8.05 | **0.013*** |
| FFM (%) | 62.25 ± 12.35 | 59.41 ± 12.06 | 59.67 ± 13.00 | 64.70 ± 12.04 | 64.70 ± 11.68 | **0.041*** |
| Physically active (%) (moderate & high) | 98 (52.12%) | 25 (53.19%) | 21 (50.0%) | 26 (52.0%) | 26 (53.06%) | 0.774** |
| SES Score | 9.96 ± 2.51 | 9.63 ± 2.65 | 10.02 ± 2.73 | 9.86 ± 2.39 | 10.33 ± 2.29 | 0.589* |
| BMR (Kcal) | 1904.99 ± 396.79 | 1808.88 ± 329.33 | 1818.39 ± 361.75 | 1975.15 ± 344.22 | 1999.83 ± 496.77 | **0.026*** |
| Energy (Kcal) | 3016.71 ± 1094.81 | 1984.97 ± 437.30 | 2660.61 ± 534.40 | 3186.88 ± 594.11 | 4263.79 ± 1123.38 | **<0.001*** |
| Appetite | 33.58 ± 8.93 | 31.82 ± 9.85 | 33.23 ± 7.78 | 32.74 ± 8.20 | 36.42 ± 9.24 | 0.062* |
| MetS status (%) | 7.14 ± 1.04 | 7.08 ± 0.97 | 6.90 ± 0.86 | 7.32 ± 1.13 | 7.22 ± 1.14 | 0.257* |
| LDL (mg/dl) | 123.68 ± 31.83 | 126.88 ± 33.77 | 130.99 ± 32.92 | 118.08 ± 28.05 | 118.45 ± 32.21 | **0.042***** |
| HDL (mg/dl) | 43.32 ± 9.52 | 43.59 ± 9.33 | 54.51 ± 10.90 | 43.14 ± 9.09 | 42.09 ± 8.58 | 0.80*** |
| TG (mg/dl) | 152.55 ± 94.13 | 145.73 ± 81.80 | 146.00 ± 103.87 | 156.36 ± 110.19 | 156.02 ± 74.68 | 0.327*** |
| TC (mg/dl) | 191.45 ± 36.64 | 195.64 ± 39.49 | 197.65 ± 38.09 | 185.54 ± 34.36 | 188.65 ± 34.81 | 0.173*** |
| FBS (mg/dl) | 92.66 ± 19.18 | 90.52 ± 12.92 | 91.96 ± 16.11 | 95.32 ± 27.57 | 93.33 ± 18.03 | 0.600*** |
| Insulin (mIU/l) | 16.17 ± 13.66 | 16.23 ± 10.94 | 14.49 ± 9.90 | 16.64 ± 11.60 | 16.17 ± 19.41 | 0.490*** |
| SBP (mmHg) | 122.99 ± 16.35 | 123.29 ± 15.04 | 120.71 ± 13.88 | 125.29 ± 16.53 | 120.77 ± 18.43 | 0.658*** |
| DBP (mmHg) | 81.78 ± 11.69 | 82.28 ± 11.53 | 80.98 ± 9.45 | 82.84 ± 13.07 | 80.02 ± 12.44 | 0.996*** |
| HOMA-IR | 3.76 ± 3.26 | 3.69 ± 2.74 | 3.44 ± 2.70 | 4.05 ± 3.04 | 3.69 ± 4.22 | 0.453*** |
| QUICKI | 0.32 ± 0.03 | 0.33 ± 0.03 | 0.33 ± 0.03 | 0.32 ± 0.03 | 0.33 ± 0.03 | 0.965*** |

