Nutrition 67–68 (2019) 110570

Contents lists available at ScienceDirect

Nutrition

journal homepage: www.nutritionjrnl.com

Applied nutritional investigation

Association of dietary acid load with cardiovascular risk factors and the prevalence of metabolic syndrome in Iranian women: A cross-sectional study

Hadis Mozaffari M.Sc. [a,b], Nazli Namazi Ph.D. [c], Bagher Larijani Ph.D. [d], Nick Bellissimo Ph.D. [e], Leila Azadbakht Ph.D. [a,c,f,*]

[a] Department of Community Nutrition, School of Nutritional Sciences and Dietetics, Tehran University of Medical Sciences, Tehran, Iran
[b] Students' Scientific Research Center, Tehran University of Medical Sciences, Tehran, Iran
[c] Diabetes Research Center, Endocrinology and Metabolism Clinical Sciences Institute, Tehran University of Medical Sciences, Tehran, Iran
[d] Endocrinology and Metabolism Research Center, Endocrinology and Metabolism Clinical Sciences Institute, Tehran University of Medical Sciences, Tehran, Iran
[e] School of Nutrition, Ryerson University, Toronto, Canada
[f] Department of Community Nutrition, School of Nutrition and Food Science, Isfahan University of Medical Science, Isfahan, Iran

ARTICLE INFO

Article History:
Received 4 August 2018
Received in revised form 11 August 2019
Accepted 13 August 2019

Keywords:
Potential renal acid load
Net endogenous acid production
Dietary acid load
Cardiovascular risks
Metabolic syndrome

ABSTRACT

Objective: Acid-base status, which can be affected by dietary acid load (DAL), has been associated with risk factors for cardiovascular disease (CVD) and metabolic syndrome (MetS). Given the limited published literature on DAL, the aim of this study was to examine the association between DAL and risk factors for CVD and prevalence of MetS in young women.

Methods: This was a cross-sectional study conducted with 371 women (20–50 y of age). Dietary intake was assessed using a food frequency questionnaire. DAL was evaluated through potential renal acid load (PRAL) and net endogenous acid production (NEAP). The associations between DAL (both PRAL and NEAP) with categories of biochemical factors (fasting blood sugar, triacylglycerol, high-density lipoprotein cholesterol, low-density lipoprotein cholesterol, total cholesterol), anthropometric parameters (body mass index and waist circumference) and the prevalence of MetS based on the National Cholesterol Education Program's Adult Treatment Panel III) were assessed using binary logistic regression in crude and adjusted models.

Results: The median values of PRAL and NEAP were 8.93 and 46.77 mEq/d, respectively. After adjustment for several covariates, a significant positive association was observed between PRAL and serum triglyceride levels (odds ratio [OR], 4.28; 95% CI, 1.67–10.99; $P = 0.002$). Moreover, there were positive associations between NEAP with overweight and obesity (body mass index $\geq$25 kg/m^2; OR, 3.07; 95% CI, 1.92–4.93; $P = 0.0001$), waist circumference (OR, 2.27; 95% CI, 1.37–3.75; $P = 0.001$), and serum triglyceride levels (OR, 4.92; 95% CI, 1.87–12.92; $P = 0.001$).

Conclusion: Compared with women with a low DAL score, women with a higher DAL score had higher weight, waist circumference, and triglyceride concentrations.

Introduction

There has been a global increase in the prevalence of cardiovascular disease (CVD) and metabolic syndrome (MetS), which has contributed to a significant increase in morbidity [1,2]. The global prevalence of CVDs is estimated to affect 422.7 million people [2]. Moreover, the worldwide prevalence of MetS is reported to affect 25% of adults, according to the International Diabetes Federation definition [1]. Both CVDs and MetS impose a substantial financial burden on health care systems [3] and contribute to both disability [2,4] and mortality [5]. Therefore, it is important to identify new approaches to treat and prevent CVDs and MetS. Dyslipidemia, insulin resistance (IR), hypertension, and central obesity are examples of risk factors for CVDs and MetS [6–9]. Unhealthy lifestyles such as physical inactivity, excessive alcohol consumption, smoking, and consumption of unhealthy diets increase the risk for CVDs or MetS [10–13].

Adherence to healthy dietary patterns can play a key role in preventing some chronic diseases including CVDs and MetS [14,15]. However, the focus should be on the dietary pattern rather than on specific foods as the dietary pattern can reflect the

This study was funded by the National Institute for Medical Research Development (grant no: 963403). The authors have no conflicts of interest to declare.
* Corresponding author: Tel.: +98 218 895 5563; Fax: +98 218 898 4861.
E-mail address: Azadbakhtleila@gmail.com (L. Azadbakht).

https://doi.org/10.1016/j.nut.2019.110570
0899-9007/

interactions between individual foods and food components [16–18]. Measuring the dietary acid load (DAL) is one approach that has been used for dietary acid–base evaluation [19]. Potential renal acid load (PRAL) and net endogenous acid production (NEAP) are two scores that provide an estimation of acid–base load from dietary intake information [20]. The PRAL score is calculated using dietary intakes of protein, potassium, calcium, magnesium, and phosphorous [20,21]. NEAP includes total protein and potassium, which are the important determinants of metabolic acidosis [22]. Both scores have been validated against objective measures of acid–base load determined from 24-h urine in healthy adults [21,22]. The median of NEAP value for a Western dietary pattern is estimated to be ~34 to 76 mEq/d [23], whereas the score for a vegan pattern is 7.26 mEq/d (formula of Remer and Manz [21]) or 15.6 mEq/d (formula of Frassetto et al. [22]) [24]. Therefore, a high DAL score may reflect a higher consumption of animal products and processed foods, which are not sufficiently compensated by the intake of fruits and vegetables. Animal products are typically high in both protein and phosphorus, thereby acting as potential inorganic acid precursors, which can affect endogenous acid production [19]. However, fruits and vegetables are categorized as alkaline foods due to their higher magnesium and potassium content [19]. If the consumption of higher acidic foods is not compensated by increased intake of alkaline foods, then this may lead to chronic metabolic acidosis [25]. The literature suggests that metabolic acidosis stimulates cortisol production [26,27], which has an adverse effect on risk factors associated with MetS and CVD [28,29].

