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Original article

Association between intakes of macro- and micro- nutrients and serum lipid profiles among Jordanian adults: a preliminary study

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Summary. Dyslipidemia is one of the most important risk factors for cardiovascular disease. Diet is considered as a major contributor for developing dyslipidemia. This study aimed to assess the intakes of macro- and micro-nutrients with serum lipid profile in disease-free adults. A convenient sample of 167 adults was recruited in this cross-sectional study. Serum lipid profile including total cholesterol, triglycerides (TG), low-density lipoprotein cholesterol LDL-C and high-density lipoprotein cholesterol HDL-C were measured. Nutrients and food groups' consumption was assessed using a validated quantitative Food Frequency Questionnaire. The findings revealed different significant differences between the levels of total energy intake and macronutrients' contribution to energy with serum lipid profiles. Cholesterol serum level was significantly higher among individuals with the highest energy intake (P=0.03), and was significantly lower with highest energy intake from trans-fatty acids (P=0.02). HDL-C tertiles were significantly associated with of percentage of energy from protein (P=0.04). Serum total cholesterol and LDL-C levels were significantly different with vitamin K intake levels (P=0.03). The intake of meat, fish, eggs, and beans was significantly different among HDL-C tertiles. (P=0.04). Many possible associations between Jordanian diet components and serum lipids were reported which indicates that diet is an important factor that should be considered when preventive or treatment strategies are implemented.

Key words: macronutrients, micronutrients, cholesterol, triglycerides (TG), low-density lipoprotein cholesterol LDL-C, high-density lipoprotein cholesterol HDL-C

Introduction

Dyslipidemia is defined as an abnormal lipid metabolism including increased levels of total cholesterol, triglycerides (TG), and low density lipoprotein cholesterol (LDL-C) and/or decreased levels of high-density lipoprotein cholesterol (HDL-C) (1). Dyslipidemia is a major risk factor for developing cardiovascular disease -the first cause of mortality worldwide (2, 3). An estimated 17.7 million people died from cardiovascular diseases (CVDs) in 2015, representing 31% of all global deaths (4). The prevalence of dyslipidemia among Jordanian adults in 2010 was high with 48.8% had high cholesterol, 40.7% had high LDL-C, 40.1% had low HDL-C, while 43.6% had high TG concentrations (5).

The underlying risk factors suggested for dyslipidemia are divided as non-modifiable (e.g., age, gender and genetics), and modifiable (e.g., diet, smoking, physical activity, and stress) (6). Diet plays a key role in the prevention and management of dyslipidemia.1 Previous studies focused on the relationship between dietary fat and cholesterol intake and serum lipids (7-9). Dietary cholesterol is a highly controversial nutrient since it increases serum cholesterol, especially among individuals who are not able to maintain plasma cholesterol homeostasis by reducing its absorption in the small intestine or by suppressing its endogenous synthesis (10). In contrast, studies conducted in young adults (11, 12) and elderly individuals (13), who were assigned to consume additional 640 mg of cholesterol for (4) weeks, found that hyper-responders raise both LDL-C and HDL-C thus the LDL-C/HDL-C ratio is maintained. In addition, those individuals who were not hyper-responders, the LDL-C/HDL-C is maintained since no significant raises in plasma cholesterol were shown (13).

Several epidemiological studies revealed the effectiveness of dietary modifications in preventing and reducing the risk of cardiovascular diseases. For example, low level of protein rich diet in saturated fat has been suggested to reduce the levels of cholesterol, TG, and LDL-C in comparison to a diet that is rich in carbohydrate and unsaturated fat (14). In addition, high intake of polyunsaturated fatty acids was negatively associated with cholesterol and LDL-C concentrations (15), but positively associated with HDL-C concentrations (16). While, excessive intake of total carbohydrates exerts a lowering effect on HDL-C concentration (14, 17), it has been reported to increase TG concentration in adults (18). Conversely, diets enriched in monounsaturated fatty acids (19-21) or both monounsaturated fatty acids and polyunsaturated fatty acids (22) showed no significant difference in serum cholesterol and LDL-C concentrations. However, consumption of fruits and vegetables was found to be associated with low LDL-C levels (23). Also, increased consumption of milk and other dairy products showed a protective effect against the increase in serum TG and decrease in HDL-C (24).

