Progress in Nutrition 2017; Vol. 19, N. 1: 48-56 - DOI: 10.23751/pn.v19i1.4856

Original Article

The effect of lentil on cholesterol-induced changes of serum lipid cardiovascular indexes in rats

Mousa Numan Ahmad

Department of Nutrition and Food Technology, Human Nutrition and Dietetics, The University of Jordan, Amman, Jordan - E-mail: mosnuman@ju.edu.jo

Summary. *Aim:* We investigated effects of lentil on serum lipemia and cardiovascular indexes in cholesterol-fed rats. *Methods:* Sixty male Sprague-Dawley rats were assigned into 2 groups fed diets with or without cholesterol. Diets contained casein, raw dehulled lentil, raw whole lentil, cooked dehulled lentil or cooked whole lentil and given to rats for 4 weeks. Serum total cholesterol (TC), low-density lipoprotein cholesterol (LDL-C), high-density lipoprotein cholesterol (HDL-C) and triglycerides (TG) were quantified and other biological parameters were assessed. Atherogenic indexes including atherogenic index of plasma (AIP), cardiac risk ratio (CRR), atherogenic coefficient (AC), HDL-C/LDL-C and TC/TG were then calculated. *Results:* Cholesterol induced marked atherogenic lipemia indicated by increased LDL-C and decreased HDL-C/LDL-C. Lentil increased ($p<0.05$) HDL-C and thus TC and TC/TG, and decreased ($p<0.05$) TG and AIP, while other lipid variables or indexes were unaffected. This effect was more evident on AIP and TC/TG than lipid variables with little or no effect for different lentil diets. In all rats, lipid indexes showed mutual correlations clearly reflecting their lipid fractions and lentil-induced changes. AIP inversely linked to TC/TG ($r=-0.901$, $p<0.0001$) and both did not correlate with AC, CRR or HDL-C/LDL-C, though AC directly correlated to CRR ($r=0.995$, $p<0.0001$) and both inversely associated with HDL-C/LDL-C ($r=-0.669$, $p<0.0001$). *Conclusions:* Results suggest that lentil reduces AIP and increases TC/TG seemingly through its beneficial effect on TG and HDL-C regardless its form or preparation method. AIP and TC/TG perhaps provide a more distinct measure for cardioprotective effect of lentil than single lipid variables

Key words: atherogenic indexes, cardioprotective activity, cholesterol, dyslipidemia, lentil, rats

Abbreviations

TC: Total cholesterol
LDL-C: Low-density lipoprotein cholesterol
HDL-C: High-density lipoprotein cholesterol
TG: Triglycerides
AIP: Atherogenic index of plasma
CRR: Cardiac risk ratio
AC: Atherogenic coefficient

Introduction

Lentil (*Lens culinaris* L.) is the most ancient cultivated crops among the legumes. It is originated in the Middle East and Mediterranean region and is central to the local diet (1). Lentil is traditionally consumed with other cereal grains such as rice or wheat products (2). Consumption of lentil is related to higher-quality diets, including higher intakes of protein, dietary fiber, antioxidants and a number of vitamins and mineral elements, and lower intakes of saturated fat, total fat and

energy (3). Pulse intake including lentil is also associated with reduced risk of cardiovascular disease (4).

The epidemic of cardiovascular disease (CVD) is a global phenomenon, and remains the leading cause of death in the world (5). Dyslipidaemia is one of the most important risk factor for CVD (6). It includes high total cholesterol (TC), high low-density lipoprotein cholesterol (LDL-C), low high-density lipoprotein cholesterol (HDL-C), and high triglycerides (TG). Dyslipidaemia is also a target of CVD risk scoring for stratification and prevention (7). Several atherogenic indexes are frequently used in human studies for assessing CVD risk beyond the routinely determined lipid profile. These include atherogenic index of plasma (AIP= log {TG/HDL-C}), cardiac risk ratio (CRR= TC/ HDL-C), atherogenic coefficient (AC= {TC-HDL-C}/HDL-C), HDL-C/LDL-C, and TC/TG (8-11). Each of these indexes is based on lipid variables which are independent risk factors for CVD. The AIP, CRR and AC have been found to be better markers of the presence of atherogenic dyslipidemia than conventional lipoprotein cholesterol ratios (8-11); nevertheless, such indexes are rarely used for evaluating dyslipidemia in animal studies.