To our knowledge, the literature on the association between DAL with risk factors of CVDs and MetS is limited. Moreover, the studies that have assessed the relationship between DAL and cardiometabolic risk factors are inconsistent [30–32]. In previous studies, DAL was positively associated with IR [30], waist circumference (WC) [31,32], triglycerides (TG) [31], low-density lipoprotein cholesterol (LDL-C) [32], and total cholesterol (TC) levels [32], and inversely associated with concentrations of high-density lipoprotein cholesterol (HDL-C) [31]. However, some of the studies failed to find any association between DAL and TG [32], HDL-C [32], or fasting blood sugar (FBS) [30–32]. Therefore, the objective of this study was to examine the association of DAL with cardiometabolic risk factors and prevalence of MetS in Iranian women.

Materials and methods

Study population

We applied cluster random sampling to 371 women for inclusion in the present cross-sectional study. Participants were between 20 and 50 y of age, and attended the Southern Health Center of Tehran from 2017 to 2018. The women had no previously diagnosed hypertension or inflammatory or metabolic diseases and were not taking any medications (including those that would influence lipid and glucose metabolism or blood pressure). Women were excluded from the study if they had been following a specialized diet during the previous year or if they had unexplained total energy intakes (<500 or >3500 kcal/day) [33]. Study sample size was determined considering mean level of serum TG (mean ± SD = 6.11 ± 2.88) as the main dependent variable, employing the following formula:

$$N = \left[(z\ 1-\alpha/2)^2 \times s^2\right]/d^2 [32].$$

Based on $\alpha = 0.05$, 356 participants were required; "d" was calculated as 6% of mean levels of TG; and a design effect of 1.5 was needed. In previous studies, ~4% of participants were excluded due to over- and underreporting of energy intake [34]. Therefore, to account for exclusion owing to under- or overreporting of energy, 371 participants were selected for inclusion in the present study. All participants provided informed consent. The study ethical protocol was approved by the National Institute for Medical Research and Development.

Dietary assessment and definition of DAL

Usual dietary intake during the previous year was determined by completing a 168-item semiquantitative food frequency questionnaire (FFQ) for each participant in a face-to-face interview. The FFQ comprised portion sizes and frequency consumption of foods on a daily, weekly, or monthly basis. The portion size of total foods was converted to grams by household measures. An adapted version of NUTRITIONIST IV corrected for Iranian food, version 7.0 (N-Squared Computing, Salem, OR, USA) was applied to calculate the average intake of energy and nutrients. The validity and reliability of the FFQ were determined in a previously published study [35].

Urinary net acid excretion is an indicator of NEAP, which is affected by dietary nutrient intake. Because it is difficult to directly measure NEAP, two indices recently have been introduced to characterize DAL from the diet. First, PRAL was estimated by applying the following formula, which was described by Remer et al. [20,21,27]:

$$\text{PRAL(mEq/d)} = 0.4888 \times \text{protein intake(g/d)} + 0.0366 \times \text{phosphorus(mg/d)} - 0.0205 \times \text{potassium(mg/d)} - 0.0125 \times \text{calcium(mg/d)} - 0.0263 \times \text{magnesium(mg/d)}.$$

Moreover, NEAP was calculated based on the following algorithm, which was developed by Frassetto et al. [22]:

$$\text{NEAP(mEq/d)} = [54.5 \times \text{protein intake(g/d)} \div \text{potassium intake(mEq/d)}] - 10.2.$$

According to this concept (NEAP), the amount of sulfuric acid and bicarbonate production owing to protein and potassium (Pro/K) metabolism are considered to be the major determinants of DAL [22].

The validity of the foregoing scores recently have been examined in comparison with 24-h urinary acid load in healthy adults [21,22]. Both PRAL and NEAP were established as reasonably valid measures for estimating DAL [21,22].

Biochemical assessment

The assessment of all biochemical factors (FBS, TG, TC, HDL-C, LDL-C) was performed on collected fasting venous blood samples. FBS concentration was assessed by the enzymatic colorimetric method and glucose oxidase (both inter- and intraassay coefficient of variation [CV]: 2.2%). For assessment of serum TG levels, an enzymatic calorimetric method with glycerol phosphate oxidase was used (interassay CV: 0.6%; intraassay CV: 1.6%). Serum TC levels were determined with cholesterol esterase and cholesterol oxidase applying the enzymatic colorimetric method (interassay CV: 1.1%; intraassay CV: 1.4%). HDL-C was measured after precipitation of other lipoproteins using phosphotungstic acid and magnesium chloride fluid (interassay CV: 1.8%; intraassay CV: 1.5%). Serum LDL-C levels were measured after precipitation of LDL-C by using heparin and sodium citrate (interassay CV: 0.95%; intraassay CV: 1.4%). All the analyses were performed using commercial enzymatic reagents (Pars Azmoon, Tehran, Iran) and by applying an auto-analyzer system (Selectra E, Vitalab, Holliston, the Netherlands).

Anthropometric assessment

Anthropometric measurements were obtained by a trained assistant. Participant weight was measured to the nearest 100 g using a portable digital scale (SECA 813; Seca, Hamburg, Germany), with participants wearing lightweight clothing. Height was recorded using unstretched meter to the nearest 0.5 cm, with participants standing against a wall with shoulders in a normal position. WC was taken at the level of umbilicus over at most a single layer of lightweight clothing to the nearest 0.5 cm. BMI was calculated as body weight in kilogram divided by the square of individual's height.

Assessment of other variables

Socioeconomic status (SES) was assessed using a valid and reliable questionnaire for the Iranian population, which was developed for measuring SES status and its association with health outcomes [36]. The total standardized score for all participants was computed (using factor analysis and summary index), then its compliance with normal summary index was examined using the κ test. This questionnaire consisted of questions about educational level, occupation, car or home ownership, having new-fashioned furniture, number of family members, and trips in or outside the country during the previous year. The reported correlation of these parameters with the total score was 0.87. In the present study, the SES of participants were reported based on the calculated scores. To collect other variables such as age, marital status (married, single, or divorced) and smoking status (non-smoker or smoker), a demographic questionnaire was used. Blood pressure (BP) was measured twice with a 30-s interval after participants were in a relaxed and seated position for ~10 min. Before measuring BP, participants were asked to empty their bladder. They did not smoke, drink caffeinated beverages, or exercise within 1 h before BP was measured.

Moreover, MetS was defined as the presence of at least three criteria based on the National Cholesterol Education Program's Adult Treatment Panel III (ATP-III) as follows: WC >88 cm for women; BP >130/85 mm Hg; fasting TG level ≥150 mg/dL and HDL-C <50 mg/dL; and FBS level ≥100 mg/dL [8]. BMI was defined according to cutoff values reported by the World Health Organization (WHO; overweight and obesity: BMI ≥25 kg/m^2) [37].