**Table 1**. General demographic characteristics of study participants by quartile of DMD. DMD, dietary methyl donors; BMI, Body mass index; WC, Waist Circumference; WHR, waist-to-hip ratio; FM, Fat Mass; FFM, Fat Free Mass; SES, socio-economic status; BMR, Basal Metabolic Rate; LDL-C, Low Density Lipoprotein Cholesterol; HDL-C, High Density Lipoprotein Cholesterol; TG, Triglyceride; TC, Total Cholesterol; FBS, fasting blood sugar; SBP, Systolic Blood Pressure; DBP, Diastolic Blood Pressure; HOMA-IR, Homeostatic Model Assessment for Insulin Resistance; QUICKI, Quantitative Insulin sensitivity Check Index; all data are mean (± SD) except Gender, marital status, education, physically active and Family size, that is presented as the number and percent of single and males respectively in each group. P* values derived from One-Way ANOVA with Tukey's post-hoc comparisons. ** P values derived from chi-squared test. P*** values derived from One-Way ANOVA with Tukey's post-hoc comparisons after adjustment for confounders (age, gender, BMI, PA and kcal).

| Variable | Quartile of DMD | | | | *P** Value | *P*** Value |
|---|---|---|---|---|---|---|
| | $Q_1$ ≤2000.88 mg | $Q_2$ 2000.88–2516.28 mg | $Q_3$ 2516.28–3184.15 mg | $Q_4$ ≥3184.15 mg | | |
| | Mean ± SD | Mean ± SD | Mean ± SD | Mean ± SD | | |
| Energy (Kcal/d) | 1984.97 ± 437.30 | 2660.61 ± 534.40 | 3186.88 ± 594.11 | 4263.79 ± 1123.38 | **< 0.001*** | **< 0.001** |
| Methyl donor nutrients | | | | | | |
| Vitamin B6 (mg/d) | 1.53 ± 0.38 | 2.09 ± 0.75 | 2.45 ± 0.50 | 3.25 ± 0.89 | **< 0.001*** | 0.422** |
| Folate (mg/d) | 476.83 ± 97.87 | 613.71 ± 132.65 | 754.35 ± 146.73 | 1045.19 ± 345.05 | **< 0.001*** | **0.021**** |
| Vitamin B12 (mg/d) | 2.92 ± 2.18 | 4.01 ± 3.04 | 5.68 ± 5.32 | 8.80 ± 9.12 | **< 0.001*** | **< 0.001**** |
| Methionine (mg/d) | 895.66 ± 173.08 | 1228.98 ± 200.41 | 1544.15 ± 233.82 | 2242.96 ± 691.61 | **< 0.001*** | **< 0.001**** |
| Choline (mg/d) | 197.85 ± 45.81 | 269.48 ± 51.76 | 336.42 ± 74.53 | 467.59 ± 118.06 | **< 0.001*** | **< 0.001**** |
| Betaine (mg/d) | 109.19 ± 58.34 | 146.32 ± 86.17 | 190.88 ± 103.00 | 301.28 ± 169.60 | **< 0.001*** | 0.189** |
| Other nutrients: | | | | | | |
| Proteins (%) | 12.64 ± 2.01 | 12.46 ± 1.83 | 13.49 ± 1.96 | 13.35 ± 1.93 | **0.023*** | **< 0.001**** |
| Fats (%) | 31.35 ± 7.29 | 32.15 ± 7.97 | 32.13 ± 6.69 | 31.09 ± 5.92 | 0.836* | 0.103** |
| Carbohydrates (%) | 58.96 ± 7.80 | 57.98 ± 7.67 | 57.01 ± 6.32 | 57.92 ± 5.88 | 0.590* | 0.554** |
| Fiber (g/d) | 43.83 ± 21.32 | 60.98 ± 28.83 | 69.66 ± 27.65 | 111.44 ± 56.01 | **< 0.001*** | 0.427** |
| n-3 fatty acids (g/d) | 0.97 ± 0.50 | 1.31 ± 0.73 | 1.67 ± 0.99 | 2.29 ± 1.49 | **< 0.001*** | 0.985** |
| Vitamin B1 (mg/d) | 1.71 ± 0.44 | 2.26 ± 0.60 | 2.74 ± 0.59 | 3.77 ± 1.16 | **< 0.001*** | 0.157** |
| Iron (mg/d) | 15.38 ± 3.90 | 21.76 ± 11.88 | 25.04 ± 5.85 | 33.07 ± 10.69 | **< 0.001*** | 0.450** |
| ***Food groups*** | | | | | | |
| Red meat (g/d) | 0.90 ± 0.82 | 1.14 ± 0.79 | 1.73 ± 1.39 | 2.16 ± 1.61 | **< 0.001*** | **0.039**** |
| Grains (g/d) | 9.03 ± 3.77 | 12.03 ± 4.71 | 14.88 ± 5.26 | 20.55 ± 7.42 | **< 0.001*** | 0.705** |
| Fruit (g/d) | 3.38 ± 3.02 | 3.90 ± 3.58 | 3.83 ± 1.99 | 5.46 ± 3.42 | **0.006*** | **0.019**** |
| Vegetables (g/d) | 2.33 ± 1.28 | 3.78 ± 1.68 | 4.08 ± 1.99 | 5.16 ± 2.79 | **< 0.001*** | 0.317** |
| Beans (g/d) | 0.48 ± 0.31 | 0. 51 ± 0.39 | 0.83 ± 0.56 | 1.09 ± 1.07 | **< 0.001*** | 0.537** |
| Dairy (g/d) | 1.31 ± 0.64 | 1.69 ± 0.95 | 2.18 ± 1.25 | 3.02 ± 1.50 | **< 0.001*** | **< 0.001**** |