Given the public health burdens of CVD, its prevention is becoming a major challenge (12). One potential countermeasure to CVD epidemic is to identify and recommend dietary factors that improve lipid profile or lessen CVD risk indexes. Evidence for possible effect of lentil on serum lipids in humans and animals is limited and inconsistent. Variable effects of lentil on serum lipid fractions have been demonstrated in pigs (13) and rats (14-17). Lentil given to type 2 diabetic patients has been shown to reduce TC without affecting other lipid fractions (18), or increase TC, HDL-C and LDL-C (19). Few studies have reported some effects of lentil on certain atherogenic ratios of LDL-C/HDL-C and TC/TG in rats (14), and of AIP in diabetic patients (20). Studies with mixed pulses including lentil have provided indirect evidence linking lentil intake with lipid profile in normal man (20-22).

In view of the apparent scarcity of studies that link the intake of lentil and cholesterol with serum CVD risk indexes in humans and animals, we investigated whether the consumption of lentil-based diets supplemented with cholesterol had any effect on AIP, CRR and AC, and selected conventional lipid ratios of HDL-C/LDL-C and TC/TG in rats fed such a dietary regimen for a period of four weeks.

Materials and methods

Lentils and diets

Whole and dehulled lentil seeds (*Lens culinaris,* L.) of Jordan-1 variety were obtained from Food Legume Improvement Project, Department of Horticulture and Crop Science, Faculty of Agriculture, The University of Jordan, Amman, Jordan. This variety is domestic to Jordan and is commonly used in the local diet (14). Part of the seeds was left raw (whole and dehulled) and another part was cooked following the home cooking method prevailing in the Middle East (2). Raw and cooked lentil powders were prepared as described elsewhere (14). Macronutrient composition of lentil powders was determined by the Weende method (23). Respective means (%) of triplicates with less than 5% coefficient of variation contained in raw dehulled, raw whole, cooked dehulled and cooked whole lentils on dry matter basis for carbohydrate were: 65.7, 65.5, 68.8 and 69.4; for protein were: 24.8, 22.5, 24.0 and 22.2; and for fat were: 1.1, 1.1, 1.3 and1.0.

Ten isocaloric and isonitrogenous diets were prepared, 5 of them were cholesterol-free and differed in the content of lentil powder (%): lentil-free (0), raw dehulled (60.5), raw whole (66.6), cooked dehulled (62.5) and cooked whole (65.6), while in the other 5, cholesterol (1%) was added to induce hypercholesterolemia. Modifications in lentil content in the lentil-containing diets were made in accordance to the macronutrient analyses described earlier. Ingredient composition of diets was described elsewhere (14). All diets contained the same amount of calories, carbohydrate, protein, fat, vitamins and mineral elements. Dietary supply of nutrients was in accordance with the dietary recommended allowances for rats from the American Institute of Nutrition (24). Macronutrient and energy contents of experimental diets are described in Table 1. Diets were freshly prepared once a week and placed desiccated in sealed polythene bags and then kept refrigerated at 4°C.

Table 1. Macronutrient and energy content of cholesterol-free and cholesterol-supplemented lentil diets

	Cholesterol-free lentil diets					Cholesterol-supplemented lentil diets				
Parameter	**Control**	**Raw dehulled**	**Raw whole**	**Cooked dehulled**	**Cooked whole**	**Control**	**Raw dehulled**	**Raw whole**	**Cooked dehulled**	**Cooked whole**
Energy (kJ/100g)	1820	1820	1820	1820	1820	1820	1820	1820	1820	1820
Carbohydrate										
(%)	66.7	66.7	66.7	66.7	66.7	66.7	66.7	66.7	66.7	66.7
(% energy)	61.4	61.4	61.4	61.4	61.4	61.4	61.4	61.4	61.4	61.4
Protein										
(%)	15.0	15.0	15.0	15.0	15.0	15.0	15.0	15.0	15.0	15.0
(% energy)	13.8	13.8	13.8	13.8	13.8	13.8	13.8	13.8	13.8	13.8
Fat										
(%)	12.0	12.0	12.0	12.0	12.0	12.0	12.0	12.0	12.0	12.0
(% energy)	24.8	24.8	24.8	24.8	24.8	24.8	24.8	24.8	24.8	24.8

Animal experimentation

Sixty male Sprague- Dawley rats were obtained from the Experimental Animal Unit of the Department of Nutrition and Food Technology, The University of Jordan, Amman, Jordan. The animals were acclimatized for 1 week before the experiment, during which they were fed on chow diet with free access to tap water. They were individually housed in plastic cages with stainless steel wire-mesh bottom (North Kent Plastic Cages, Ltd, Dartford, England) under controlled temperature (22±2°C) and hygienic conditions with 12-hour light, 12-hour dark cycle. All experiments involving animals were approved by Institutional Animal Ethics Committee and carried out according to the recommended guidelines for animal use (25).