The equivocal results regarding the association between dietary factors and serum lipids may be due to the influence of several confounding factors which could have been inconsistently taken into account. Genetic differences arise among the factors that may cause variation in serum lipid levels through various gene loci in which a direct or indirect involvement in lipid metabolism and/or transport may take place (25, 26). Gender-specific differences may influence serum lipids; women have higher levels of cholesterol and HDL-C, while men have higher LDL-C levels. HDL-C and LDL-C are also influenced by age (27) and body mass index (BMI) (28). The physical activity level mostly increases HDL-C, but findings for cholesterol and LDL-C are less consistent (29). Although the examination of the dietary factors associated with lipid profiles is essential to prevent dyslipidemia, cardiovascular diseases, type 2 diabetes and another chronic disease; the effects of diet on lipid profiles are not yet fully understood. Therefore, the aim of the present study was to investigate the differences in macro and micro-nutrient intake and serum lipids in apparently healthy Jordanian adults.

Methods

Study design and participants

A total number of 167 (83 males and 84 females) disease-free Jordanian volunteers (students and employees), aged 18-51 years were recruited conveniently from the King Hussein Medical Center (KHMC) during the period of October 2014 to July 2015. Based on an alpha probability of 0.05 and power of 0.8 the sample size was calculated. Eligibility criteria to be enrolled in the study were: being Jordanian and above 18 years old. Pregnant and lactating women and individuals with eating disorders, major surgeries or any chronic diseases were excluded from this study. A consent form was obtained from each participant. The study protocol was approved by the Jordanian Royal Medical Services (JRMS) ethics committee.

Measurement of lipid profiles

Blood samples were drawn from participants after overnight fasting by a specialized medical laboratory technician. Serum samples were centrifuged and separated from the whole blood and stored at −80°C until

further analysis. Fasting blood lipid profiles, including total cholesterol, LDL-C, HDL-C, and TG levels were measured by Jenway 6305 UV/Visible Spectrophotometer, USA using commercially available Enzymatic kits (TECO DIAGNOSTICS, USA).

Assessment of nutrient intake

A validated quantitative Food Frequency Questionnaire (FFQ) was used for dietary assessment (30). The FFQ was completed by a trained dietitian through face-to-face interviews. Participants were asked how frequently, on average, during the past year they had consumed one standard serving of specific food items in nine categories (<1/month, 2-3/month, 1-2/week, 3-4/week, 5-6/week, 1/day, 2-3/day, 4-5/day, or 6/day). Food lists in the modified FFQ questions were classified based on types of food: 8 items of cereals; 9 items of milk and dairy products; 21 items of fruits and juices; 21 items of vegetables; 16 items of meat such as red meat (lamb and beef), chicken, fish, cold meat, and others; 4 items of beans; 4 items of soups and sauces; 5 items of drinks; 9 items of snacks and sweets; and 14 items of herbs and spices (30). Portion size estimation was carried out using food models and standard measuring tools. Dietary intakes were analyzed using dietary analysis software (ESHA Food Processor SQL version 10.1.1; ESHA, Salem, OR, USA) with additional data on foods consumed in Jordan (31).

Physical activity level

7-Day physical activity recall (PAR) is a questionnaire that focuses on a participant's recall of time spent doing the physical activity over a seven-days period (32). The validated PAR questionnaire was used to measure physical activity level. Our study participants were asked to respond to the PAR questions with an emphasis on their personal exercise pattern and behavior during the noted time period. Physical activity level was calculated according to the Sallis et al., (1985) protocol (32).

Statistical analysis

The data were analyzed using SPSS statistical package version 20. Energy, macronutrients and micronutrients were presented as a mean ± standard error. Cholesterol, TG, HDL-C and LDL-C were grouped into tertiles. Post-hoc Analysis of Variance (ANOVA) was used to assess the impact of energy, macronutrients and micronutrients intake on serum lipid profiles after adjustment for age, sex, BMI, energy intake, physical activity and smoking. Different letters denote significant differences among the tertiles. *P*-values<0.05 were considered statistically significant.