**Table 2**. Dietary intakes of energy, macro and several micronutrients of study participants by quartile of DMD. DMD, dietary methyl donors. P* values derived from unadjusted ANCOVA P** values derived from ANCOVA after adjustment for confounders (age, gender, BMI, PA and energy intake).

## Discussion

The results of the present study which designed to examine the effect of DMD on cardiometabolic factors in obese individuals, showed more favorable body composition profile (lower fat mass and higher fat free mass) in higher quartiles of DMD. Our findings also revealed that the intake of DMD was inversely associated with LDL-C and TC levels. These associations were strengthened even after considering the potential confounders. However, no relation was found between DMD with other lipid profiles, FBS, insulin level and blood pressure. Furthermore, participants in the higher DMD quartile showed higher appetite level.

Obesity, as a multifactorial disorder, contributes to the increased risk of chronic diseases such as T2D, CVD, MetS and cancer[37]. Also, an increase in fasting plasma TG, high LDL-C, HDL-C, increased blood glucose and insulin levels, and high blood pressure occur following obesity[38,39]. These cardiometabolic factors can be modified using some dietary factors. There are a variety of sources of methyl donor micronutrients (vitamin B6 and B12, folate, betaine, choline and methionine[14]) among foods. For example, animal food sources like meats (including red meat, white meat and fish), eggs and dairy products which are the main sources of vitamin B6, B12, methionine and choline[40–42]. Folate is highly found in fruits, green leafy vegetables and legumes[40]. In addition, wheat bran, wheat germ, spinach and wheat bread are rich in betaine[42]. These nutrients might also be found in other food sources; thus, the total intake of these nutrients was obtained from the whole diet.

The beneficial effect of DMD on body composition and lipid profile (lower LDL-C and TC) was demonstrated in current study. In the study by Gao et al.[2]. , better body composition was indicated in individuals with higher consume of dietary choline and betaine in both women and men. Also, some other studies showed that lower serum vitamin-B12 and folate levels are associated with overweight and obesity[43–45]. A study by Rojas Cano has indicated that betaine as a methyl donor can improve lean body mass and reduce fat deposition of animals[46]. The mechanism of reducing fat deposition might be related to the modification of DNA methylation. Betaine supplementation has been shown to regulate the expression of genes (downregulated ACC and FAS mRNA expression) that lead to reduced fat deposition[47]. Additionally, betaine can provide methyl groups for the body to produce lecithin and carnitine, which can promote the metabolism of fat in the liver[46,48]. Another study by Gao indicated an inverse association between betaine and TG, TC and LDL-C levels[49]. Similarly, choline supplementation has been shown to normalize cholesterol metabolism and regulate the expression of genes involved in cholesterol transport and esterification[50]. In a separate study, serum betaine, but not choline, was