At the beginning of the experiment, animals were assigned into the 5 cholesterol-free or 5 cholesterol-supplemented diets described above. During the experimental period, which lasted for 4 weeks, experimental diets and tap water were given *ad libitum.* Body weight and food intake were monitored weekly. Food conversion ratio as body weight gain per food intake was also calculated. On the termination day and after an overnight fast, animals were anesthetized using chloroform. Blood was collected by performing cardiac puncture and serum was isolated and stored frozen at −20°C until chemical analysis.

Biochemical analysis

Concentrations of serum lipids and lipoproteins were determined by using commercial kits and in accordance to manufacturer's instructions (Boehringer Mannheim GmBH, Germany). The lipid variables included TC, LDL-C, HDL-C, and TG. Analysis was performed at the Medical Laboratories of the Islamic Hospital, Amman, Jordan, using a pre-calibrated automated clinical chemistry analyzer (Roche/Hitachi 912 chemistry analyzer). Serum lipid atherogenic indexes were computed according to formulas where the serum lipid variables were expressed in molar concentrations as documented by previous reports (8-11). These indexes were AIP= log {TG/HDL-C}, CRR= TC/HDL-C, AC= {TC-HDL-C}/HDL-C, and ratios of HDL-C/LDL-C, and TC/TG.

Statistical analysis

Data analysis was performed using statistical analysis software (SAS version 9, USA). Statistical significance was assessed by two-way ANOVA followed by the Duncan's multiple range tests. Data were expressed as means ±standard deviation. Partial correlations were used to test the relationship between each of the serum lipid fractions and cardiovascular indexes for the whole study animals. Significance was set at $p<0.05$.

Results

At the start of the experiment, body weights of the rat groups assigned for different diets were essentially similar ($p>0.05$). They were (mean ± standard deviation in g) 89.7±6.1, 93.0±5.1, 88.8±9.1, 90.8±9.3, and 89.2±7.1 for cholesterol-free control, raw dehulled, raw whole, cooked dehulled and cooked whole lentil groups respectively, and 99.0±13.9, 97.0±8.3, 95.7±6.1, 95.2±8.1, and 95.7±6.8 for cholesterol-supplemented control, raw dehulled, raw whole, cooked dehulled and cooked whole lentil groups respectively.

Table 2 shows body weight, food intake and food conversion ratio of rats fed the lentil diets. These variables were not significantly ($p>0.05$) affected by cholesterol feeding. Compared to control, raw dehulled, raw whole and cooked whole lentils caused significant ($p<0.05$) decrease in weight gain, food intake and food conversion ratio in cholesterol-free groups, whereas these variables were unaffected ($p>0.05$) by cooked dehulled lentil. The lentil diets produced similar pattern of effects on the aforementioned variables when compared to control in cholesterol-supplemented groups. Compared to other lentil diets, cooked dehulled lentil significantly ($p<0.05$) increased weight gain, food intake and food conversion ratio in cholesterol-free and cholesterol-supplemented groups. There were minimal differences in these variables due to raw dehulled, raw whole and cooked whole lentils within or between rat groups fed cholesterol-free or cholesterol-supplemented diets.

Concentrations of serum lipids and lipoproteins of rats fed the lentil diets are presented in Table 3. In contrast to cholesterol-free control, cholesterol significantly ($p<0.05$) increased LDL-C and decreased TG, whereas TC and HDL-C were unchanged. In cholesterol-free groups, compared to control, lentil diets resulted in significant ($p<0.05$) increase in HDL-C and thus TC, and decrease in TG without influencing LDL-C. In cholesterol-supplemented groups, compared to control, lentil diets caused significant ($p<0.05$) increase in TC and HDL-C with less noticeable influence on TG and LDL-C. In cholesterol-free and cholesterol-supplemented groups, there were little or no differences in TC, HDL-C, LDL-C, and TG as a result of feeding different lentil diets.

