

The Effect of Dehulling and Cooking of Lentils (*Lens Culinaris*, L.) on Serum Glucose and Lipid and Lipoprotein Concentrations in Rats Fed Cholesterol- Supplemented Diets

Mousa Numan Ahmad

Department of Nutrition and Food Technology/ Human Nutrition and Dietetics, Faculty of Agriculture,
The University of Jordan, Amman 11942, Jordan
mosnuman@ju.edu.jo; mousanuman@gmail.com

Abstract: Lentil (*Lens culinaris*, L.) is one of the most important pulse crops worldwide and has been known for many health benefits, but its antihyperlipidemic effect still remains unclear. The aim of this study was to investigate effects of lentils on serum lipids and glucose, body weight and food intake in cholesterol-fed rats. Sixty male Sprague–Dawley rats were assigned into five cholesterol-free or five cholesterol-supplemented diets containing casein (control), raw dehulled lentil, raw whole lentil, cooked dehulled lentil and cooked whole lentil and given *ad libitum* to the rats for four weeks. Serum total cholesterol (TC), low- density lipoprotein cholesterol (LDL-C), high-density lipoprotein cholesterol (HDL-C), triglycerides (TG) and glucose were then quantified and other biological parameters were assessed. Compared to control, cholesterol significantly ($p<0.05$) increased LDL-C, LDL-C/HDL-C and TC/TG ratios and liver weight, and decreased TG, whereas TC, HDL-C, glucose, weight gain and food intake were unchanged. In normal and cholesterol-fed groups, compared to control, lentil diets significantly ($p<0.05$) increased TC, notably due to increased HDL-C, while glucose was decreased and LDL-C and LDL-C/HDL-C ratio were unaffected. Triglyceride was decreased in normal groups and was maintained in cholesterol-fed groups resulting in increased or decreased TC/TG ratio in these groups respectively. In all rats, with exception of cooked dehulled lentil diet, lentil diets markedly ($p<0.05$) decreased weight gain, liver weight and food intake. Among all rats, there were little or no differences in studied variables due to lentil diets. These results show that lentil equally maintains its favorable lipid modifying activity and low glycemic property in normal and hypercholesterolemic rats with little or no effect for cooking and dehulling processes.

[Ahmad MN. The Effect of Dehulling and Cooking of Lentils (*Lens Culinaris*, L.) on Serum Glucose and Lipid and Lipoprotein Concentrations in Rats Fed Cholesterol- Supplemented Diets. *Life Sci J* 2014;11(11):924-931] (ISSN: 1097-8135). <http://www.lifesciencesite.com>. 165

Key words: Lentil, cholesterol, lipemia, glycemia, dehulling, cooking, rats

1. Introduction

Atherosclerosis, the basis of diseases of cardiovascular system, is closely related to the elevated plasma cholesterol concentration, particularly low-density lipoprotein cholesterol [1, 2]. Cholesterol plaque is a characteristic feature of the atherosclerotic lesions in animals and humans [3, 4]. The epidemic of cardiovascular disease is a global issue, and continues to be the most prevalent cause of death and disability [5-7]. Given the public health burdens of this disease, its prevention is becoming a major challenge, urging the need to identify the factors that may favorably affect plasma cholesterol [1].

Diet plays a crucial role in the prevention or management of cardiovascular disease and other related disorders [8, 9]. It is well appreciated that the reduction of plasma cholesterol levels could reverse atherosclerosis in animals and humans [2, 8-10]. Nowadays, there is a growing interest in the intake of plant foods, especially pulse crops for reducing plasma cholesterol level and the risk of cardiovascular disease [11-14].

Lentil (*Lens culinaris* L.), a species belonging to the family Leguminosae (Fabaceae), is one of the

well known and oldest pulse crops in the Near East and Mediterranean region and constitutes an essential part of the local diet [15, 16]. Lentil is usually soaked, boiled, sprouted, fermented, fried or mixed with other cereal grains, particularly rice or wheat in preparation of several traditional dishes [17, 18]. Lentil is known to have a good nutritional value due to its high protein content, besides it contains several active phytochemicals such as polyphenols, dietary fiber, defensin and phytosterols [19]. Further, lentil is reported to possess a number of biological activities mainly antimicrobial, anti-inflammations, anticancer and antioxidant [19].

Evidence for possible antihyperlipidemic activity of lentil in humans and animals is very limited. An early study in pigs has revealed that lentil proteins exert a hypocholesterolemic effect [20]. Further, hypocholesterolemic and hypotriglyceridemic effects of lentil and several other pulses have been reported in rats [21]. However, a study in rats failed to support these effects [22]. In humans, cooked lentil given to type 2 diabetic patients has been shown to reduce total cholesterol without affecting other lipid fractions [23]. Increasing evidence has accumulated in recent years

showing that addition of mixed pulses including lentil to diets of healthy subjects reduces total cholesterol or triglycerides or low-density lipoprotein cholesterol but not the high-density lipoprotein cholesterol [24-26]. Reductions in the total cholesterol and low-density lipoprotein cholesterol have been concluded from a meta-analysis review involving ten clinical trials studying non-soya pulses including lentil [27].

The evidence that links lentil consumption with plasma glucose is consistent. It has been shown that lentil fiber is able to hinder the carbohydrate-induced deterioration in the metabolic control of diabetic rats [28]. Similar results have been documented in healthy subjects and in patients with type 1 and type 2 diabetes mellitus [29, 30]. Lentil has been repeatedly described to have a low glycemic index in different experimental settings [31, 32].