| Variable | | Quartile of DMD | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | | $Q_1$ ≤2000.88 mg | $Q_2$ 2000.88–2516.28 mg | | $Q_3$ 2516.28–3184.15 mg | | $Q_4$ ≥3184.15 mg | |
| | | | OR (CI) | P-value | OR (CI) | P-value | OR (CI) | P-value |
| LDL (mg/dl) | Model I | 1 REF | 1.004 (0.995–1.013) | 0.421 | 0.991 (0.981–1.001) | 0.071 | 0.991 (0.981–1.001) | 0.086 |
| | Model II | | 1.003 (0.994–1.013) | 0.477 | 0.991 (0.981–1.001) | 0.065 | 0.991 (0.981–1.001) | 0.075 |
| | Model III | | 0.996 (0.980–1.012) | 0.589 | 0.978 (0.957–0.998) | **0.033** | 0.968 (0.943–0.994) | **0.015** |
| HDL (mg/dl) | Model I | 1REF | 1.020 (0.989–1.053) | 0.208 | 0.995 (0.963–1.027) | 0.754 | 0.983 (0.951–1.016) | 0.296 |
| | Model II | | 1.021 (0.988–1.055) | 0.209 | 1.000 (0.967–1.035) | 0.982 | 0.992 (0.958–1.027) | 0.651 |
| | Model III | | 0.998 (0.939–1.061) | 0.955 | 0.987 (0.919–1.060) | 0.726 | 0.943 (0.860–1.034) | 0.213 |
| TG (mg/dl) | Model I | 1REF | 1.000 (0.997–1.004) | 0.984 | 1.001 (0.998–1.005 | 0.461 | 1.001 (0.998–1.005) | 0.476 |
| | Model II | | 1.000 (0.996–1.003) | 0.931 | 1.001 (0.998–1.004) | 0.589 | 1.001 (0.997–1.004) | 0.711 |
| | Model III | | 0.997 (0.987–1.007) | 0.522 | 1.003 (0.992–1.015) | 0.560 | 1.006 (0.993–1.020) | 0.375 |
| TC (mg/dl) | Model I | 1REF | 1.001 (0.993–1.010) | 0.725 | 0.992 (0.984–1.001) | 0.074 | 0.995 (0.986–1.003) | 0.218 |
| | Model II | | 1.001 (0.993–1.009) | 0.825 | 0.992 (0.984–1.001) | 0.068 | 0.995 (0.986–1.003) | 0.206 |
| | Model III | | 0.994 (0.979–1.010) | 0.474 | 0.983 (0.964–1.001) | 0.067 | 0.974 (0.951–0.997) | **0.029** |
| FBS (mg/dl) | Model I | 1REF | 1.006 (0.986–1.027) | 0.542 | 1.015 (0.996–1.034) | 0.128 | 1.011 (0.991–1.030) | 0.285 |
| | Model II | | 1.005 (0.984–1.027) | 0.613 | 1.015 (0.996–1.035) | 0.129 | 1.011 (0.991–1.013) | 0.280 |
| | Model III | | 1.029 (0.985–1.075) | 0.195 | 1.027 (0.981–1.075) | 0.249 | 1.022 (0.973–1.073) | 0.383 |
| Insulin (mIU/l) | Model I | 1REF | 0.987 (0.955–1.020) | 0.438 | 1.003 (0.979–1.028) | 0.788 | 1.000 (0.974–1.026) | 0.980 |
| | Model II | | 0.985 (0.951–1.020) | 0.397 | 1.003 (0.978–1.029) | 0.798 | 1.000 (0.974–1.028) | 0.979 |
| | Model III | | 0.957 (0.897–1.021) | 0.181 | 1.000 (0.930–1.076) | 0.998 | 0.989 (0.904–1.081) | 0.807 |
| SBP (mmHg) | Model I | 1REF | 0.990 (0.971–1.009) | 0.297 | 1.008 (0.989–1.028) | 0.401 | 0.990 (0.971–1.009) | 0.308 |
| | Model II | | 0.985 (0.965–1.006) | 0.162 | 1.008 (0.987–1.029) | 0.479 | 0.986 (0.966–1.007) | 0.191 |
| | Model III | | 0.999 (0.957–1.043) | 0.960 | 1.020 (0.974–1.067) | 0.402 | 1.000 (0.944–1.060) | 0.997 |
| DBP (mmHg) | Model I | 1REF | 0.990 (0.965–1.017) | 0.469 | 1.004 (0.978–1.031) | 0.748 | 0.984 (0.958–1.010) | 0.216 |
| | Model II | | 0.985 (0.958–1.013) | 0.300 | 1.004 (0.976–1.032) | 0.798 | 0.981 (0.954–1.009) | 0.179 |
| | Model III | | 1.020 (0.971–1.073) | 0.424 | 1.039 (0.983–1.099) | 0.179 | 1.042 (0.975–1.114) | 0.221 |
| HOMA-IR | Model I | 1REF | 0.969 (0.850–1.104) | 0.634 | 1.031 (0.929–1.144) | 0.568 | 1.000 (0.890–1.122) | 0.995 |
| | Model II | | 0.964 (0.841–1.105) | 0.599 | 1.031 (0.924–1.150) | 0.584 | 1.003 (0.891–1.129) | 0.963 |
| | Model III | | 0.864 (0.663–1.127) | 0.282 | 1.012 (0.753–1.361) | 0.935 | 0.935 (0.658–1.329) | 0.708 |
| QUICKI | Model I | 1REF | 1.030 (0.871–1.173) | 0.789 | 1.010 (0.971–1.073) | 0.450 | 1.010 (0.881–1.103) | 0.814 |
| | Model II | | 1.020 (0.981–1.083) | 0.789 | 0.969 (0.860–1.114) | 0.415 | 1.030 (0.991–1.163) | 0.938 |
| | Model III | | 1.030 (0.981–1.063) | 0.620 | 1.039 (0.950–1.104) | 0.936 | 1.020 (0.971–1.169) | 0.941 |
| Appetite | Model I | 1REF | 1.019 (0.971–1.069) | 0.448 | 1.012 (0.967–1.059) | 0.607 | 1.062 (1.013–1.113) | **0.013** |
| | Model II | | 1.017 (0.968–1.068) | 0.511 | 1.010 (0.964–1.058) | 0.675 | 1.055 (1.006–1.107) | **0.027** |
| | Model III | | 0.996 (0.939–1.057) | 0.900 | 0.967 (0.904–1.035) | 0.333 | 0.996 (0.911–1.088) | 0.928 |