Results

Participants' demographic and anthropometric data, the concentration of serum cholesterol, TG, HDL-C, and LDL-C were published previously in another publication (33). They revealed that serum levels of IL-6, cholesterol, LDL and HDL were significantly ($P<0.05$) greater in obese participants than those reported for overweight and normal body weight participants. TG serum concentration was the only biochemical variable which was significantly higher ($P<0.05$) in overweight and obese participants when compared with normal body weight participants. However, the values of the all variables were within the normal levels as shown in table 1.

Table 2 shows the possible associations between participants' daily total energy intake and macronutrients' contribution to energy intake with serum lipid profiles. Cholesterol serum level was the only lipid profile which was significantly higher among individuals with the highest total energy intake ($P=0.03$), and was significantly lower among individuals with highest energy intake from trans-fatty acids ($P=0.02$). Similarly, HDL-C serum concentration was the only lipid profile that was significantly different with energy intake from protein ($P=0.04$). There was no significant relationship between energy intake from carbohydrate, fat and saturated fat across all tertiles for serum cholesterol, TG, HDL-C and LDL-C.

Table 3 shows participants' daily intake of different macronutrients through different tertiles of serum cholesterol, TG, HDL-C, and LDL-C. Individuals with the lowest level of serum cholesterol reported increased consumption of trans fatty acids as compared to the middle and highest level of serum cholesterol ($P=0.02$).

There was no statistically significant difference between fat soluble and water soluble vitamins as well

Table 1. Anthropometric and biochemical parameters of the study sample based on BMI[ſ].

Parameter	Normal			Overweight			Obese			*p-value
	N	Mean	SEM	N	Mean	SEM	N	Mean	SEM	
Total Cholesterol (mg/ml)	68	149.5[a]	3.5	40	158.8[a]	5.1	42	171.5[b]	5.9	**0.005**
TG (mg/ml)	68	84.2[a]	5.1	40	109.7[b]	11.4	42	125.9[b]	10.2	**0.008**
HDL (mg/ml)	68	44.5[a]	1.3	40	40.5[a]	1.2	42	38.8[b]	1.3	**0.022**
LDL (mg/ml)	68	101.0[a]	2.9	40	109.9[a]	3.7	42	123.1[b]	5.5	**0.001**

[ſ] *Al-Radaideh et al, 2016*

Abbreviations: SEM - standard error of mean; BMI - body mass index; TG - triglycerides; HDL- high-density lipoprotein cholesterol; LDL - low-density lipoprotein cholesterol.

** p<0.05: those were significantly different based on LSD analysis are labeled with different letters.*

Table 2 - Percentages of energy intakes and distribution across different tertiles of cholesterol, TG, HDL-C and LDL-C

Nutrients		Serum cholesterol		TG		HDL-C		LDL-C	
		Mean±SEM	*P*-value	Mean±SEM	*P*-value	Mean±SEM	*P*-value	Mean±SEM	*P*-value
Energy (kcal)	T_1	2985.0±183.6[ab]		2838.5±159.4		2997.9±156.2		2930.8±180.5	
	T_2	2615.1±151.9[a]	**0.03**	2853.2±172.9	0.36	3019.5±201.2	.67	2806.6±156.2	0.47
	T_3	3248.1±175.1[b]		3151.9±188.2		2815.2±167.1		3106.6±184.3	
Percent Energy from Carbohydrates (%)	T_1	61.2±1.2		61.5±1.1		62.1±1.2		61.8±1.2	
	T_2	63.4±1.3	0.29	62.6±1.4	0.29	63.2±1.5	0.78	62.1±1.4	0.24
	T_3	63.9±1.4		64.4±1.4		63.3±1.3		64.7±1.3	
Percent Energy from Fats (%)	T_1	29.7±1.1		29.8±0.9		28.8±0.9		29.37±1.06	
	T_2	28.5±1.1	0.30	28.91±1.2	0.11	28.9±1.2	0.76	29.16±1.05	0.21
	T_3	27.4±1.1		26.7±1.0		27.8±1.1		26.97±1.08	
Percent Energy from Protein (%)	T_1	12.2±0.4	0.76	12.1±0.3	0.71	12.6±0.4[b]	**0.04**	11.9±0.4	0.37
	T_2	11.9±0.3		11.9±0.4		11.3±0.4[a]		12.5±0.4	
	T_3	12.2±0.4		12.3±0.4		12.3±0.3[b]		11.9±0.3	
Saturated Fatty Acids. Kcal	T_1	292.5±14.9	0.90	300.3±12.8	0.87	286.1±12.6	0.12	291.9±14.4	0.91
	T_2	304.4±14.5		303.7±14.5		328.9±25.8		300.7±14.8	
	T_3	297.6±23.1		290.7±24.4		281.6±12.6		302.1±23.2	
Trans-Fatty Acids. kcal	T_1	37.1±4.8[b]	**0.02**	30.7±3.1	0.82	31.9±4.4	0.54	35.0±4.9	0.12
	T_2	23.5±2.3[a]		27.5±3.4		27.9±3.3		25.1±2.5	
	T_3	26.6±3.0[a]		28.7±4.3		26.6±2.7		27.0±3.0	