Noteworthy, the focus of previous researches so far has been mainly on the effect of mixed pulse diets on lipid profile [21-27]. Thus, distinction of the effect of lentil in most of these researches is difficult, and the evidence derived from them is relatively not consistent. We have previously shown that lentils administered to streptozotocin-induced diabetic rats improve fasting glucose and increase high-density lipoprotein cholesterol with no influence on the other lipid variables [33]. Nevertheless, the effects of processed lentils on lipid profile and glucose in normal and hypercholesterolemic animals or humans are yet to be studied. Therefore, this study aimed at investigating effects of raw and cooked lentils, whole and dehulled, on serum glucose and lipids and lipoproteins, body weight and food intake in rats fed cholesterol-supplemented or cholesterol-free diets.

2. Materials and Methods

Preparation of lentil flours

One batch (25kg) of "Jordan 1" whole and dehulled lentil seeds (*Lens culinaris*, L.) was obtained from the 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 [34]. The seeds were freed from broken stones and other foreign materials, cleaned and then divided into four parts, two were left as raw (whole and dehulled) and the other two were cooked as described elsewhere [33]. Whole and dehulled lentil seeds were washed thoroughly with tap water, drained and then boiled in an excess of tap water for 1-2 hours until they became soft. This process is similar to the home cooking method of lentils described in the Middle East [18]. The cooked lentils were spread on aluminum trays, left to cool at room temperature ($25 \pm 2^\circ\text{C}$) and then dried in a drying oven ((Mettert, Karl lob, Germany)) at 70°C for 16 hours. Cooked and raw lentils (whole and

dehulled) were ground to pass through five mm mesh (Retsch GmbH, Haan, Germany) to obtain homogenous flours. The resultant flours were then placed desiccated in sealed polythene bags and kept refrigerated at 4°C until further use.

Animals

Male Sprague-Dawley rats ($n=60$) were obtained from the Experimental Animal Unit of the Department of Nutrition and Food Technology, The University of Jordan, Amman, Jordan. 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 ($22 \pm 2^\circ\text{C}$) and hygienic conditions with 12-hour light, 12-hour dark cycle. All the experiments involving animals were approved by the Institutional Animal Ethics Committee and carried out according to the recommended guidelines for animal use.

Diets

Ten isocaloric and isonitrogenous diets were prepared, five of them were cholesterol-free and differed in the type and content of lentil flour: lentil-free, raw dehulled (60.5%), raw whole (66.6%), cooked dehulled (62.5%) and cooked whole (65.6%), while in the other five, cholesterol (1%) was added to induce hypercholesterolemia. Modifications in lentil content in the lentil-containing diets were made in accordance to macronutrient content analyses. The composition of experimental diets is described in Table 1. All diets contained the same amount of calories, protein, carbohydrate, fat, vitamins and minerals. Dietary supplies of nutrients were in accordance with the dietary recommended allowances for rats from the American Institute of Nutrition [35]. The experimental diets were freshly prepared once a week and stored refrigerated at 4°C . Proximate nutrient composition of cooked and raw lentil flours is shown in Table 2.

Experimental protocol

At the beginning of the experiment, animals weighed $93.4 \pm 1.1\text{g}$ ($n=60$) and they were assigned into the five cholesterol-free or the five cholesterol-supplemented groups (6 rats/group). During the experimental period, which lasted for four weeks, experimental diets and tap water were given *ad libitum*. Body weight and food intake were monitored weekly. Food efficiency ratio as body weight gain per 100g 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.

Table 1. Composition of the experimental diets

Ingredient	Cholesterol-free diets (g. kg ⁻¹) ¹					Cholesterol-supplemented diets (g. kg ⁻¹) ¹				
	Control	RDL	RWL	CDL	CWL	Control	RDL	RWL	CDL	CWL
Cholesterol	0	0	0	0	0	10	10	10	10	10
Lentil flour	0	604.8	666.6	625.0	675.6	0	604.8	666.6	625.0	675.6
Cornstarch	667.0	269.6	230.4	237.0	198.2	667.0	269.6	230.4	237.0	198.2
Casein	150.0	0	0	0	0	150.0	0	0	0	0
Corn oil	120.0	113.4	112.6	111.8	113.2	120.0	113.4	112.6	111.8	113.2
Vitamin mix (AIN-93) ²	20	20	20	20	20	20	20	20	20	20
Mineral mix (AIN-93) ²	40	40	40	40	40	40	40	40	40	40
DL-Methionine	3	3	3	3	3	3	3	3	3	3
Carbohydrate (%)	66.7	66.7	66.7	66.7	66.7	66.7	66.7	66.7	66.7	66.7
Protein (%)	15.0	15.0	15.0	15.0	15.0	15.0	15.0	15.0	15.0	15.0
Fat (%)	12.0	12.0	12.0	12.0	12.0	12.0	12.0	12.0	12.0	12.0
Fiber (%)	0	3.1	5.5	1.8	3.0	0	3.1	5.5	1.8	3.0
Energy (kcal. 100g ⁻¹)	434.8	434.8	434.8	434.8	434.8	434.8	434.8	434.8	434.8	434.8

¹RDL: Raw dehulled lentil; RWL: Raw whole lentil; CDL: Cooked dehulled lentil; CWL: Cooked whole lentil²AIN: American Institute of Nutrition [35]Table 2. Nutrient composition of lentil flours¹