**Table 3**. Biochemical variables of study participants by quartile of DMD. OR, odds ratio, CI, confidence interval, DMD, dietary methyl donors; LDL-C, Low Density Lipoprotein Cholesterol; HDL-C, High Density Lipoprotein Cholesterol; TG, Triglyceride; TC, Total Cholesterol; FBS, fasting blood sugar; SBP, Systolic Blood Pressure; DBP, Diastolic Blood Pressure; HOMA-IR, Homeostatic Model Assessment for Insulin Resistance; QUICKI, Quantitative Insulin sensitivity Check Index; The multivariate multinomial logistic regression was used for estimation of ORs and confidence interval (CI). Model I: crude, Model II: adjusted for age and sex, Model III: adjusted for age, BMI, sex, physical activity, SES and energy intake.

associated with improved cardio-metabolic risk factors, such as lower LDL-C and TG levels among older adults[51]. Additionally, in another study, choline supplementation was found to reduce serum TC and LDL-C in patients with T2DM[52]. Betaine supplementation may increase insulin- like growth factor (IGF-1) production as a result of stimulation of growth hormone secretion, leading to increased protein synthesis[53,54]. DMD can stimulate fat degradation in adipose tissue due to increased activity of hormone-sensitive lipase in pigs[55]. Also, the reduction of lipoprotein lipase mRNA expression decreases uptake of TG from circulating lipoproteins following betaine consumption[56]. Methyl donor micronutrients may also be related to fatty acid β-oxidation due to their role in carnitine biosynthesis by N-methylation of lysine[57]. In previous studies, betaine supplementation as a methyl donor had a decreasing effect on TC levels in the liver[47,58]. Some possible mechanistic pathways of the role of DMD in modifying cardiometabolic factors are presented in Fig. 1.