Statistical analysis

The Kolmogorov–Smirnov test showed that all variables were normally distributed. We first categorized participants based on their median PRAL (<8.93 versus ≥8.93 mEq/d) and NEAP (<46.77 versus ≥46.77 mEq/d) values. Participant characteristics were described for the total population and for categories of PRAL (median: 8.93; interquartile range [IQR], 19.91 mEq/d [Q3 versus Q1: 19.68 versus −0.23]) and NEAP (median: 46.77; IQR, 18.82 mEq/d [Q3 versus Q1: 55.83 versus 37.01]). To test differences in characteristics between categories, χ^2 and independent-sample *t* tests were used for qualitative and quantitative variables, respectively. Dietary variables (micro- and macronutrients) were described for categories of PRAL and NEAP. Then, the categories of PRAL and NEAP were compared using one-way analysis of covariance (ANCOVA) to adjust for energy intake. Levels of anthropometric indices (weight and BMI) and biochemical parameters (FBS, HDL-C, LDL-C, and TC) in the two categories of PRAL and NEAP were compared using independent-sample *t* test in a crude model and ANCOVA in an adjusted model (energy, age, marital status, SES, and BMI). Before performing binary logistic regression, participants were categorized for cardiometabolic risk factors based on the reported cutoffs by ATP-III as follows: WC >88 cm, BP >130/85 mm Hg, FBS ≥100 mg/dL, TG ≥150 mg/dL, HDL-C <50 mg/dL, LDL-C >130 mg/dL, and TC >200 mg/dL [38]. Moreover, participants were categorized for BMI according to cutoff values defined by the WHO (overweight and obesity: BMI ≥25 kg/m^2) [37]. To find the association of DAL (PRAL, NEAP) with CVD risk factors (BMI, WC, FBS, TG, HDL-C, LDL-C, and TC) and the prevalence of MetS, binary logistic regression in both crude and adjusted models (model 1: energy, age, marital status, and SES; model 2: all mentioned variables plus BMI) was used. We chose binary regression analysis for a few reasons. First, the results may be easier for non-statisticians to understand and interpret [39] (i.e., some clinicians find obesity risks presented relative to a reference group easier to interpret than linear regression or correlation coefficients). Furthermore, health care professionals often prefer dichotomous outcomes (like normal/abnormal, risky/benign, treat/do not treat) [40]. Second, this method allowed us to obtain reliable risk estimates, not just to ensure statistically significant findings. Third, this approach let a "never" or "zero" exposed category be easily incorporated into a categorical analysis [39]. To conduct categorical analyses, we determined the cutoffs for the exposure and outcome variables based on theoretic or clinical considerations and avoided data-driven cut-points [40]. Although it would have been preferable to have more categories, we made sure there were sufficient individuals in each category when selecting category boundaries [40]. SPSS software version 13 (SPSS Inc., Chicago, IL, USA) was used for statistical analyses and $P < 0.05$ was considered statistically significant.

Results

General characteristic of participants (N = 371) for the total population and for each category of PRAL and NEAP are described in Table 1. Participants assigned to the highest PRAL category were more likely to be married ($P = 0.009$). Moreover, participants in the highest category of NEAP were younger ($P = 0.0001$), had lower BMI ($P = 0.005$), were more likely to be married ($P = 0.009$), and were more likely to be university-educated ($P = 0.002$). However, for other characteristics, there was no difference between participants in the highest or lowest categories.

Dietary intake of participants for each category of PRAL and NEAP are presented in Table 2. Participants included in the highest category of PRAL were characterized by higher energy intakes ($P = 0.0001$), protein ($P = 0.0001$), fat ($P = 0.05$), dietary cholesterol ($P = 0.0001$), phosphorus ($P = 0.0001$), calcium ($P = 0.001$), sodium/potassium ($P = 0.0001$), and meat ($P = 0.0001$). Furthermore, they showed lower intake of carbohydrates ($P = 0.0001$), fiber ($P = 0.0001$), potassium ($P = 0.0001$), magnesium ($P = 0.003$), fruit ($P = 0.0001$), and vegetables ($P = 0.0001$).

Participants in the highest category of NEAP also were characterized by higher intake of protein ($P = 0.0001$), phosphorus ($P = 0.0001$), sodium/potassium ($P = 0.0001$), and meat ($P = 0.0001$). However, they had lower intake of carbohydrates ($P = 0.009$), fiber ($P = 0.0001$), potassium ($P = 0.0001$), calcium ($P = 0.0001$), magnesium ($P = 0.0001$), fruit ($P = 0.0001$), and vegetables ($P = 0.0001$).

Anthropometric measurements and biochemical markers for each category of PRAL and NEAP are reported in Table 3. Participants in the highest category of NEAP had higher WC ($P = 0.01$). The differences between other anthropometric measurements, FBS, and lipid profiles in women included in the high compared with the low category of PRAL and NEAP were not significant in adjusted models 1 or 2.

Odds ratio (OR) and 95% confidence intervals (CI) for CVD risk factors for each category of PRAL and NEAP are provided in Table 4. After adjustment for a wide range of confounding variables, a significant positive association was observed between high NEAP with overweight and obesity (BMI ≥25 kg/m^2; OR, 3.07; 95% CI, 1.92–4.93; $P = 0.0001$) and WC (OR, 2.27; 95% CI, 1.37–3.75; $P = 0.001$). Moreover, there was a strong positive association between the highest DAL score (both PRAL and NEAP) and serum TG levels (PRAL: OR, 4.28; 95% CI, 1.67–10.99; $P = 0.002$; NEAP: OR, 4.92; 95% CI, 1.87–12.92; $P = 0.001$). No significant association with any of the other CVD risk factors or the prevalence of MetS was found.