Component	Raw dehulled lentil (g. 100g ⁻¹)	Raw whole lentil (g. 100g ⁻¹)	Cooked dehulled lentil (g. 100g ⁻¹)	Cooked whole lentil (g. 100g ⁻¹)
Moisture	9.0± 0.04	9.7± 0.02	5.3± 0.02	5.8± 0.05
Carbohydrate	65.7± 0.05	65.5± 0.03	68.8± 0.04	69.4± 0.05
Protein	24.8± 0.04	22.5± 0.04	24.0± 0.05	22.2± 0.02
Fat	1.1± 0.04	1.1± 0.06	1.3± 0.07	1.0± 0.03
Ash	3.3± 0.06	3.2± 0.05	3.0± 0.07	3.0± 0.05
Fiber	5.1± 0.07	8.2± 0.07	2.9± 0.06	4.4± 0.04
Energy (kcal.100g ⁻¹)	371.9	361.9	382.9	375.4

¹Mean of three determinations ± SEM on dry matter basis

Chemical analysis

Concentrations of serum glucose and lipids and lipoproteins including triglycerides, total cholesterol, low-density lipoprotein cholesterol and high-density lipoprotein cholesterol were determined by using commercial kits and according to the manufacturer's directives (Boehringer Mannheim GmbH, Germany). Analysis was performed at the Islamic Hospital Medical Laboratories, Amman, Jordan, using a pre-calibrated automated clinical chemistry analyzer

(Roche/Hitachi 912 chemistry analyzer). The ratios of low-density lipoprotein cholesterol/ high-density lipoprotein cholesterol and total cholesterol/ triglycerides were calculated. Proximate nutrient composition of the cooked and raw lentil flours (whole and dehulled) used in the feeding experiments was determined by the Weende method [36]. Proximate analyses included the determination of moisture, carbohydrate, protein, fat, ash and fiber.

Table 3. Body weight, liver weight, food intake and food efficiency ratio of rats fed lentil diets for 4 weeks^{1, 2}

Variable	Cholesterol-free groups ³					Cholesterol-supplemented groups ³				
	Control	RDL	RWL	CDL	CWL	Control	RDL	RWL	CDL	CWL
Initial body weight (g)	89.7± 2.5 ^a	93.0± 2.1 ^a	88.8± 3.7 ^a	90.8± 3.8 ^a	89.2± 2.9 ^a	99.0± 5.7 ^a	97.0± 3.4 ^a	95.7± 2.5 ^a	95.2± 3.3 ^a	95.7± 2.8 ^a
Final body weight (g)	198.2± 8.6 ^a	143.8± 6.5 ^b	126.0± 4.6 ^b	181.2± 5.1 ^b	137.0± 4.7 ^b	210.2± 14.9 ^a	143.2± 6.1 ^b	125.8± 6.1 ^b	177.8± 11.2 ^b	141.6± 7.6 ^b
Weight gain (g. day ⁻¹)	3.86± 0.35 ^a	1.81± 0.16 ^b	1.33± 0.11 ^b	3.24± 0.23 ^a	1.71± 0.12 ^b	3.97± 0.39 ^a	1.65± 0.13 ^b	1.08± 0.22 ^b	2.95± 0.32 ^a	1.65± 0.09 ^b
Food intake (g. day ⁻¹)	10.32± 0.26 ^a	7.88± 0.36 ^{bc}	6.76± 0.30 ^c	10.10± 0.40 ^a	8.11± 0.41 ^b	11.32± 1.06 ^a	6.98± 0.30 ^{bc}	6.54± 0.37 ^c	9.6± 0.95 ^{ab}	8.12± 0.52 ^b
FER ⁴	37.2± 2.5 ^a	22.7± 1.0 ^b	19.6± 1.4 ^b	31.9± 1.8 ^a	21.0± 1.0 ^b	35.0± 1.7 ^a	23.4± 1.0 ^b	15.9± 2.8 ^b	30.4± 1.0 ^a	19.7± 1.6 ^b
Liver weight (g)	3.66± 0.20 ^b	2.44± 0.18 ^c	2.10± 0.08 ^c	3.28± 0.06 ^{bc}	2.58± 0.17 ^c	7.16± 0.36 ^a	2.84± 0.29 ^c	2.42± 0.14 ^c	4.94± 0.45 ^b	3.96± 0.33 ^b

¹ Values are means ± SEM² Means within a row with different superscripts are significantly different ($p < 0.05$)³RDL: Raw dehulled lentil; RWL: Raw whole lentil; CDL: Cooked dehulled lentil; CWL: Cooked whole lentil⁴FER: Food efficiency ratio, body weight gain (g)/100g food intake

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, and the significance was set at $p < 0.05$. Data were expressed as means \pm standard errors of the mean (SEM).

3. Results

The proximate nutrient composition of cooked and raw lentils used this study is given in Table 2. The moisture contents of raw whole and raw dehulled lentils were almost similar and higher than those for cooked whole and cooked dehulled lentils. On dry matter basis, dehulling process resulted in marked decrease in fiber and increase in protein without appreciable influence on carbohydrate, fat, and ash contents of lentils. Cooking process noticeably decreased fiber and increased carbohydrate in both whole and dehulled lentils without affecting the contents of other components.