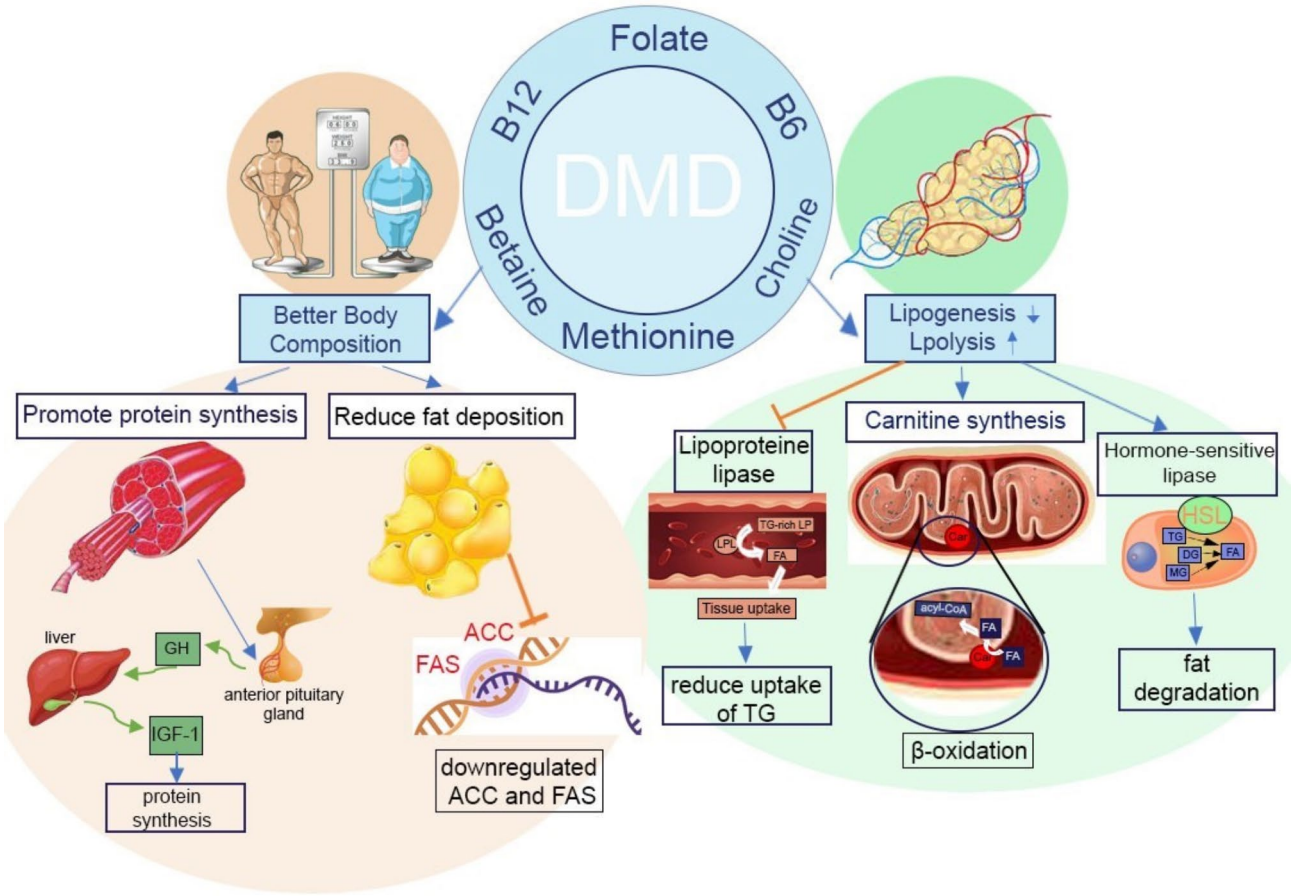


**Fig. 1**. Mechanistic pathways of the possible effects of dietary methyl donors (DMD) in body composition and lipid metabolism. DMD: Dietary methyl donors, HSL: Hormone-sensitive lipase, LPL: Lipoprotein lipase, TG: Triglyceride, DG: Diglyceride, MD: Monoglyceride, FA: Fatty acid, Car: Carnitine, ACC: Acetyl-CoA carboxylase, FAS: Fatty acid synthase, GH: Growth hormone, IGF-1: Insulin-like growth factor 1.