- Statistical significant difference (P< 0.050)

- Different letters to denote significant differences among the tertiles.

T stands for Tertile.

as calcium, copper, iron and zinc intake through all tertiles of the analyzed serum lipid profiles, except for total cholesterol and LDL-C levels, which were positively affected by vitamin K intake (*P*=0.03) (Table 4).

Table 5 presents the differences in food groups' intake according to serum lipid levels. The highest intake of meat, fish, eggs, and beans was inversely affected by HDL-C (*P*=0.04). No significant differences

Table 3 - Mean±SEM for adjusted macronutrients intake in different tertiles of cholesterol, TG, HDL-C and LDL-C

Nutrients		Serum cholesterol		TG		HDL-C		LDL-C	
		Mean±SEM	*P*-value	Mean±SEM	*P*-value	Mean±SEM	*P*-value	Mean±SEM	*P*-value
Total Carbohydrate (g)	T_1	511.4±12.2		510.1±9.9		518.4±10.5		515.4±12.1	
	T_2	518.6±12.4	0.34	518.9±13.4	0.26	525.2±16.1	0.91	512.8±12.8	0.27
	T_3	538.1±14.7		539.6±15.7		525.2±13.4		540.1±14.4	
Fiber (g)	T_1	56.9±2.4		58.2±1.9		59.19±2.07		57.2±2.2	
	T_2	57.4±1.8	0.43	56.3±2.2	0.42	55.2±2.3	0.21	57.9±2.0	0.69
	T_3	60.6±2.1		60.3±2.2		60.3±1.9		59.4±2.2	
Soluble Fiber (g)	T_1	6.9±0.6		7.1±0.6		7.6±0.6		6.7±0.6	
	T_2	7.5±0.56	0.53	7.0±0.7	0.59	6.8±0.7	0.51	8.1±0.6	0.32
	T_3	7.9±0.7		7.9±0.6		7.8±0.7		7.5±0.7	
Insoluble Fiber (g)	T_1	12.0±1.0		12.7±0.9		13.4±1.0		11.9±1.0	
	T_2	12.4±0.9	0.40	11.8±1.1	0.45	11.8±1.1	0.55	12.9±0.9	0.62
	T_3	13.8±1.0		13.6±1.0		12.9±0.9		13.3±1.1	