Table 4 presents serum lipid cardiovascular indexes of experimental groups. Cholesterol induced significant ($p<0.05$) increase in AC and decrease in HDL-C/LDL-C, whereas AIP, CRR and TC/TG were not changed as compared to cholesterol-free control. In cholesterol-free groups, compared to control, different lentil diets significantly ($p<0.05$) decreased AIP and increased TC/TG, whereas AC, CRR and HDL-C/LDL-C were almost not affected. Noteworthy, almost similar pattern of effects of lentil diets on the serum lipid cardiovascular indexes were observed in cholesterol-supplemented groups. However, differences in these indexes of rats fed the lentil diets in both cholesterol-free and cholesterol-supplemented groups were less noticeable.

Table 2. Body weight, food intake and food conversion ratio of rats fed lentil-based diets for 4 weeks.

	Cholesterol-free lentil groups[§*]					Cholesterol-supplemented lentil groups[§*]				
Parameter	**Control**	**Raw dehulled**	**Raw whole**	**Cooked dehulled**	**Cooked whole**	**Control**	**Raw dehulled**	**Raw whole**	**Cooked dehulled**	**Cooked whole**
Weight gain (g)	108.0± 24.0[a]	50.8± 11.1[b]	37.2± 7.7[b]	90.6± 16.3[a]	47.8± 8.6[b]	111.2± 27.3[a]	46.2± 8.9[b]	30.2± 15.4[b]	82.6± 22.2[a]	46.2± 16.1[b]
Food intake (g)	289.0± 17.9[a]	220.6± 25.1[bc]	189.3± 20.9[c]	282.8± 28.0[a]	227.2± 28.4[b]	317.1± 73.3[a]	195.5± 21.0[bc]	183.3± 25.5[c]	269.0± 65.6[ab]	227.5± 35.9[b]
FCR[#]	0.373± 0.063[a]	0.228± 0.026[b]	0.196± 0.035[b]	0.319± 0.045[a]	0.210± 0.025[b]	0.350± 0.042[a]	0.234± 0.022[b]	0.160± 0.071[b]	0.305± 0.024[a]	0.198± 0.040[b]

*[§]Values are means ± standard deviation; [#]FCR: food conversion ratio (weight gain/food intake); *Values in rows with different superscripts are significantly different (p< 0.05)*

Table 3. Serum lipid and lipoprotein concentrations of rats fed lentil-based diets for 4 weeks

	Cholesterol-free lentil groups[$*]					Cholesterol-supplemented lentil groups[$*]				
Parameter	**Control**	**Raw dehulled**	**Raw whole**	**Cooked dehulled**	**Cooked whole**	**Control**	**Raw dehulled**	**Raw whole**	**Cooked dehulled**	**Cooked whole**
TC	1.21± 0.24^{b}	1.59± 0.10^{a}	1.62± 0.23^{a}	1.70± 0.24^{a}	1.78± 0.21^{a}	1.36± 0.26^{b}	1.88± 0.34^{a}	2.18± 0.54^{a}	1.94± 0.34^{a}	1.88± 0.29^{a}
TG	1.20± 0.26^{a}	0.77± 0.16^{cd}	0.64± 0.15^{cd}	0.99± 0.25^{b}	0.51± 0.16^{d}	0.87± 0.22^{bc}	0.57± 0.16^{cd}	0.54± 0.17^{d}	0.73± 0.30^{cd}	0.59± 0.21^{cd}
HDL-C	0.98± 0.09^{b}	1.35± 0.04^{a}	1.23± 0.25^{a}	1.32± 0.15^{a}	1.41± 0.21^{a}	0.93± 0.12^{b}	1.42± 0.24^{a}	1..37± 0.15^{a}	1.07± 0.19^{a}	1.29± 0.35^{a}
LDL-C	0.25± 0.19^{c}	0.25± 0.12^{c}	0.39± 0.08^{bc}	0.38± 0.12^{bc}	0.37± 0.08^{bc}	0.43± 0.18^{b}	0.45± 0.31^{b}	0.80± 0.62^{ab}	0.87± 0.32^{a}	0.53± 0.38^{ab}

*[$]Values are means ± standard deviation (mmol/l); [#]Abbreviations: TC: total cholesterol; TG: triglycerides; HDL-C: high- density lipoprotein cholesterol; LDL-C: low- density lipoprotein cholesterol; * Values in rows with different superscripts are significantly different (p< 0.05)*