Body weight, liver weight, and food intake and food efficiency ratio of rats fed lentil diets are presented in Table 3. Initial body weights were essentially similar ($p \geq 0.05$) in all experimental groups. Compared to the cholesterol-free control, cholesterol feeding did not significantly ($p \geq 0.05$) influence body weight, weight gain, and food intake and food efficiency ratio, but it induced a significant ($p < 0.05$) increase in liver weight. In cholesterol-free groups, compared to control, raw dehulled, raw whole and cooked whole lentils resulted in significant ($p < 0.05$) decrease in weight gain, liver weight and food intake and food efficiency ratio, whereas these

variables were unaffected ($p \geq 0.05$) by cooked dehulled lentil. The different lentil diets produced similar pattern of effects on the aforementioned variables when compared to control in cholesterol-supplemented groups. Noteworthy, when compared to other lentil diets, cooked dehulled lentil significantly ($p < 0.05$) increased weight gain, liver weight and food intake, as well as food efficiency ratio in the cholesterol-free and cholesterol-supplemented groups. Differences in these variables due to raw dehulled, raw whole and cooked whole lentils within or between cholesterol-free and cholesterol-supplemented groups were minimal.

Concentrations of serum glucose and lipids and lipoproteins of rats fed lentil diets are shown in Table 4. In contrast to cholesterol-free control, cholesterol feeding induced significant ($p < 0.05$) increase in LDL-C concentration and ratios LDL-C/ HDL-C and total TC/ TG and decrease in TG concentration, whereas TC, HDL-C and glucose concentrations were unchanged.

In cholesterol-free groups, compared to control, raw dehulled, raw whole, cooked dehulled and cooked whole lentils produced significant ($p < 0.05$) increase in TC and HDL-C concentrations and ratio of TC/ TG, and significant ($p < 0.05$) decrease in TG and glucose concentrations, whereas LDL-C concentration and ratio of LDL-C/ HDL-C were unaffected (Table 4). With the exception of TG concentration and TC/TG ratio, glucose and the other lipid fractions exhibited similar pattern of changes due to the different lentil diets when compared to control in cholesterol-supplemented groups. In the latter groups,

Table 4. Serum glucose and lipid and lipoprotein concentrations of rats fed lentil diets for 4 weeks^{1, 2}

Variable	Cholesterol-free groups ³					Cholesterol-supplemented groups ³				
	Control	RDL	RWL	CDL	CWL	Control	RDL	RWL	CDL	CWL
TC ⁴ (mg.dl ⁻¹)	46.5 \pm 3.9 ^b	61.5 \pm 1.6 ^a	62.0 \pm 3.6 ^a	65.7 \pm 3.9 ^a	68.7 \pm 3.3 ^a	52.6 \pm 4.2 ^b	74.5 \pm 5.3 ^a	75.9 \pm 3.5 ^a	73.3 \pm 5.3 ^a	70.3 \pm 4.6 ^a
HDL-C ⁵ (mg.dl ⁻¹)	37.9 \pm 1.4 ^b	52.0 \pm 0.7 ^a	47.4 \pm 4.0 ^a	51.1 \pm 1.3 ^a	54.4 \pm 3.4 ^a	36.0 \pm 1.9 ^b	55.0 \pm 3.9 ^a	53.0 \pm 2.4 ^a	43.2 \pm 2.1 ^a	50.6 \pm 3.6 ^a
LDL-C ⁶ (mg.dl ⁻¹)	8.3 \pm 3.4 ^c	9.4 \pm 2.0 ^c	14.5 \pm 1.7 ^{bc}	14.6 \pm 2.0 ^{bc}	14.3 \pm 1.2 ^{bc}	16.6 \pm 3.0 ^b	17.4 \pm 4.9 ^b	22.6 \pm 4.2 ^{ab}	33.6 \pm 5.1 ^a	21.8 \pm 4.3 ^{ab}
Triglycerides (mg.dl ⁻¹)	101.3 \pm 6.9 ^a	62.1 \pm 5.1 ^{cd}	56.5 \pm 5.6 ^{cd}	82.1 \pm 6.3 ^b	50.1 \pm 5.2 ^d	76.3 \pm 8.0 ^{bc}	54.5 \pm 3.0 ^{cd}	47.1 \pm 6.1 ^d	60.4 \pm 8.9 ^{cd}	57.2 \pm 5.7 ^{cd}
LDL-C/ HDL-C	0.22 \pm 0.08 ^c	0.18 \pm 0.04 ^c	0.33 \pm 0.05 ^{bc}	0.28 \pm 0.03 ^{bc}	0.27 \pm 0.03 ^{bc}	0.55 \pm 0.07 ^{ab}	0.34 \pm 0.12 ^{bc}	0.45 \pm 0.10 ^{bc}	0.78 \pm 0.12 ^a	0.46 \pm 0.11 ^{abc}
TC/ Triglycerides	0.46 \pm 0.03 ^c	0.98 \pm 0.10 ^b	1.15 \pm 0.12 ^b	0.85 \pm 0.12 ^{bc}	1.42 \pm 0.11 ^{ab}	0.78 \pm 0.17 ^{bc}	1.36 \pm 0.14 ^{ab}	1.83 \pm 0.12 ^a	1.39 \pm 0.20 ^{ab}	1.32 \pm 0.12 ^{ab}
Glucose (mg.dl ⁻¹)	120.3 \pm 7.8 ^a	81.1 \pm 5.7 ^b	57.9 \pm 3.5 ^c	90.2 \pm 5.2 ^b	70.1 \pm 8.5 ^{bc}	119.9 \pm 10.0 ^a	90.1 \pm 4.5 ^b	74.7 \pm 4.2 ^b	69.1 \pm 5.9 ^{bc}	60.6 \pm 4.5 ^c