This dyslipidemia is one of the most common risk factors for CVD, which aggravates the atherosclerosis process[59]. A study of Ronco et al.. indicated a protective effect of 5-methyltetrahydrofolate and vitamin B-12 against LDL-oxidation[60]. Also, Pancharuniti et al.. suggested that Hcy may act as an initiator of LDL-C oxidation, which can lead to atherosclerosis by endothelial dysfunction[61].

The statistically significant effect size we observed, shows a relatively small association between the variables examined. However, we do not expect to see very large effects regarding the effect of food. Foods and nutrients often exert their influence on the body through subtle, chronic effects that may accumulate over time[62]. DMD may have seemingly small effects on health, but their impact can be significant. These nutrients play a crucial role in a process called methylation, which is essential for the regulation of gene expression, DNA repair, neurotransmitter production and many other vital functions in the body[63]. Inadequate intake of methyl donors can lead to impaired methylation processes, which has been linked to an increased risk of chronic disease[64,65]. Therefore, even though the effects of dietary methyl donors may seem subtle, their impact on health can be profound.

Our study has several strengths and weaknesses. It was the first study investigating the association between a combination of DMD intake and cardio-metabolic factors among Iranian obese adults. Plus, a trained nutritionist performed all phases of recruitment and data collection which made the evaluations more accurate. A remarkable number of potential confounding factors were also considered in the analysis to reach an autonomous relationship between DMD and cardio-metabolic factors. Nevertheless, some limitations should be considered while interpreting our findings. First, the cross-sectional design of the study makes it difficult to infer a causal and direction of the relationship; longitudinal studies are needed to better illustrate the cause-effect associations. Second, controlling for confounding factors were impeded in the absence of a control group. Third, our use of questionnaire-based data including FFQ raises the possibility of recall bias by subjects. However, the most widely used questionnaire for the assessment of dietary intake at the population level is FFQ and has been successfully adapted for the Iranian population[33].

The findings from this cross-sectional study suggest a slight favorable cardiometabolic effects of DMD. These results indicate that promoting increased consumption of DMD-rich foods could be a useful dietary strategy to help manage cardiometabolic factors. Also, if longitudinal studies can better illustrate the cause-effect association,

DMD supplementation could be explored as a potential adjunct therapy alongside lifestyle modifications. Overall, these findings warrant further investigation, but they provide initial evidence that integrating DMD intake into clinical practice could contribute to more effective approaches to the metabolic conditions of obesity.

## Conclusion

In conclusion, we witnessed more favorable body composition (lower FM and higher FFM) and lipid profile (lower LDL-C and TC levels) in higher quartiles of DMD among 335 individuals with obesity. However, further well-designed studies are warranted to illustrate better results.

## Data availability

The datasets generated and/or analyzed during the current study are not publicly available due to privacy and ethical considerations, but can be available from the corresponding author on reasonable request.

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## Author contributions

All authors approved the final version of the article. MAF contributed to study design, supervision, statistical analysis, and manuscript writing. FA was involved in writing the first draft of manuscript and English language revision. She also performed the statistical analysis. MM was involved in figure illustration and also edition.

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## Declarations

### Competing interests
The authors declare no competing interests.

### Ethics approval and consent to participate
All subjects provided a written informed consent before participation in the study. The study protocol was approved and registered by the ethics committee of Tabriz University of Medical Sciences (registration code: IR.TBZMED.REC.1403.370). We confirm that methods were performed in accordance with declaration of Helsinki's guidelines and regulations. Also, legal guardians of the illiterate participants provided a written informed consent.

### Consent for publication
Not applicable.

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