Table 4. Serum lipid cardiovascular indexes of rats fed lentil-based diets for 4 weeks

	Cholesterol-free lentil groups[$*]					Cholesterol-supplemented lentil groups[$*]				
Parameter	**Control**	**Raw dehulled**	**Raw whole**	**Cooked dehulled**	**Cooked whole**	**Control**	**Raw dehulled**	**Raw whole**	**Cooked dehulled**	**Cooked whole**
AIP	0.08± 0.05^{a}	-0.25± 0.05^{be}	-0.28± 0.03^{bf}	-0.14± 0.03^{bd}	-0.46± 0.05^{c}	-0.04± 0.04^{ad}	-0.42± 0.07^{cef}	-0.42± 0.54^{cf}	-0.19± 0.02^{def}	-0.35± 0.12^{cef}
AC	0.25± 0.19^{c}	0.19± 0.10^{c}	0.34± 0.10^{bc}	0.29± 0.07^{bc}	0.27± 0.08^{bc}	0.47± 0.18^{ab}	0.34± 0.28^{bc}	0.62± 0.54^{abc}	0.86± 0.46^{a}	0.54± 0.49^{abc}
CRR	1.23± 0.20^{bc}	1.19± 0.10^{ce}	1.34± 0.10^{bd}	1.29± 0.07^{bce}	1.27± 0.08^{dc}	1.47± 0.18^{ab}	1.34± 0.28^{bc}	1.61± 0.54^{abc}	1.86± 0.46^{a}	1.53± 0.49^{abc}
HDL-C/ LDL-C	5.62± 3.07^{ad}	6.90± 3.66^{ae}	3.34± 1.45^{af}	3.76± 1.26^{af}	3.91± 0.96^{af}	2.55± 1.24^{bcf}	5.12± 3.91^{cde}	2.71± 1.90^{bd}	1.39± 0.55^{b}	3.29± 1.69^{cde}
TC/ TG	1.02± 0.18^{c}	2.16± 0.63^{b}	2.64± 0.75^{b}	1.86± 0.72^{bc}	3.77± 1.04^{ab}	2.00± 1.10^{bc}	3.61± 1.25^{ab}	4.15± 0.72^{a}	3.00± 1.26^{ab}	3.46± 1.09^{ab}

*[$]Values are means ± standard deviation (mmol/l); [#]Abbreviations: AIP: atherogenic index of plasma [log (TG/HDL-C)]; AC: atherogenic coefficient [TC- HDL-C/HDL-C]; CRR: cardiac risk ratio (TC/HDL-C); HDL-C: high-density lipoprotein cholesterol; LDL-C: low-density lipoprotein cholesterol; TC: total cholesterol; TG: triglycerides; * Values in rows with different superscripts are significantly different (p< 0.05)*

Overall correlations for serum lipid fractions and cardiovascular indexes of all study animals (n=60) are shown in Table 5. Positive correlations (p<0.0001) were found between TC and HDL-C and LDL-C, while negative correlations (p<0.01) were found between TG and TC and HDL-C. AIP was negatively correlated (p<0.001) with TC and HDL-C and TC/TG, and positively (p<0.0001) with TG. No correlations (p>0.05) were observed between AIP and AC, CRR and HDL-C/LDL-C. AC and CRR were strongly linked to each other (p<0.0001), and were positively correlated (p<0.0001) with TC and LDL-C and nega-

Table 5. Overall correlations for serum lipid fractions and cardiovascular indexes of all study rats (n=60)

Variable[#]	TG	HDL-C	LDL-C	AIP	AC	CRR	HDL-C/ LDL-C	TC/TG
TC	-0.287[d]	0.515[a]	0.750[a]	-0.410[b]	0.561[a]	0.561[a]	-0.402[b]	0.571[a]
TG		-0.346[c]	-0.057	0.909[a]	0.046	0.046	0.078	-0.863[a]
HDL-C			-0.171	-0.663[a]	-0.395[c]	-0.395[c]	0.301[d]	0.449[b]
LDL-C				0.040	0.941[a]	0.941[a]	-0.689[a]	0.307[c]
AIP					0.213	0.213	-0.072	-0.901[a]
AC						0.995[a]	-0.669[a]	0.153
CRR							-0.669[a]	0.153
HDL-C/LDL-C								-0.184