Discussion

In the present cross-sectional study, a significant positive association was demonstrated between high DAL (both PRAL and NEAP

Table 1
General participant characteristics by median-split dietary acid load*

Variables	Total N = 371	PRAL (mEq/d)		*P*-value†	NEAP (mEq/d)		*P*-value†
		Low category <8.93 n = 185	High category >8.93 n = 186		Low category <46.77 n = 185	High category >46.77 n = 186	
Age, y	30.67 ± 6.92	31.23 ± 7.50	30.11 ± 6.26	0.12	32.30 ± 7.72	29.05 ± 5.60	0.0001
BMI, kg/m^2	24.26 ± 4.03	24.32 ± 3.95	24.19 ± 4.13	0.74	24.99 ± 4.43	23.52 ± 3.45	0.005
Socioeconomic status	32.01 ± 6.19	32.11 ± 6.69	31.91 ± 5.66	0.75	32.42 ± 6.88	31.61 ± 5.40	0.20
Marital status, n (%)				0.009			0.009
Married	230 (62)	107 (58)	123 (66)		107 (58)	123 (66)	
Single/Divorced	141 (38)	78 (42)	63 (34)		78 (42)	63 (34)	
Educational status, n (%)				0.08			0.002
Illiterate/Underdiploma	33 (9)	15 (8)	18 (10)		20 (11)	13 (7)	
Diploma	68 (18)	40 (22)	28 (15)		32 (17)	36 (19)	
Academic degree	270 (73)	130 (70)	140 (75)		133 (72)	137 (74)	
Smoking n (%)				0.56			0.25
Yes	4 (1)	3 (2)	1 (1)		3 (2)	1 (1)	
No	367 (99)	182 (98)	185 (99)		182 (98)	185 (99)	

BMI, body mass index; NEAP, net endogenous acid production; PRAL, potential renal acid load.
*Mean ± SD.
†Calculated by χ^2 and *t* test for qualitative and quantitative variables, respectively.

Table 2
Energy-adjusted dietary intakes by median-split dietary acid load in Iranian women*

Variables	PRAL (mEq/d)		P-value†	NEAP (mEq/d)		P-value†
	Low category <8.93 n = 185	High category >8.93 n = 186		Low category <46.77 n = 185	High category >46.77 n = 186	
Energy, kcal/d	2163.77 ± 653.08	2859.19 ± 708.77	0.0001	2469.70 ± 794.24	2554.91 ± 733.47	0.28
Protein, g/d	82.87 ± 15.64	98.14 ± 15.64	0.0001	86.20 ± 15.67	94.83 ± 15.53	0.0001
Fat, g/d	75.99 ± 15.50	79.03 ± 15.50	0.05	78.02 ± 14.72	77.30 ± 14.72	0.63
Carbohydrate, g/d	390.65 ± 39.71	362.60 ± 39.57	0.0001	381.93 ± 39.11	371.26 ± 38.98	0.009
Fiber, g/d	7.68 ± 2.17	5.71 ± 2.17	0.0001	7.98 ± 1.77	5.42 ± 1.77	0.0001
Cholesterol, mg/d	209.17 ± 129.33	270.03 ± 128.92	0.0001	234.31 ± 124.85	245.03 ± 124.44	0.40
Phosphorus, mg/d	1249.42 ± 296.75	1492.27 ± 295.80	0.0001	1321.92 ± 295.22	1420.70 ± 296.04	0.0001
Potassium, mg/d	3811.08 ± 869.04	3185.02 ± 866.32	0.0001	4036.40 ± 676.72	2960.92 ± 674.95	0.0001
Calcium, mg/d	985.72 ± 302.46	1098.20 ± 301.51	0.001	1119.67 ± 279.00	964.97 ± 278.18	0.0001
Magnesium, mg/d	302.38 ± 60.38	282.78 ± 60.24	0.003	320.63 ± 50.29	264.62 ± 50.15	0.0001
Sodium, mg/d	4985.17 ± 2384.76	5044.50 ± 2377.55	0.82	5122.11 ± 2246.49	4908.30 ± 2240.36	0.35
Sodium/Potassium, g/mEq	1.38 ± 0.68	1.67 ± 0.68	0.0001	1.35 ± 0.68	1.70 ± 0.68	0.0001
Grains, g/d	515.47 ± 181.01	537.61 ± 180.47	0.26	488.83 ± 166.83	564.10 ± 166.42	0.0001
Fruits, g/d	400.21 ± 228.48	181.24 ± 227.80	0.0001	393.75 ± 212.90	187.66 ± 212.35	0.0001
Vegetables, g/d	436.89 ± 227.12	312.45 ± 226.44	0.0001	454.95 ± 206.08	294.49 ± 205.54	0.0001
Meat, g/d	102.20 ± 74.52	141.32 ± 74.25	0.0001	105.35 ± 206.76	138.19 ± 206.63	0.0001

PRAL, potential renal acid load; NEAP, net endogenous acid production.
*Mean ± SD.
†Calculated by *t* test for energy intake and multivariate analysis of covariance for other variables (other variables adjusted for energy intake).

Table 3
Anthropometric indices, and biochemical markers by median-split dietary acid load in Iranian women*

Variables	PRAL (mEq/d)		P-value†	NEAP (mEq/d)		P-value†
	Low category <8.93 n = 185	High category >8.93 n = 186		Low category <46.77 n = 185	High category >46.77 n = 186	
Weight (kg)						
Crude	63.90 ± 10.29	64.36 ± 11.02	0.67	65.13 ± 11.27	63.13 ± 9.93	0.07
Model 1‡	66.21 ± 11.01	64.05 ± 11.01	0.90	64.61 ± 10.35	63.66 ± 10.35	0.39
BMI (kg/m^2)						
Crude	24.32 ± 3.95	24.19 ± 4.13	0.74	24.99 ± 4.43	23.52 ± 3.45	0.005
Model 1‡	24.38 ± 3.94	24.13 ± 3.95	0.58	24.71 ± 3.80	23.80 ± 3.68	0.05
WC (cm)						
Crude	83.02 ± 7.76	84.34 ± 9.19	0.13	83.09 ± 8.07	84.27 ± 8.93	0.18
Model 1‡	83.08 ± 8.84	84.28 ± 8.85	0.22	82.54 ± 8.29	84.82 ± 8.31	0.01
FBS (mg/dL)						
Crude	91.37 ± 33.37	88.42 ± 11.64	0.25	93.18 ± 33.96	86.62 ± 18.90	0.01
Model 1‡	91.93 ± 24.20	87.87 ± 24.20	0.13	91.71 ± 22.98	88.09 ± 22.84	0.13
Model 2§	91.93 ± 24.34	87.87 ± 24.20	0.13	91.71 ± 23.12	88.09 ± 22.98	0.14
TG (mg/dL)						
Crude	104.43 ± 22.60	100.68 ± 24.80	0.11	103.82 ± 26.24	101.09 ± 21.02	0.26
Model 1*	103.80 ± 25.29	101.11 ± 25.35	0.33	103.19 ± 24.07	101.73 ± 23.98	0.56
Model 2§	103.75 ± 25.29	101.17 ± 25.35	0.35	102.99 ± 24.07	101.92 ± 24.12	0.67
HDL-C (mg/dL)						
Crude	47.10 ± 9.85	48.79 ± 8.89	0.08	46.66 ± 9.90	49.23 ± 8.73	0.008
Model 1‡	47.31 ± 9.92	48.58 ± 9.82	0.24	47.02 ± 9.38	48.87 ± 9.28	0.06
Model 2§	47.36 ± 9.79	48.53 ± 9.79	0.28	47.20 ± 9.24	48.69 ± 9.28	0.13
LDL-C (mg/dL)						
Crude	78.69 ± 18.56	81.13 ± 20.30	0.22	80.19 ± 20.83	79.65 ± 18.05	0.78
Model 1‡	80.71 ± 19.85	79.13 ± 19.92	0.47	79.45 ± 18.90	80.38 ± 18.83	0.64
Model 2§	80.68 ± 19.85	79.16 ± 19.85	0.49	79.32 ± 18.94	80.51 ± 18.94	0.55
TC (mg/dL)						
Crude	175.88 ± 36.60	172.36 ± 32.23	0.32	179.68 ± 34.64	168.58 ± 33.50	0.002
Model 1‡	177.00 ± 34.27	171.24 ± 34.26	0.13	177.04 ± 32.36	171.21 ± 32.48	0.09
Model 2§	177.00 ± 34.27	171.24 ± 34.27	0.13	177.06 ± 32.57	171.18 ± 32.57	0.09