Protein (g)	T_1	98.9±2.9		98.2±2.3		101.2±2.6		97.2±2.8	
	T_2	95.5±2.2	0.49	95.5±2.6	0.43	93.6±3.0	0.12	100.5±2.8	0.52
	T_3	99.9±3.0		100.5±3.1		98.9±2.4		96.4±2.5	
Fat (g)	T_1	116.1±4.7		117.1±3.9		113.0±4.0		115.0±4.7	
	T_2	115.6±4.5	0.21	114.9±5.2	0.18	113.5±5.5	0.91	116.0±4.6	0.28
	T_3	105.5±5.1		105.1±5.2		110.7±5.1		106.2±5.1	
Saturated Fat (g)	T_1	32.5±1.7		33.4±1.4		31.8±1.4		32.4±1.6	
	T_2	33.8±1.6	0.89	33.8±1.6	0.87	36.6±2.9	0.12	33.4±1.7	0.91
	T_3	33.1±2.6		32.3±2.7		31.3±1.4		33.6±2.6	
Monounsaturated Fat (g)	T_1	28.9±1.8		28.9±1.6		28.3±1.5		28.2±1.7	
	T_2	26.9±1.4	0.11	27.2±1.7	0.07	23.6±1.7	0.09	27.8±1.7	0.14
	T_3	24.1±1.7		23.7±1.6		27.8±1.6		24.0±1.6	
Polyunsaturated Fat (g)	T_1	21.8±1.8		23.0±1.7		21.9± 1.6		21.9±1.8	
	T_2	23.3±1.7	0.24	22.8±1.9	0.12	19.3± 1.8	0.29	23.6±1.7	0.13
	T_3	19.2±1.7		18.6±1.6		23.2±1.8		18.8±1.6	
Trans-Fatty Acids (g)	T_1	4.2±0.5[b]		3.4±0.3		3.6±0.5		3.9±0.5	
	T_2	2.7±0.3[a]	**0.02**	3.1±0.4	0.82	3.1±0.4	0.55	2.8±0.3	0.12
	T_3	2.9±0.3[a]		3.2±0.5		3.0±0.3		3.0±0.3	
Cholesterol (mg)	T_1	228.4±26.5		201.9±14.3		226.6±22.9		226.7±25.9	
	T_2	205.7±18.4	0.67	200.4±20.1	0.36	217.7±23.3	0.50	216.4±20.5	0.58
	T_3	205.7±16.0		237.5±26.3		193.1±13.1		196.5±14.1	
Omega 6 Fatty Acids (g)	T_1	18.9±1.8		20.2±1.7		19.2±1.6		18.8±1.8	
	T_2	20.8±1.6	0.19	20.2±1.9	0.96	15.9±2.1	0.19	20.9±1.7	0.17
	T_3	16.1±2.0		15.4±1.9		20.7±1.9		16.1±1.9	
Omega 3 Fatty Acids (g)	T_1	1.4±0.2		1.5±0.1		1.4±0.1		1.4±0.2	
	T_2	1.7±0.1	0.08	1.5±0.2	0.15	1.2±0.2	0.29	1.6±0.1	0.23
	T_3	1.2±0.2		1.2±0.2		1.6±0.2		1.2±0.2	