[#]Abbreviations: TC: total cholesterol; TG: triglycerides; HDL-C: high-density lipoprotein cholesterol; LDL-C: low-density lipoprotein cholesterol; AIP: atherogenic index of plasma [log (TG/HDL-C)]; AC: atherogenic coefficient [TC- HDL-C/HDL-C]; CRR: cardiac risk ratio (TC/HDL-C). [a]p< 0.0001; [b]p< 0.001; [c]p< 0.01; [d]p< 0.05

tively (p<0.001) with HDL-C and HDL-C/LDL-C. The HDL-C/LDL-C was also positively linked (p<0.05) to HDL-C, and negatively (p<0.001) to TC and LDL-C. Furthermore, TC/TG was positively associated (p<0.01) with TC, HDL-C and LDL-C and negatively (p<0.0001) with TG.

Discussion

The present study shows that in rats, the inclusion of lentil to cholesterol-free and cholesterol-containing diets induces changes in serum TC, HDL-C, TG, AIP and TC/TG and weight gain, food intake and food conversion ratio. The different lentil diets had only apparently random effects on these variables. This study also shows little or no influence of lentil diets on serum LDL-C, AC, CRR and HDL-C/LDL-C**.** Unlike the other serum lipid fractions and indexes, TG and AIP decreased and TC, HDL-C and TC/TG increased in response to lentil diets. Each of serum cardiovascular indexes exhibited characteristic correlations reflecting the content of their lipid fractions and the changes induced by experimental feeding. The AC was positively linked to CRR and both were negatively associated with HDL-C/LDL-C, while AIP was negatively linked to TC/TG and both were not associated with AC, CRR or HDL-C/LDL-C.

In this study, cholesterol feeding increased LDL-C and decreased TG with a parallel rise in AC and a fall in HDL-C/LDL-C, though TC, HDL-C, AIP, CRR and TC/TG were unaffected. This indicates the development of dyslipidemia in cholesterol-fed rats. In fact, dyslipidemia has been widely induced by cholesterol administration in several animal models (26,27). There is also a general agreement regarding the lack of influence of cholesterol on weight gain, food intake and food conversion ratio (28), a matter that is consistent with the findings of the current study.

The lower weight gain of rats fed lentil diets compared with those fed cholesterol-free and cholesterol-containing control diets may be attributed to the lower food intake and thus food conversion ratio observed in these rats. However, the recorded values of these variables were still comparable with those reported for normal rats (29). The effect of legumes including lentil in lessening weight gain has been documented in animals (21, 22). Consistently, it has been found that lentil-based meals were more potent at increasing satiety and lowering food intake, and thus controlling body weight than meals based on chickpeas, navy beans and yellow peas (30). Furthermore, epidemiological observations have provided evidence that consumption of pulse foods confers several benefits, particularly their potential in combating overweight and obesity (31).

Several studies have investigated the effect of lentil on serum lipids in humans (18, 20) and animals (13-17), but findings are controversial. This might be due to the large discrepancy between the various experimental protocols used. In fact, the type and complexity of the lentil source, raw, cooked or dehulled or genotype, the level in the diet, feeding duration, energy intake, basal diet composition and the experimental model used, are among many potential confounders that may contribute to this inconsistency. It may be noticed that the aim of most of the previous studies was to evaluate the effect of legumes or mixed pulses including lentil when added to cholesterol- or fat-modified diets in normal or other experimental models or pathological conditions. However, studies involving lentil inclusion to cholesterol-containing diets or those investigating such diets on serum lipid cardiovascular indexes are generally scarce. This certainly limits the comparison of the present results with those of the other studies.