¹ Values are means \pm SEM

² Means within a row with different superscripts are significantly different ($p < 0.05$)

³RDL: Raw dehulled lentil; RWL: Raw whole lentil; CDL: Cooked dehulled lentil; CWL: Cooked whole lentil

⁴TC: Total cholesterol

⁵HDL-C: High- density lipoprotein cholesterol

⁶LDL-C: Low- density lipoprotein cholesterol

compared to control, the different lentil diets lead to significant ($p < 0.05$) decrease in TG concentration without affecting TC/TG ratio. There were little or no differences in glucose and lipid profile within and between cholesterol-free or cholesterol-supplemented groups as a result of feeding different lentil diets (Table 4).

4. Discussion

We used "Jordan 1" variety of lentil which is domestic to Jordan [34]. Although macronutrient content of lentil has been previously reported, data are not always comparable [33, 37-39]. Compared to documented values, the presently recorded protein content of raw whole lentil was lower [38] or higher [33, 39], whereas that of raw dehulled lentil was close [33] or lower [38]. The carbohydrate content of raw whole lentil was close [38] or lower [33], whereas that of raw dehulled lentil was close [33] or higher [38] than those reported. Among the macronutrient content of lentil, fiber showed the greatest variability [33, 37-39]. The contents of fat and ash were almost close to documented values [33, 38]. This variability can be attributed to a number of factors, such as differences in genotype, product quality, postharvest handling, dehulling techniques and storage conditions and methods of analysis [33, 37].

Cooking and dehulling perhaps cause changes in the macronutrient content of lentils. However, little information is available regarding the effect of these processes on the chemical composition of lentils [38, 39]. In the current study, dehulling slightly increased the protein content and markedly decreased the fiber content in both raw and cooked lentils, whereas other components were unchanged. These results are in agreement with those of previous studies [33, 37]. It is possible that the fiber-rich seed coat contains little protein, thus dehulled lentil seeds would contain proportionally less fiber and more protein, a matter which explains these findings [33, 37]. Compared to raw whole and dehulled lentils, cooking increased the carbohydrate content and decreased the fiber content without affecting protein, fat and ash contents. Consistently, the decrease in the fiber content has been reported and attributed to softening of soluble fiber due to cooking that might lead to subsequent loss and reduction of its content [33, 37]. It has been claimed that the loss of soluble solids during cooking could be the reason behind the documented change in the macronutrient content of lentils, particularly carbohydrate and protein [37].

Cholesterol has been widely used in animals to induce hyperlipidemia [40-42]. Consistently, after 4 weeks of cholesterol feeding, defective lipid profile was established in this study. Cholesterol feeding induced elevation in LDL-C paralleled by marked rise in ratios of LDL-C/ HDL-C and TC/ TG and a fall in

TG concentration, though the concentrations of TC and HDL-C were not affected. This indicates impaired lipid metabolism in the cholesterol-fed rats. These findings are in close agreement with those of several reports [43, 44]. However, TC concentration has been shown to increase [44, 45] or remain unchanged [46] as a result of cholesterol feeding in animals. In fact, this may reflect differences in experimental protocols and procedures, particularly animal model, diet composition and duration of feeding. The recorded increase in liver weight has been considered an evident marker of cholesterol-induced hyperlipidemia that could be a consequence of increased fat content in rats [21, 47]. There is also a general agreement regarding the lack of influence of cholesterol on weight gain, food intake and food efficiency ratio [43, 46], a matter that is consistent with the results of the current study.

To the best of our knowledge, this study is perhaps the first demonstration that links processed lentils with lipid parameters in rats. We examined the effect of feeding raw and cooked, whole and dehulled, lentils on serum glucose and lipid and lipoprotein concentrations in the cholesterol-fed rat. This model is known to have abnormal lipid profile and functional atherosclerosis [40- 47]. Authentic variety of lentil was used and included into the isocaloric and isonitrogenous semi-purified diets and fed *ad libitum* for four weeks. The prevailing custom of lentil preparation, at least in the Middle East, was closely followed [17, 18]. Male rats were used and changes in body weight and food intake were also considered.

The lower food intake of rats fed raw whole, raw dehulled and cooked whole lentil diets compared with the cooked dehulled lentil diet and normal and hypercholesterolemic controls could explain the lower weight gain and liver weight observed in these rats. Despite these changes, the recorded food intake, food efficiency ratio, weight gain and liver weight were still comparable with those reported for normal rats [48-50]. However, in contrast to controls, these variables were maintained unchanged in the cooked dehulled lentil diet of both normal and cholesterol-fed groups. The reason for these findings is not quite clear. Few studies on lentils and other legumes have dealt with this aspect in humans and animals. The effect of bean diets on weight gain compared with control has been documented in several animal species [20, 21] and in human subjects [51] which accord with our findings. Consistently, it has been also found that lentil-based meals were more potent at increasing satiety and lowering food intake, and thus controlling body weight than chickpeas, navy beans and yellow peas meals [52]. Further, epidemiological observations have provided marked evidence that consumption of pulses confers numerous benefits,

particularly low incidence of obesity [53]. However, this study is the first to demonstrate differences in weight gain and food intake of rats fed different processed lentil diets.