BMI, body mass index; FBS, fasting blood sugar; HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol; NEAP, net endogenous acid production; PRAL, potential renal acid load; TC, total cholesterol; TG, triacylglycerol; WC, waist circumference.
*Mean ± SD.
†Calculated by independent *t* test in crude model, and analysis of covariance in adjusted model.
‡Model 1 sdjusted for energy intake, age, marital status, socioeconomic status.
§Model 2 adjusted for energy intake, age, marital status, socioeconomic status and BMI.

scores) and serum TG levels. Moreover, a positive relationship was observed between NEAP score with overweight and obesity (BMI ≥25 kg/m^2) and WC. No significant association with any of the other CVD risk factors or the prevalence of MetS was found. Findings of the present study provide further insight into the relationship between DAL and CVD risk factors.

Table 4
Cardiovascular risk factors and the prevalence of metabolic syndrome based on median-split of dietary acid load in Iranian women*

Variables	PRAL (mEq/d)		P-value†	NEAP (mEq/d)		P-value†
	Low category <8.93 n = 185	High category >8.93 n = 186		Low category <46.77 n = 185	High category >46.77 n = 186	
Overweight and obese (BMI ≥25 kg/m^2)						
Crude	1	1.21 (0.79–1.84)	0.37	1	3.48 (2.23–5.42)	0.0001
Model 1‡	1	1.35 (0.80–2.27)	0.24	1	3.07 (1.92–4.93)	0.0001
WC (>88 cm)						
Crude	1	1.65 (1.06–2.57)	0.02	1	3.10 (1.95–4.90)	0.0001
Model 1‡	1	1.67 (0.95–2.93)	0.07	1	2.27 (1.37–3.75)	0.001
FBS (≥100 mg/dL)						
Crude	1	1.85 (0.88–3.89)	0.10	1	2.14 (1.01–4.56)	0.04
Model 1‡	1	0.91 (0.30–2.73)	0.86	1	0.49 (0.17–1.38)	0.18
Model 2§	1	0.86 (0.28–2.62)	0.79	1	0.39 (0.13–1.20)	0.10
TG (≥150 mg/dL)						
Crude	1	2.66 (1.37–5.17)	0.004	1	8.54 (3.52–20.68)	0.0001
Model 1‡	1	4.07 (1.65–10.01)	0.002	1	5.79 (2.25–14.91)	0.0001
Model 2§	1	4.28 (1.67–10.99)	0.002	1	4.92 (1.87–12.92)	0.001
HDL-C (<50 mg/dL)						
Crude	1	1.70 (1.04–2.78)	0.03	1	1.93 (1.17–3.17)	0.009
Model 1‡	1	0.93 (0.51–1.69)	0.81	1	1.49 (0.85–2.58)	0.15
Model 2§	1	0.49 (0.08–2.71)	0.41	1	1.29 (0.73–2.29)	0.37
LDL-C (>130 mg/dL)						
Crude	1	0.19 (0.04–0.89)	0.03	1	2.05 (0.60–6.95)	0.24
Model 1‡	1	0.42 (0.08,2.30)	0.32	1	1.79 (0.49–6.55)	0.37
Model 2§	1	0.88 (0.48,1.62)	0.68	1	2.05 (0.55–7.63)	0.28
TC (>200 mg/dL)						
Crude	1	1.27 (0.76–2.11)	0.35	1	1.79 (1.06–3.00)	0.02
Model 1‡	1	1.31 (0.70–2.45)	0.39	1	1.32 (0.75–2.32)	0.32
Model 2§	1	1.32 (0.70–2.46)	0.38	1	1.35 (0.76–2.38)	0.29
MetS						
Crude	1	3.43 (1.10–10.75)	0.03	1	8.11 (1.82–36.02)	0.006
Model 1‡	1	4.06 (0.88–18.74)	0.07	1	3.77 (0.77–18.40)	0.10

BMI, body mass index; FBS, fasting blood sugar; HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol; MetS, metabolic syndrome; NEAP, net endogenous acid production; PRAL, potential renal acid load; TC, total cholesterol; TG, triacylglycerol; WC, waist circumference.
*Odds ratio (95% CI).
†Calculated by logistic regression.
‡Model 1 adjusted for energy intake, age, marital status, and socioeconomic status.
§Model 2 adjusted for energy intake, age, marital status, socioeconomic status. and BMI.