The present study was specifically performed to test the effect of lentil when included to cholesterol-free and cholesterol-containing diets and administered to rats for 4 weeks. Under the present experimental conditions, a significant fall in serum TG and a marked rise in TC and HDL-C occurred in response to lentil diets in all experimental groups. The rise in TC was evidently due to the rise in HDL-C. The other lipid fractions and indexes were barely influenced by lentil diets. In this respect, very few studies have investigated lentil-cholesterol interaction. Red lentils, baked beans, peas and butter beans when fed to pigs for 42 days, have been shown to inhibit cholesterol- induced hypercholesterolemia without affecting HDL-C (13). In contrast to baked beans, butter beans, marrowfat peas and Bambara groundnuts, lentil has been shown to reduce TC, LDL-C and TG and increase HDL-C in rats fed these legumes with added cholesterol and cholic acid for 4 or 8 weeks (16). Moreover, it has been reported that lentil did not influence TC, while peas increased it in rats fed cholesterol-free diet containing these legumes (17). In streptozotocin-induced diabetic rats, cooked dehulled lentil has been demonstrated to be more effective in improving HDL-C than cooked whole, raw dehulled and raw whole lentils without effecting TC, HDL-C and TG (15). Among lipid fractions, reduction in TC has been reported in a randomized cross-over clinical trial on 30 patients with type 2 diabetes given a diet containing 50g cooked lentil for 6 weeks (18). Lentil sprouts (60 g/day) administered to 39 obese-type 2 diabetics for 8 weeks have been shown to increase TC, HDL-C and LDL-C and decrease TG (20). Apparently, some of these reported results are consistent with our findings; however, a remarkable variation in the experimental protocols still exists.

To the best of our knowledge, this study is perhaps the first demonstration that links lentil intake with lipid cardiovascular indexes in cholesterol-fed rats. It is generally accepted that this model has abnormal lipid metabolism with characteristic features of atherosclerosis (26). Interestingly, compared to control, lentil caused marked decrease in AIP and increase in TC/TG without affecting other atherogenic indexes. This effect came in line with changes observed in some lipid fractions, particularly HDL-C, TC and TG. Preliminary data in rats have shown that raw whole, raw dehulled, cooked whole or cooked dehulled lentils when included in cholesterol-free or cholesterol-supplemented diets did not influence serum LDL-C/HDL-C, but lead to an increase in TC/TG (14). A decrease in AIP has been reported in obese- diabetic patients given a diet containing lentil sprouts (20). Noteworthy, along with these findings, an increase in HDL-C and a decrease in TG have been demonstrated (14, 20), which accord with those of the present study.

Nowadays, there is an increasing body of evidence indicating the importance of lipid indexes in optimizing the predictive power of lipid profile through their ability to provide integrative information regarding metabolic interactions between the different lipid variables (32). By expressing imbalances between atherogenic and cardioprotective lipoproteins, such indexes are powerful indicators of CVD risk in humans (8, 32). AIP has been described as a biomarker of plasma atherogenicity (8). This state has been reported to be a function of LDL-C and HDL-C particle sizes, with small particle size exhibiting great atherogenic potential (33). Indeed, the link between CVD and AIP, cholesterol etherification rate in HDL-C and lipoprotein particle sizes has been well demonstrated (34). In this study, AIP and TC/TG were the only lipid indexes that responded to lentil diets, though such finding has

not been yet documented in rats. Unlike humans, almost 80% of rat serum cholesterol is found in HDL-C (35), thus TC and HDL-C are closely related. This may explain the recorded strong negative correlations between TC/TG and each of AIP and TG.

It is apparent from the current results that lentil has a favorable effect on the CVD indexes: AIP and TC/TG in rats. This effect was more evident than that observed on the lipid variables: TC, HDL-C and TG. Hence, for describing the cardioprotective effect of lentil, it may be noted that the use of either AIP or TC/TG is better than the use of TC, HDL-C or TG alone. This view is compatible with that of human studies reporting that lipid indexes may provide a more integrated explanation for CVD risks than single lipid measures (32-34).

However, some limitations to the present study need to be noted. The possible bioactive component in lentil neither was determined nor was its serum level assessed. Thus, it would be of great importance to explore the mechanisms by which lentil and cholesterol interact and modify lipid assimilation and metabolism in humans and animals

In conclusion, when incorporated into cholesterol-free and cholesterol-supplemented diets in rats, lentil markedly reduces AIP and increases TC/TG apparently through its favorable effect on TG and HDL-C. Lentil appears to exert such effect regardless its form or preparation method. The cardioprotective effect of lentil seems to be more evident when considering AIP and TC/TG than TG or HDL-C. These results suggest that incorporation of lentils in human diets may help in protection and management of CVD and related disorders.

Acknowledgments

The author would like to thank the Deanship of Scientific Research at The University of Jordan for their financial support.

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Correspondence:
Mousa Numan Ahmad
Department of Nutrition and Food Technology,
Human Nutrition and Dietetics,
The University of Jordan,
Amman11942, Jordan
Phone: (962-6) 5355000 Ext. 22412
Fax: (962-6) 5300806
E-mail: mosnuman@ju.edu.jo; mousanuman@gmail.com