- Statistical significant difference (P<0.05)

- Different letters denotes significant differences among the tertiles.

- T stands for Tertile.

Table 4 - Mean±SEM for adjusted micro-nutrients intake for different of cholesterol, TG, HDL-C and LDL-C

Nutrients		Serum cholesterol		TG		HDL-C		LDL-C	
		Mean±SEM	*P*-value	Mean±SEM	*P*-value	Mean±SEM	*P*-value	Mean±SEM	*P*-value
Vitamin A. IU	T_1	17773.9±2117.9		17768.4±1642.7		20193.9±1916.6		17522.2±1993.4	
	T_2	19501.7±2425.7	0.38	18165.7±2352.2	0.12	21035.8±2744.1	0.62	19288.8±2454.3	0.26
	T_3	22065.8±1995.1		23454.7±2477.0		18037.9±1905.4		22535.2±2065.1	
β-carotene (µg)	T_1	7566.4±1084.1	0.41	7519.9±827.5	0.17	9154.0±1012.2	0.43	7315.3±1040.9	0.24
	T_2	8578.5±1353.2		8001.1±1420.0		9422.9±1613.4		8481.5±1337.1	
	T_3	9854.9±1161.9		10512.9±1306.3		7341.5±937.2		10205.2±1204.5	
Vitamin D (µg)	T_1	1.0±0.3		0.7±0.2		0.7±0.1		1.1±0.3	
	T_2	0.9±0.2	0.78	0.8±0.2	0.44	1.1±0.3	0.43	0.8±0.2	0.57
	T_3	0.7±0.2		1.1±0.3		0.8±0.3		0.7±0.2	
Vitamin E (mg)	T_1	16.5±4.1		15.4±2.6		11.8±0.9		17.8±4.2	
	T_2	16.8±2.8	0.82	16.3±2.8	0.98	18.1±3.6	0.25	15.0±2.7	0.75
	T_3	14.2±2.4		15.90±4.1		18.3±4.4		14.7±2.4	
Vitamin K (µg)	T_1a	143.8±19.7		179.8±22.7		221.2±37.1		141.2±18.9[a]	
	T_2b	173.1±27.9	**0.04**	241.4±73.1	0.67	227.4±71.5	0.72	171. 7±28.9[a]	**0.03**
	T_3c	309.7±75.5		206.6±39.3		175.2±30.6		313.7±75.1[b]	
Folate (µg)	T_1	507.8±125.3		463.2±72.8		369.5±26.0		548.2±128.2	
	T_2	510.9±78.9	0.90	511.2±81.2	0.90	576.1±101.9	0.20	461.4±73.9	0.80
	T_3	474.3±71.1		520.7±123.1		568.6±133.8		484.5±70.9	
Vitamin B12 (µg)	T_1	7.6±1.8		6.4±1.1		4.9±0.5		8.13±1.9	
	T_2	7.1±1.3	0.73	7.1±1.2	0.90	7.9±1.6	0.18	6.31±1.2	0.57
	T_3	5.9±1.1		7.2±1.8		8.2±1.9		6.21±1.1	
Vitamin C (mg)	T_1	281.6±46.7		268.2±39.5		277.1±36.7		285.8±45.8	
	T_2	262.3±40.3	0.37	287.4±73.1	0.54	309.9±48.4	0.84	265.5±43.5	0.48
	T_3	344.7±41.3		333.4±46.3		303.9±45.1		337.2±39.1	
Calcium (mg)	T_1	1468.9±333.4		1386.0±199.5		1095.6±88.7		1582.2±335.0	
	T_2	1441.5±202.5	0.99	1423.9±235.1	0.93	1680.0±286.2	0.20	1325.5±198.8	0.77
	T_3	1417.5±216.3		1520.0±323.0		1608.8±349.7		1422.7±215.7	
Copper (mg)	T_1	1.5±0.1		1.5±0.1		1.5±0.1		1.4±0.1	
	T_2	1.3±0.1	0.09	1.4±0.1	0.80	1.3±0.1	0.34	1.4±0.1	0.74
	T_3	1.6±0.1		1.5±0.1		1.4±0.1		1.5±0.1	
Iron (mg)	T_1	32.3±5.7		29.7±3.3		26.1±1.2		34.1±5.8	
	T_2	32.3±3.5	0.85	30.8±3.5	0.81	34.1±4.6	0.26	30.3±3.3	0.72
	T_3	29.3±3.1		33.4±5.6		34.6±6.0		29.5±3.0	
Zinc (mg)	T_1	19.8±4.5		17.8±2.9		14.4±0.9		20.9±4.7	
	T_2	20.0±3.0	0.72	19.4±2.9	0.94	20.8±3.9	0.24	18.6±2.9	0.71
	T_3	16.5±2.6		19.2±4.5		21.9±4.8		16.8±2.6	

- Statistical significant difference (P<0.05)
- Different letters to denote significant differences among the tertiles.
- Abbreviations: Retinol Equivalent, RE; International Unit, IU; Tertiles, T

Table 5 - Food groups distribution across different tertiles of cholesterol, TG, HDL-C and LDL-C