In this study, proportional modifications in the lipid profile were observed with all experimental diets. In this respect, raw and cooked, whole and dehulled, lentil diets were equally potent with little or no differences between them. The increase in TC concentration induced by lentil diets relative to normal and cholesterol-fed controls was in effect a consequence of increased HDL-C concentration. Similarly, in all rats, it was evident that the recorded change in the ratio of TC/ TG was essentially due to the changes in TG concentration. It was also notable that LDL-C concentration and LDL-C/ HDL-C ratio remained unchanged. It follows that regardless its form in the diet, lentil equally improves lipid profile in normal and cholesterol-fed rats through increasing HDL-C, decreasing TG and maintaining LDL-C concentrations.

Cholesterol- induced hypercholesterolaemia has been inhibited in pigs consuming red lentils, baked beans, peas and butter beans for 42 days, without affecting HDL-C concentrations [20]. Among five legume species, namely baked beans, butter beans, marrowfat peas and Bambara groundnuts, lentils have been shown to reduce the TC, LDL-C and TG concentrations and increase HDL-C concentration in rats fed these legumes for 4 and 8 weeks [21]. On the other hand, peas-based diet has been reported to increase TC, while lentils maintain it [22]. It is apparent from our results and from those of other studies that lentil has a favorable effect on lipid fractions in animals, though some controversy exists. The experimental conditions of previous studies were quite different from those given here. Experimental diets were either with added cholesterol alone [20], or with added cholesterol and cholic acid [21], or were low fat- diets with no added cholesterol [22]. These studies have used cooked legumes including lentil, but they did not specify its form, whether whole or dehulled; besides animal models were different [20-22].

Some evidence is now available which indicates that regular inclusion of mixed pulses including lentil to the diet of normal individuals could favorably modify lipid profile, particularly reduction in TC, TG or LDL-C concentrations [24-27]; however effects of the lentil cannot be isolated from those of other pulses. Among the lipid fractions, reduction in TC has been reported in a randomized cross-over clinical trial on 30 patients with type 2 diabetes mellitus given an experimental diet containing 50g cooked lentil for 6 weeks [23]. In streptozotocin-induced diabetic rats, we have demonstrated that cooked dehulled lentil was

more effective in improving HDL-C concentration than cooked whole, raw dehulled and raw whole lentils with no noticeable effects on TC, HDL-C and TG concentrations [33]. These observations are in agreement with the present findings which show that lentil has an improving effect on serum lipids and possibly has a role in the dietary management of diabetes.

It was evident that lentil diets equally reduced fasting serum glucose concentration in normal and cholesterol-fed rats with little or no differences among the different lentil diets. The available evidence that links lentil with glucose concentration is consistent and accords with our findings. It is well known that lentil and meals or diets containing lentil have low glycemic indices in healthy [29, 31] and diabetic [29, 30] humans and are able to reduce blood glucose concentration in diabetic rats [28, 33]. Compared to cooked whole, raw dehulled and raw whole lentils, cooked dehulled lentil has been shown to be more potent in reducing glucose in streptozotocin-induced diabetic rats [33].

Taken together, when incorporated into normal diets or those with high cholesterol content, cooked and raw lentils (whole or dehulled) exhibit similar anti-atherogenic and hypoglycemic activities, and have an appreciable effect on food intake and body weight control in rats. Thus, regular inclusion of lentils in the diet is recommended, especially in regimens involving lipid, glucose and weight control. However, the present hypothesis demands further investigations in humans.

Acknowledgment

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

Corresponding Author:

Mousa Numan Ahmad, Department of Nutrition and Food Technology/ Human Nutrition and Dietetics, The University of Jordan, Amman 11942, Jordan.

Phones: 962-6-535-5000 ext. 22412.

E-mail: mosnuman@ju.edu.jo,
mousanuman@gmail.com

References

1. Mendis S, Puska P, Norrving B. Global Atlas on Cardiovascular Disease Prevention and Control. World Health Organization in collaboration with the World Heart Federation and World Stroke Organization. Geneva; 2011.
2. NCEP. Third Report of the National Cholesterol Education Program (NCEP) Expert Panel on Detection, Evaluation, and Treatment of High Blood Cholesterol in Adults (Adult Treatment