DAL is a relatively new dietary concept that reflects the effects of diet on body acid–base status [19]. PRAL and NEAP are two indices that have been applied to estimate DAL from dietary intake [19,32,41]. Higher values of either PRAL or NEAP indicate higher diet acidity. In line with previously published studies, higher intake of grains, meat, and dairy products were related to higher DAL, whereas higher intake of fruits and vegetables was associated with lower DAL levels [23,31]. In the present study, PRAL (mean, 9.61 mEq/d; median, 8.93; IQR, 19.91 mEq/d [Q3 versus Q1: 19.68 versus −0.23]) and NEAP (mean, 47.46 mEq/d; median, 46.77; IQR, 18.82 mEq/d [Q3 versus Q1: 55.83 versus 37.01]) values showed higher acidity compared with other studies in healthy Iranian adults (mean, PRAL −11.2 mEq/d; mean, NEAP 35.6 mEq/d) [31, 42] and patients with diabetic nephropathy (median, PRAL −0.32 mEq/d; median, NEAP −0.28 mEq/d) [43]; however, the values were almost similar to that of young Japanese women (mean, PRAL 10.4 mEq/d) [32], adult Japanese (median, PRAL 8.95 mEq/d; median, NEAP 52.35 mEq/d) [30], elderly Chinese (median, NEAP 47.3; IQR, 25.5 mEq/d [Q3 versus Q1: 61 versus 35.5]) [44], and elderly Swedish men (median, NEAP 40.7 mEq/d) [45].

The present study showed a positive association between high DAL (NEAP score) with overweight and obesity (BMI 25 kg/m^2) and WC. In line with the present findings, a cross-sectional study by Bahadoran et al. (N = 5620 adults, 20–70 y of age) showed that both PRAL (mean, −22 mEq/d) and Pro/K (mean, −0.02 mEq/d) were associated with higher WC and body weight [31]. Moreover, in a cross-sectional study with 1154 Japanese women 18 to 22 y of age, a positive association was found between Pro/K (mean, 1.2 mEq/d) and WC and obesity, whereas PRAL score (mean, 10.4 mEq/d) showed no association [32]. One possible explanation for the association between DAL and anthropometric measurements might be the effects of metabolic acidosis on muscle metabolism or IR. For example, metabolic acidosis can result in increased proteolysis through increased mRNA encoding of the ubiquitin proteasome pathway proteins (including ubiquinone, and the subunits of proteasome) [46]. A cross-sectional study by Welch et al. showed that women (18–79 y of age) with the lowest acidic diet (PRAL, −24.44 mEq/d) had higher body fat-free mass [47]. Moreover, another explanation could be related to the positive association between serum markers of metabolic acidosis (including low bicarbonate, increased lactate, and high anion gap) and IR [48–50]. Following IR, the risk for obesity can increase [51].

It is noteworthy that although studies differed in several important factors such as age, sex, race, exposure and outcome assessment tools, and adjustments, the findings regarding obesity were consistent across the majority of studies [31,32,47]. The positive association between DAL and obesity was reported in both sexes and in different age groups such as young adults (18-22 years old) [32], adults (19-70 years old), and the elderly (>55 years old) [31,41]. Furthermore, this association was observed in a wide range of PRAL scores, ranging from −22 mEg/d in the study by Bahadoran et al. [31] to 9.61 mEq/d in the present study. However,

to the best of our knowledge, most of the studies were performed with Asian populations [31,32,42,44] and there are few studies from Western countries.

The present study did not find an association between DAL and FBS. In line with our findings, Bahadoran et al. found no significant association between higher DAL diet (both PRAL and Pro/K) and FBS in adults [31]. In the cross-sectional study by Murakami et al., no association was revealed between a high DAL diet (PRAL, 18.7 versus 1.3 mEq/d; Pro/K, 1.51 versus 0.96 mEq/d) and both FBS and hemoglobin A1c in young Japanese women [32]. In a study by Akter et al., no association between PRAL (17.1 versus −1.3 mEq/d) and NEAP (66.4 versus 40 mEq/d) with FBS level was found [30]. These findings are in contrast to a previously published prospective study by Fagherazzi et al. that found a strong association between high DAL and increased risk for type 2 diabetes [52]. Moreover, other studies reported a positive association between DAL and IR [25,30].

Different findings across studies may be due to differences in DAL. In the study by Fagherazzi et al. [52], there was a wider range of DAL (PRAL, 14.3 versus −23.0 mEq/d; NEAP, 58.2 versus 31.5 mEq/d) compared with other studies that did not find an association between DAL and FBS [31,32]. Metabolic acidosis that is induced by higher DAL is associated with disturbed binding of insulin to its receptor, inhibition of the insulin signaling pathway, and an increase in hepatic gluconeogenesis [25]. Several plausible mechanisms that reduce the rate of glucose absorption in the intestinal lumen [53], increase secretion of bile acids (may induce the production of glucagon-like peptide-1) [54], and conserve the composition of gut flora may have improved IR among participants [55].

A significant positive association was observed between high DAL (both PRAL and NEAP) and TG. In agreement with this finding, Bahadoran et al. showed that both PRAL (mean, −22 mEq/d) and Pro/K (mean, 0.02 mEq/d) scores were positively associated with TG and inversely associated with HDL-C [31]. The underlying mechanism that relates high DAL with elevated TG levels seems to be high cortisol secretion in the presence of metabolic acidosis [56]. High cortisol concentrations have been shown to induce lipase activity (lipoprotein lipase and hormone-sensitive lipase), which in turn results in increased efflux of free-fatty acids to the bloodstream and augment production of very low-density lipoproteins (high in TG concentration) in the liver [57,58]. In contrast with the present study, in the study by Murakami et al., PRAL (18.7 versus 1.3 mEq/d) was positively associated with LDL-C and TC in young adult Japanese women, whereas no association regarding TG was detected [32]. Although the DAL in the present study was similar to that found by Murakami et al. [32], participants in the present study had higher intake of fruits and vegetables. Consumption of fermentable fiber from plant sources, which control metabolic activity of the intestinal flora and also their metabolites [59], could be an explanation for the differences between the present findings and those of Murakami et al.

To our knowledge, this is the first study to investigate the relationship between DAL and a range of CVD risk factors and the prevalence of MetS among Iranian women. However, there were several limitations to the present study. First, the cross-sectional study design did not allow us to determine cause and effect. Second, owing to the closed-ended response nature of FFQs, the possibility of dietary misclassification was increased. Third, it is unknown whether the underlying association between DAL and CVD risk factors was caused by acidity of the diet or if it was due to minerals present in DAL formula. Fourth, although cortisol may be the factor that links the association between DAL and CVD risk factors, it was not measured in this study.

Conclusion

A significant positive association was found between DAL (both PRAL and NEAP scores) with serum TG concentrations. Moreover, a positive relationship was observed between NEAP score and overweight and obesity (BMI $\geq$ 25 kg/m^2) and WC. Further prospective studies in different populations are required to elucidate the role of DAL in the development of CVDs.