Nutrients		Serum cholesterol		TG		HDL-C		LDL-C	
		Mean±SEM	*P*-value	Mean±SEM	*P*-value	Mean±SEM	*P*-value	Mean±SEM	*P*-value
Grain group (oz)	T_1	13.1±1.1		13.1±0.9		13.0±0.9		13.1±1.1	
	T_2	12.6±0.9	0.63	12.4±0.9	0.50	13.3±1.1	0.98	13.5±0.9	0.92
	T_3	13.8±0.8		14.0±1.0		13.1±0.9		12.9±0.8	
Vegetables group (cup)	T_1	4.9±0.6		4.3±0.5		5.0±0.5		4.9±0.6	
	T_2	3.8±0.4	0.12	4.2±0.4	0.27	4.1±0.4	0.39	3.9±0.4	0.21
	T_3	5.1±0.5		5.2±0.6		4.6±0.6		5.0±0.5	
Fruit group (cup)	T_1	3.7±0.8		3.9±0.8		4.3±0.7		3.6±0.7	
	T_2	3.8±0.9	0.20	4.0±1.0	0.49	4.9±1.2	0.77	4.1±1.1	0.31
	T_3	5.8±1.0		5.3±0.9		4.0±0.8		5.5±0.9	
Dairy group (cup)	T_1	0.6±0.1		0.6±0.1		0.6±0.1		0.5±0.1	
	T_2	0.5±0.1	0.60	0.6±0.1	0.97	0.7±0.1	0.26	0.6±0.1	0.57
	T_3	0.7±0.1		0.6±0.1		0.5±0.1		0.7±0.1	
Meat group (meat, fish, eggs, and beans) (oz)	T1	4.6±0.5		4.0±0.4		4.7±0.4[b]		4.1±0.5	
	T2	3.3±0.3	0.06	3.8±0.4	0.72	3.3±0.3[a]	**0.04**	3.9±0.4	0.91
	T3	4.1±0.4		4.2±0.4		3.9±0.4[ab]		4.0±0.3	
Fats and oils (tsp)	T_1	10.4±0.9		10.0±0.8		10.2±0.9		9.9±0.8	
	T_2	8.9±0.8	0.23	10.4±1.1	0.92	10.8±1.1	0.56	9.9±0.9	0.88
	T_3	11.1±1.1		9.9±1.0		9.4±0.8		10.5±1.1	

- Statistical significant difference (P<0.050)
- Different letters to denote significant differences among the tertiles.
- T stands for Tertile; C strands for Cup; oz strands for ounces; tsp stands for teaspoon.

between grains, vegetables, fruits, milk, fats and oils intake and cholesterol, TG, HDL-C and LDL-C concentrations were observed.

Discussion

Dyslipidemia is the abnormal lipid metabolism which is regarded as a strong predictor for the development of cardiovascular diseases. Little is known about the associations between serum lipid profiles and dietary factors. In this study, we examined the associations between macronutrient and micronutrient intake and serum lipid profile among healthy Jordanian adults.

The present study showed that total energy intake was associated with serum total cholesterol. Extra caloric intake is not recommended, whether the extra calories are from fat, protein, or carbohydrate (34). In contrast to our finding, Song et al. (2016) did not find an association between total energy intake and TC and LDL-C pattern (35). This could be attributed to the highest energy intake among our study tertiles (around 3250 calories) compared to what was found by other researchers. Total energy intake among our study group when stratified according to the BMI was significantly higher among obese participants as compared to overweight and normal body weight (3369.2±173.9, 2806.6±142.6, 2801.8±195.6 kcal for obese, overweight and normal body weight, respectively; p<0.03). These results are not presented in the tables. Excessive energy intake leads to obesity which associated with states of hyperinsulinemia and insulin resistance (36). Insulin resistance was linked to the increase in cholesterol synthesis. Insulin stimulates lipid synthesis, in particular, cholesterol synthesis, thus raising the total cholesterol level (37, 38).

Additionally, serum cholesterol was significantly lower among Jordanian adults who consumed the highest amount of trans-fatty acid. This can be interpreted as the highest amount of trans-fatty acid that can be found in hydrogenated vegetable oils, which were considered free of cholesterol (39). As a consequence, serum cholesterol could be lower for those who consumed a considerable amount of hydrogenated fats and oils. On the other hand, it is well known that trans-fatty acid intake is highly associated with elevated level of serum LDL-C and reduced level of HDL-C (39). However, the present study did not report any significant differences between trans-fatty acid intake levels and levels of serum LDL-C and that was consistent with a reported randomized crossover study in healthy young Japanese which also showed no significant effects of 0.6% energy trans-fatty acid intake on total serum cholesterol concentrations (40). Therefore, it could be concluded that the intake of trans-fatty acids among this study sample is lower than the level which may affect serum lipids.