- Panel III) Final Report. *Circulation* 2002; 106: 3143–3421.
3. Faggiotto A, Ross R. Studies of hypercholesterolemia in the nonhuman primate. II. Fatty streak conversion to fibrous plaque. *Atherosclerosis* 1984; 4:341–356.
 4. Freedman D, Newman WI, Tracy R, Voors AE, Srinivasan SR, Webber LS, Restrepo C, Strong JP, Berenson GS. Black-white differences in aortic fatty streaks in adolescence and early adulthood: the Bogalusa heart study. *Circulation* 1988; 77:856–864.
 5. World Health Organization. Global Status Report on Noncommunicable Diseases 2010. WHO. Geneva; 2011.
 6. European Society of Cardiology: ESC guide-lines on the management of stable coronary artery disease. the Task Force on the management of stable coronary artery disease of the European Society of Cardiology (ESC). *Eur Heart J* 2013, doi:10.1093/eurheartj/eh296.
 7. American Heart Association. Heart disease and stroke statistics—2014 update: a report from the American Heart Association. *Circulation* 2014, 128DOI:10.1161/01.cir.0000441139.02102.80.
 8. American Heart Association. Diet and lifestyle recommendations revision 2006: a scientific statement from the American Heart Association Nutrition Committee. *Circulation* 2006; 114:82-96.
 9. Dalen JE, Stephen D: Diets to prevent coronary heart disease 1957-2013. what have we learned? *Am J Med* 2014; 127: 364-369.
 10. Ichihashi T, Izawa M, Miyata K, Mizui T, Hirano K, Takagishi Y. Mechanisms of hypercholesterolemic action of S-89211 in rats: S-8921 inhibits ileal bile acid absorption. *J Pharmacol Exper Therapeutics* 1998; 284: 43-50.
 11. Ros E, Tapsell LC, Sabaté J. Nuts and berries for heart health. *Curr Ather Rep* 2010; 12:397-406.
 12. Anderson JW, Major AW. Pulses and lipemia, short- and long-term effect: potential in the prevention of cardiovascular disease. *Br J Nutr* 2002; 88:S263–S271.
 13. Flight I, Clifton P. Cereal grains and legumes in the prevention of coronary heart disease and stroke: a review of the literature. *Eur J Clin Nutr* 2006; 60:1145–1159.
 14. Estruch R, Ros E, Salas-Salvadó J, Covas MI, Corella D, Arós F, Gómez-Gracia E, Ruiz-Gutiérrez V, Foil M, Lapetra J, Lamuela-Raventos, RM, Serra-Majem L, Pintó X, Basora J, Muñoz MN, Sorlí JV, Martínez JV, Martínez-González MN. Primary prevention of cardio-vascular disease with a Mediterranean diet. *N Engl J Med* 2013; 368:1279-1290.
 15. Jin L, Jian-Ping G, Dong-Xu X, Xiao-Yan Z, Jing G, Xu-Xiao Z. Genetic diversity and population structure in lentil (*Lens culinaris* Medik.) germplasm detected by SSR markers. *Acta Agron Sinica* 2008; 34: 1901-1909.
 16. Cubero JI. Origin, taxonomy and domestication. In *Lentils*. Edited by Webb C, Hawlin G. London: CAB; 1981.
 17. Food and Agriculture Organization. *Traditional Food Plants*. FAO. Geneva; 1988.
 18. Dagher SM. *Traditional Foods in the Middle East: Preliminary Report*. Food and Agriculture Organization. Geneva; 1985.
 19. Mo'ez Al-Islam EF, Takruri HR, Issa AY. Role of lentils (*Lens culinaris* L.) in human health and nutrition: a review. *Mediterr J Nutr Metab* 2013; 6:3–16.
 20. Kingman SM, Walker AF, Low AG, Sambrook IE. Comparative effect of four legume species on plasma lipids and fecal steroid excretion in hypocholesterolemic pigs. *Br J Nutr* 1993; 69: 409–421.
 21. Dabai FD, Walker AF, Sambrook IE, Welch VA, Owen RW, Abeyasekera S. Comparative effects on blood lipids and fecal steroids of five legume species incorporated into a semipurified, hypercholesterolaemic rat diet. *Br J Nutr* 1996; 75:557–571.
 22. Soni GI, Sohal BS, Rattan S. Comparative effect of pulses on tissue and plasma cholesterol levels in albino rats. *Indian J Biochem Biophys* 1979; 16: 444-446.
 23. Shams H, Tahbaz F, Entezari M, Abadi A. Effects of cooked lentils on glycaemic control and blood lipids of patients with type 2 diabetes. *ARYA Athero J* 2008; 3:215–218.
 24. Jenkins DJA, Wong GS, Patten R, Bird J, Hall M, Buckley GC, McGuire V, Reichert R, Little JA. Leguminous seeds in the dietary management of hyperlipidemia. *Am J Clin Nutr* 1983; 38:567–573.
 25. Duane WC. Effects of legume consumption on serum cholesterol, biliary lipids, and sterol metabolism in humans. *J Lipid Res* 1997; 38:1120–1128.
 26. Kingman SM. The influence of legume seeds on human plasma lipid concentrations. *Nutr Res Rev* 1991; 4:97–123.
 27. Bazzano LA, Thompson AM, Tees MT, Nguyen CH, Winham DM. Non-soy legume consumption lowers cholesterol levels: a meta-analysis of randomized controlled trials. *Nutr Metab Cardiovasc Dis* 2011; 21:94–103.
 28. Calle-Pascual AL, Marengo G, Asis MJ, Bordiu E, Romeo S, Martin PJ, Maranes JP, Charro AL. Effects of different proportions of carbo-hydrates, polysaccharides/ mono-saccharides, and different fibers on the metabolic control in diabetic rats. *Metabolism* 1986; 35:919–923.
 29. Rizkalla SW, Bellisle F, Slama G. Health benefits of low glycaemic index foods, such as pulses, in diabetic patients and healthy individuals. *Br J Nutr* 2002; 88:S255–S262.