References

[1] O'neill S, O'driscoll L. Metabolic syndrome: a closer look at the growing epidemic and its associated pathologies. Obes Rev 2015;16:1–12.
[2] Roth GA, Johnson C, Abajobir A, Abd-Allah F, Abera SF, Abyu G, et al. Global, regional, and national burden of cardiovascular diseases for 10 causes, 1990 to 2015. J Am Coll Cardiol 2017;70:1–25.
[3] Boutayeb A, Boutayeb S. The burden of non communicable diseases in developing countries. Int J Equity Health 2005;4:2.
[4] Carriere I, Peres K, Ancelin ML, Gourlet V, Berr C, Barberger-Gateau P, et al. Metabolic syndrome and disability: findings from the prospective three-city study. J Gerontol A Biol Sci Med Sci 2013;69:79–86.
[5] Lakka HM, Laaksonen DE, Lakka TA, Niskanen LK, Kumpusalo E, Tuomilehto J, et al. The metabolic syndrome and total and cardiovascular disease mortality in middle-aged men. JAMA 2002;288:2709–16.
[6] DeFronzo RA, Ferrannini E. Insulin resistance: a multifaceted syndrome responsible for NIDDM, obesity, hypertension, dyslipidemia, and atherosclerotic cardiovascular disease. Diabetes Care 1991;14:173–94.
[7] Han T, van Leer EM, Seidell JC, Lean ME. Waist circumference action levels in the identification of cardiovascular risk factors: prevalence study in a random sample. BMJ 1995;311:1401–5.
[8] Huang PL. A comprehensive definition for metabolic syndrome. Dis Model Mech 2009;2:231–7.
[9] Mirzababaei A, Mozaffari H, Shab-Bidar S, Milajerdi A, Djafarian K. Risk of hypertension among different metabolic phenotypes: a systematic review and meta-analysis of prospective cohort studies. J Hum Hypertens 2019;33:365–77.
[10] Kohl HW. Physical activity and cardiovascular disease: evidence for a dose response. Medicine and science in sports and exercise 2001;33:S472–83.
[11] Pyrgakis VN. Smoking and cardiovascular disease. Hellenic J Cardiol 2009;50:231–4.
[12] Verschuren WM. Diet and cardiovascular disease. CurrCardiol Rep 2012;14:701–8.
[13] Park YW, et al. The metabolic syndrome: prevalence and associated risk factor findings in the US population from the Third National Health and Nutrition Examination Survey, 1988-1994. Arch Intern Med 2003;163:427–36.
[14] Joint WHO/FAO Expert Consultation. Diet, nutrition and the prevention of chronic diseases: Report of a joint WH. Geneva, Switzerland: Author; 2002.
[15] Setorki M, et al. Anti atherosclerotic effects of verjuice on hypocholesterolemic rabbits. Afr J Pharm Pharmacol 2011;5:1038–45.
[16] Mozaffari H, Daneshzad E, Surkan PJ, Azadbakht L. Dietary total antioxidant capacity and cardiovascular disease risk factors: a systematic review of observational studies. J Am Coll Nutr 2018;37:533–45.
[17] Mozaffari H, Namazi N, Larijani B, Surkan PJ, Azadbakht L. Associations between dietary insulin load with cardiovascular risk factors and inflammatory parameters in elderly men: a cross-sectional study. Br J Nutr 2019;121:773–81.
[18] Abshirini M, Siassi F, Koohdani F, Qorbani M, Mozaffari H, Aslani Z, et al. Dietary total antioxidant capacity is inversely associated with depression, anxiety and some oxidative stress biomarkers in postmenopausal women: a cross-sectional study. Ann Gen Psychiatry 2019;18:3.
[19] Scialla JJ, Anderson CA. Dietary acid load: a novel nutritional target in chronic kidney disease? Adv Chronic Kidney Dis 2013;20:141–9.
[20] Remer T, Dimitriou T, Manz F. Dietary potential renal acid load and renal net acid excretion in healthy, free-living children and adolescents. Am J Clin Nutr 2003;77:1255–60.
[21] Remer T, Manz F. Estimation of the renal net acid excretion by adults consuming diets containing variable amounts of protein. Am J Clin Nutr 1994;59:1356–61.
[22] Frassetto LA, Todd KM, Morris RC Jr., Sebastian A. Estimation of net endogenous noncarbonic acid production in humans from diet potassium and protein contents. Am J Clin Nutr 1998;68:576–83.
[23] Zhang L, Curhan GC, Forman JP. Diet-dependent net acid load and risk of incident hypertension in United States women. Hypertension 2009;54:751–5.
[24] Ströhle A, Waldmann A, Koschizke J, Leitzmann C, Hahn A. Diet-dependent net endogenous acid load of vegan diets in relation to food groups and bone health-related nutrients: results from the German Vegan Study. Ann Nutr Metab 2011;59:117–26.