The 3 tertiles of HDL-C were significantly associated with percentage of energy from protein, irrespective to protein sources: plants or animals. However, no specific trend could be detected in the 3 tertiles. In a study conducted in Tehran, Bahadoran et al. (2013) reported that the higher dietary intake of protein as % of energy and g/kg body weight in men was inversely related with 3-year changes in HDL-C levels (41). However, Song et al. (2016) did not find any association between % of energy from protein with TG and HDL-C pattern or even with TC and LDL-C pattern (35). The mechanism by which protein is associated with upregulated HDL cholesterol production and the extent to which body weight status modulates this response requires further study. However, because consuming a high protein diet regularly was associated with higher HDL cholesterol (and lower adiposity) regardless of total dietary energy, carbohydrate, and fat intake, the intrinsic properties of protein appear to be partially responsible for these effects (42). In addition, a higher ratio of dietary protein to carbohydrates is related to more efficient glycemic control and satiety during weight loss which may enhance HDL (43).

Regarding the results of calcium, copper, iron and zinc intake and their associations with serum lipid profile, no significant associations have been detected. In a Québec Family Study, Jacqmain et al. (2003), found that the plasma lipoprotein-lipid profile in both women and men is apparently affected by a low dietary calcium intake (<600 mg) compared to a high dietary calcium intake (>1000 mg) (44). Inconsistent with our findings, Song et al. (2016) observed an inverse association between calcium intake and TG and HDL-C pattern (35). In a cross-sectional study on healthy females, Zaribaf et al. (2014) reported that there was no significant correlation between total amount of iron, heme iron, and non-heme dietary iron with serum lipid profile (45). On the other hand, an inverse association between iron intake and total cholesterol and LDL-C pattern was observed by other (35). Additionally, a negative association between dietary zinc intake and total cholesterol and triglycerides was noticed in a cross-sectional study (41). Regarding dietary copper intake, limited cross-sectional studies tend to suggest that dietary copper is associated with a better lipoprotein profile (46).

Both water and fat soluble vitamins, except vitamin K, were not significantly associated with serum lipid profile. In this study, the differences between vitamin K intake levels were significant with serum cholesterol and LDL-C values. The effect of vitamin k intake and blood lipid profile in the literature is equivocal and depends on its sources if they are plant (vitamin K1: phylloquinone) or animal (vitamin K2: menaquinones) sources. Indeed, vitamin K1 was found to be inversely associated with a fatal heart attack (47), while vitamin K2 was not associated with increased incidence of heart disease and increased fatal heart attack (48). In a prospective cohort study of Dutch men and women, neither vitamin K1 nor vitamin K2 was found to be associated with stroke risk (49). In our study, total vitamin K is estimated irrespective of its source, so further studies are warranted to assess the association between vitamin K from the two sources and its effect on serum lipids.

It was observed that number of servings consumed from the following groups: grain, vegetables, fruits, milk and dairy products and fats and oils were not associated with serum lipid profile. Similar results were obtained in another study, except for vegetable group, which was inversely associated with total cholesterol

and LDL-C pattern and milk as well as dairy products, which was inversely associated with TG and HDL-C pattern (35). In the present study, food groups that were inversely associated with HDL-C included meat, fish, eggs and beans. In consistent with our findings, Daoud et al. (2014) reported that high protein diets resulted in a reduction in HDL-C and LDL-C (50).

Limitations

Our study has several limitations including the cross-sectional design that did not determine a causal relationship between dietary factors and dyslipidemia. Furthermore, the one year dietary recall period, which may be affected by changes in memory and bias, is one of the major limitations of the present study. However, we believe that because food selection and the taste are mostly based on availability and habits that influence deliberate choices, including endemic cultural biases, we accept that the recall period of one year is very likely to be reflective of the previous years. In addition, our sample size is small due to the limited financial support for the biochemical analyses but within the recommended size as calculated using the sample size power calculations. Additionally, the subjects' lipid profile is generally within the normal levels which showed few associations with some nutrients. Therefore, another study on a large-scale is warranted to generalize these findings.

Conclusion

In conclusion, there are many possible associations between Jordanian diet and its components and serum lipids, even after adjustment for potential confounding factors. The consumption of meat, fish, eggs and beans should be within the recommendations.

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