30. Wolever TMS, Katzman-Relle L, Jenkins AL, Vuksna V, Josse RG, Jenkins DJA. Glycaemic index of 102 complex carbohydrate foods in patients with diabetes. *Nutr Res* 1994; 14:651–669.
31. Germaine KA, Samman S, Fryirs CG, Griffiths PJ, Johnson SK, Quail KJ: Comparison of in vitro starch digestibility methods for predicting the glycaemic index of grain foods. *J Sci Food Agric* 2008; 88:652–658.
32. Chung HJ, Liu Q, Hoover R, Tom D, Warkentin C, Vandenberg A. In vitro starch digestibility, expected glycaemic index, and thermal and pasting properties of flours from pea, lentil and chickpea cultivars. *Food Chem* 2008; 111:316–321.
33. Al-Tibi AMH, Takruri HR, Ahmad MN. Effect of dehulling and cooking of lentils (*Lens culinaris* L.) on serum glucose and lipoprotein levels in streptozotocin-induced diabetic rats. *Malays J Nutr* 2010; 16:83–92.
34. Haddad N, Snober B, Masadeh A. Food Legume Improvement Project in Collaboration with IDRC. Amman: The University of Jordan; 1989.
35. Reeves PG. Components of the AIN-93 diets as improvements in the AIN-76A diet. *J Nutr* 1997; 127: 838S-841S
36. Association of Official Agricultural Chemists. Official Methods of Analysis of the Association of the Official Analytical Chemists. AOAC. Virginia; 1995.
37. Wang N, Hatcher DW, Toews R, Gawalko EJ. Influence of cooking and dehulling on nutritional composition of several varieties of lentils (*Lens culinaris*, L.). *LWT- Food Sci Technol* 2009; 42: 842–848.
38. United States Department of Agriculture. USDA National Nutrient Database for Standard Reference (Release, 23). USDA: Washington DC; 2011. (http://www.ars.usda.gov/research/publications/publications.htm?seq_no_115=243584).
39. Costa GEA, Queriros-Monici KS, Reis SMPM, de Oliveira AC. Chemical composition, dietary fiber and resistant starch contents of raw and cooked pea, common bean, chickpea and lentil legumes. *Food Chem* 2006; 94: 327–330.
40. Csont T, Balogh G, Csonka C, Boros I, Horva' th I, Vigh L, Ferdinandy P. Hyperlipidemia induced by high cholesterol diet inhibits heat shock response in rat hearts. *Biochem Biophys Res Comm* 2002; 290: 1535-1538.
41. Horton JD, Cuthbert JA, Spady DK. Regulation of hepatic 7 α -hydroxylase expression and response to dietary cholesterol in the rat and hamster. *J Biol Chem* 1995; 270: 5381-5387.
42. Lichtman AH, Clinton SK, Iiyama K, Connelly PW, Libby P, Cybulsky MI. Hyperlipidemia and atherosclerotic lesion development in LDL receptor-deficient mice fed defined semi-purified diets with and without cholate. *Arterioscler Thromb Vasc Biol* 1999; 19: 1938-1944.
43. Martin-Carron N, Goni I, Larrauri JA, Garcia-Alonso A, Saura-Calixto F. Reduction in serum total and LDL cholesterol concentrations by a dietary fiber and polyphenol-rich grape product in hypercholesterolemic rats. *Nutr Res* 1999; 19: 1371-1381.
44. Gorinstein S, Yamamoto K, Katrich E, Loeontowicz H, Lojek A, Loeontowicz M, Ciz M, Shalev U, Trakhtenberg S. Antioxidative properties of jaffa sweets and grapefruit and their influence on lipid metabolism and plasma antioxidative potential in rats. *Biosci Biotech Biochem* 2003; 67: 907-910.
45. Otunola GA, Oloyede OB, Oladiji AT, Afolayan AA. Effects of diet-induced hypercholesterolemia on the lipid profile and some enzyme activities in female Wistar rats. *Afr J Biochem Res* 2010; 4:149-154.
46. Hexeberg S, Hexeberg E, Willumsen N, Berge RK. A study on lipid metabolism in heart and liver of cholesterol- and pectin-fed rats. *Br J Nutr* 1994; 71:181-192.
47. Matos SL, de Paula H, Pedrosa ML, dos Santos RC, de Oliveira EL, Junior DAC, Silva ME. Dietary models for inducing hypercholesterolemia in rats. *Braz Arch Biol Technol* 2005; 48:203-209.
48. Al-Jada DN, Ahmad MN. Effect of dietary fats on the development of insulin resistance in relation to PPAR γ activation and leptin concentration in rats. *J Am Sci* 2014; 10(08): 59-66.
49. Ahmad MN, Yaghi GA, Ajarma KG. Cinnamomum zeylanicum aqueous extract is superior to bark powder in ameliorating fasting glycaemia in cornstarch and fructose fed rats. *J Am Sci* 2014; 10(09): 264-271.
50. Ahmad MN, Abdoh MJ. The Effect of date palm fruit (*Phoenix dactylifera* L.) on serum lipid and lipoprotein concentrations in rats fed cholesterol-supplemented diet. *Mediterr J Nutr Metab* 2014; accepted for publication.
51. Anderson JW, Gustafson NJ, Spencer DB, Tietzen J, Bryant CA. Serum lipid response of hypercholesterolemic men to single and divided doses of canned beans. *Am J Clin Nutr* 1990; 51:1013-1019.
52. Mollard RC, Zykus A, Luhovyy BL, Nunez MF, Wong CL, Anderson GH. The acute effects of a pulse-containing meal on glycaemic responses and measures of satiety and satiation within and at a later meal. *Br J Nutr* 2012; 108:509–517.
53. McCrory MA, Hamaker BR, Lovejoy JC, Eichelsdoerfer PE. Pulse consumption, satiety, and weight management. *Adv Nutr* 2010; 1:17–30.

10/20/2014