[25] Williams RS, Kozan P, Samocha-Bonet D. The role of dietary acid load and mild metabolic acidosis in insulin resistance in humans. Biochimie 2016;124:171–7.
[26] Maurer M, Riesen W, Muser J, Hulter HN, Krapf R. Neutralization of Western diet inhibits bone resorption independently of K intake and reduces cortisol secretion in humans. Am J Physiol Renal Physiol 2003;284:F32–40.
[27] Remer T, Pietrzik K, Manz F. Short-term impact of a lactovegetarian diet on adrenocortical activity and adrenal androgens. J Clin Endocrinol Metab 1998;83:2132–7.
[28] Fraser R, Ingram MC, Anderson NH, Morrison C, Davies E, Connell JM. Cortisol effects on body mass, blood pressure, and cholesterol in the general population. Hypertension 1999;33:1364–8.
[29] Mårin P, Darin N, Amemiya T, Andersson B, Jern S, Björntorp P. Cortisol secretion in relation to body fat distribution in obese premenopausal women. Metabolism 1992;41:882–6.
[30] Akter S, Eguchi M, Kuwahara K, Kochi T, Ito R, Kurotani K. High dietary acid load is associated with insulin resistance: the Furukawa Nutrition and Health Study. Clin Nutr 2016;35:453–9.
[31] Bahadoran Z, Mirmiran P, Khosravi H, Azizi F. Associations between dietary acid-base load and cardiometabolic risk factors in adults: the Tehran Lipid and Glucose Study. Endocrinol Metab 2015;30:201–7.
[32] Murakami K, Sasaki S, Takahashi Y, Uenishi K, Japan Dietetic Students' Study for Nutrition and Biomarkers Group. Association between dietary acid–base load and cardiometabolic risk factors in young Japanese women. Br J Nutr 2008;100:642–51.
[33] Saraf-Bank S, Haghighatdoost F, Esmaillzadeh A, Larijani B, Azadbakht L. Adherence to Healthy Eating Index-2010 is inversely associated with metabolic syndrome and its features among Iranian adult women. Eur J Clin Nutr 2017;71:425–30.
[34] Darooghegi Mofrad M, Siassi F, Guilani B, Bellissimo N, Azadbakht L. Association of dietary phytochemical index and mental health in women: a cross-sectional study. Br J Nutr 2019:1–24.
[35] Mirmiran P, Esfahani FH, Mehrabi Y, Hedayati M, Azizi F. Reliability and relative validity of an FFQ for nutrients in the Tehran lipid and glucose study. Public Health Nutr 2010;13:654–62.
[36] Garmaroudi GR, Moradi A. Socio-economic status in Iran: a study of measurement index. Payesh 2010;9:137–44.
[37] World Health Organization. Obesity: preventing and managing the global epidemic. Geneva, Switzerland: Author; 2000.
[38] Brewer HB Jr. New features of the National Cholesterol Education Program Adult Treatment Panel III lipid–lowering guidelines. Clin Cardiol 2003;26:19–24.
[39] Turner EL, Dobson JE, Pocock SJ. Categorisation of continuous risk factors in epidemiological publications: a survey of current practice. Epidemiol Perspect Innov 2010;7:9.
[40] Naggara O, Raymond J, Guilbert F, Roy D, Weill A, Altman DG. Analysis by categorizing or dichotomizing continuous variables is inadvisable: an example from the natural history of unruptured aneurysms. AJNR Am J Neuroradiol 2011;32:437–40.
[41] Engberink MF, Bakker SJ, Brink EJ, van Baak MA, van Rooij FJ, Hofman A, et al. Dietary acid load and risk of hypertension: the Rotterdam Study. Am J Clin Nutr 2012;95:1438–44.
[42] Moghadam SK, Bahadoran Z, Mirmiran P, Tohidi M, Azizi F. Association between dietary acid load and insulin resistance: Tehran Lipid and Glucose Study. Prev Nutr Food Sci 2016;21:104–9.
[43] Haghighatdoost F, Najafabadi MM, Bellissimo N, Azadbakht L. Association of dietary acid load with cardiovascular disease risk factors in patients with diabetic nephropathy. Nutrition 2015;31:697–702.
[44] Chan R, Leung J, Woo J. Association between estimated net endogenous acid production and subsequent decline in muscle mass over four years in ambulatory older Chinese people in Hong Kong: a prospective cohort study. J Gerontol A Biol Sci Med Sci 2014;70:905–11.
[45] Luis D, Huang X, Riserus U, Sjøogren P, Lindholm B, Arnløov J, et al. Estimated dietary acid load is not associated with blood pressure or hypertension incidence in men who are approximately 70 years old, 2. J Nutr 2014;145:315–21.
[46] Bailey JL, Wang X, England BK, Price SR, Ding X, Mitch WE. The acidosis of chronic renal failure activates muscle proteolysis in rats by augmenting transcription of genes encoding proteins of the ATP-dependent ubiquitin-proteasome pathway. J Clin Invest 1996;97:1447–53.
[47] Welch A, MacGregor AJ, Skinner J, Spector TD, Moayyeri A, Cassidy A. A higher alkaline dietary load is associated with greater indexes of skeletal muscle mass in women. Osteoporos Int 2013;24:1899–908.
[48] Crawford SO, Hoogeveen RC, Brancati FL, Astor BC, Ballantyne CM, Schmidt MI, et al. Association of blood lactate with type 2 diabetes: the Atherosclerosis Risk in Communities Carotid MRI Study. Int J Epidemiol 2010;39:1647–55.
[49] Farwell W, Taylor E. Serum bicarbonate, anion gap and insulin resistance in the National Health and Nutrition Examination Survey. Diabet Med 2008;25:798–804.
[50] Souto G, Donapetry C, Calvino J, Adeva MM. Metabolic acidosis-induced insulin resistance and cardiovascular risk. Metab Syndr Relat Disord 2011;9:247–53.
[51] McKeigue P, Shah B, Marmot M. Relation of central obesity and insulin resistance with high diabetes prevalence and cardiovascular risk in South Asians. Lancet 1991;337:382–6.
[52] Fagherazzi G, Vilier A, Bonnet F, Lajous M, Balkau B, Boutron-Rualt MC, et al. Dietary acid load and risk of type 2 diabetes: the E3 N-EPIC cohort study. Diabetologia 2014;57:313–20.
[53] Livesey G, Tagami H. Interventions to lower the glycemic response to carbohydrate foods with a low-viscosity fiber (resistant maltodextrin): meta-analysis of randomized controlled trials. Am J Clin Nutr 2008;89:114–25.
[54] Ma H, Patti ME. Bile acids, obesity, and the metabolic syndrome. Best Pract Res Clin Gastroenterol 2014;28:573–83.
[55] Vrieze A, Van Nood E, Holleman F, Salojarvi J, Kootte RS, Bartelsman JF, et al. Transfer of intestinal microbiota from lean donors increases insulin sensitivity in individuals with metabolic syndrome. Gastroenterology 2012;143.913–6.e7.
[56] Whitworth JA, Williamson PM, Mangos G, Kelly JJ. Cardiovascular consequences of cortisol excess. Vasc Health Risk Manag 2005;1:291.
[57] Xu C, He J, Jiang H, Zu L, Zhai W, Pu S, et al. Direct effect of glucocorticoids on lipolysis in adipocytes. Mol Endocrinol 2009;23:1161–70.
[58] Djurhuus C, Gravholt CH, Nielsen S, Mengel A, Christiansen S, Schmitz OE, et al. Effects of cortisol on lipolysis and regional interstitial glycerol levels in humans. Am J Physiol Endocrinol Metab 2002;283:E172–7.
[59] Tuohy KM, Fava F, Viola R. 'The way to a man's heart is through his gut microbiota'—dietary pro- and prebiotics for the management of cardiovascular risk. Proc Nutr Soc 2014;73:172–85.