Effect of Dehulling and Cooking of Lentils (Lens Culinaris, L.) on Serum Glucose and Lipoprotein Levels in Streptozotocin-Induced Diabetic Rats

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ABSTRACT

This study was conducted to investigate the effect of lentils on serum glucose and serum lipid levels in diabetic rats. Forty adult male Sprague-Dawley rats, 12 weeks of age weighing 220-290g, were used. Diabetes was induced by streptozotocin at a level of 35 mg/kg intra-peritoneally. The animals were randomly divided into five groups, eight animals each: a casein diet (control), raw whole lentil (RWL), cooked whole lentil (CWL), raw dehulled lentil (RDL) and cooked dehulled lentil (CDL). Animals were fed with experimental diets for six weeks, sacrificed and blood samples were taken. Serum glucose level of the CDL group (387.9 ± 53.3 mg/dl) was significantly lower (P<0.05) than that of the control, RDL and RWL groups (529.0 ± 11.7, 538.6 ± 45.0, 542.1 ± 32.2 mg/dl respectively). In addition, HDL concentration of CWL group (66.3 ± 1.9 mg/dl) was significantly higher (P<0.01) than that of the control, RWL and RDL groups (54.9 ± 3.5, 50.8 ± 4.2, 54.0 ± 3.4 mg/dl respectively). However, there was no significant difference in serum glucose and serum HDL between the CDL and CWL groups. No significant differences (p>0.05) were detected in triglycerides, total cholesterol and LDL cholesterol among the experimental groups. It is concluded that cooked lentils rather than raw lentils was more effective in lowering blood glucose and improving HDL cholesterol in diabetic rats. There was no difference between whole and dehulled lentils with regard to effects on blood glucose and HDL cholesterol levels.

Keywords: Diabetes mellitus, lentils, lipids, serum glucose

INTRODUCTION

Diabetes mellitus (DM) is currently a major health problem all over the world (Daisy et al., 2009). It is a chronic metabolic disorder resulting from interaction of hereditary and environmental factors (Daisy et al., 2009). It is associated with reduced life expectancy, significant morbidity due to specific diabetes related microvascular complications, increased risk of macrovascular complications (ischaemic heart disease, stroke and peripheral vascular disease), and a diminished quality of life (WHO, 2006).

The number of adults with diabetes in the world is expected to increase from 171 million in 2000 to 366 million in 2030 (WHO, 2006). Numerous studies have demon-
Amro MH Al-Tibi, Hamed R Takruri & Mousa N Ahmad 410

strated that the risk and incidence of coronary heart disease (CHD) and vascular disease in patients with DM are higher than those of non-diabetics (Barakat et al., 1996). In fact, vascular disease accounts for more than 60% of morbidity and mortality of diabetes (Barakat et al., 1996).

In order to control health problems related to DM, persons with diabetes should develop a prevention strategy for normalising serum lipids and minimising other factors such as overweight, physical inactivity and hypertension (Miller, 1994). Food and nutrition interventions that reduce post-prandial blood glucose level are important, since dietary carbohydrate is the major determinant of post-prandial glucose levels (American Diabetes Association, 2008). Monitoring carbohydrate intake via carbohydrate counting or exchanges has been the mainstay in achieving glycemic control, but new research has shown that the choice of carbohydrate consumed, not just the amount, also has an impact on blood glucose levels (McGonigal & Kapustin, 2008). There is evidence that slowly digested and absorbed carbohydrates are favorable in the dietary management of metabolic disorders such as diabetes and hyperlipidemia. Previous studies have shown that low glycemic index (GI) diets such as legumes, including lentil, had useful effects on lipid profiles and glycemic control in diabetic patients (Giacco & Parillo, 2000). Moreover, there are data suggesting that consuming a high-fibre diet (50 g fibre/day) reduces glycemia in subjects with type 1 diabetes and glycermia, hyperinsulinemia, and lipemia in subjects with type 2 diabetes (American Diabetes Association, 2008).

Lentil is now considered as one of the most beneficial legumes for health (Combe et al., 2004). It is a valuable source of proteins, starch and non-starchy carbohydrates, minerals and micronutrients (Combe et al., 2004). The glycemic responses of lentil soup are significantly lower than that of the standard white bread (Wolever et al., 1994). It could be used to develop GI control and serum total cholesterol level in diabetic patients. Documented studies on the effect of lentils as one of low GI foods on glycemic control are limited. Thus, it is expected that the inclusion of dehulled or whole lentils in the diet would help in the management of DM, and prevention of its complications.

MATERIALS AND METHODS

Selection and preparation of lentils

Whole and dehulled lentils (Lens culinaris, L.) of Canadian origin used in the present experiment were purchased from the local market. The seeds were cleared off broken stones and other foreign materials, cleaned and then divided into four parts, two were left as raw (whole and dehulled) and two groups were cooked.

Cooking was done as follows: cleaned whole and dehulled lentils were washed with tap water for 2 to 3 times and then drained. Whole lentils were cooked by boiling in an excess of tap water from 1-2 hours at boiling temperature, until they became soft when felt between fingers. Dehulled lentils were cooked in the same manner until they formed a thick slurry. The cooked lentils were then spread on aluminum trays and left to cool. After cooling at ambient room temperature (25 ± 2°C), cooked lentils were dried in an air-circulated drying oven (Blue M, Blue Islands, IL, USA) at 70 °C for 16 hours.

Both raw and cooked lentils were then ground separately to pass through 5 mm mesh (Retsch GubH, Haan, Germany), then placed in polyethylene bags and kept in the refrigerator at 4 °C to prevent spoilage, until used for preparation of experimental diets.

Chemical analysis

Proximate analyses of raw and cooked lentils (Lens culinaris, L.) used in the experiment were determined; proximate analyses included determination of moisture, crude fat, crude protein, ash, crude fibre and soluble carbohydrate content.
Effect of Dehulling and Cooking of Lentils on Serum Glucose & Lipoprotein Levels in Diabetic Rats

According to the Official Methods of the Association of Official Analytical Chemists (AOAC, 1995).

Animal experimentation

This research was approved by the Department of Nutrition and Food Technology Committee for Animal Experimentation/University of Jordan. Forty adult male Sprague-Dawley rats, 10-13 weeks of age weighing 220-290g, were used in the experiment. Each rat was housed individually in a plastic cage with a stainless steel wire floor and front (North Kent Plastic Cages, Ltd, Dartford, UK). A tray was placed under each cage to collect the faeces and spilled food. Rats were maintained under standard environmental conditions: lighting in 12:12 hours light: dark cycles and temperature at 22±2 ºC. They were fed ad libitum with a standard diet and water at the Animal Unit of the Faculty of Agriculture, The University of Jordan.

Induction of diabetes

After acclimatisation, 40 rats were subjected to a 18-hour fast. Diabetes was partially induced in 40 rats with a single dose of streptozotocin (Sigma Chemical Co., Mo, USA) at a level of 35 mg / kg body weight intra-peritoneally. The streptozotocin was dissolved in 0.1M fresh cold citrate buffer at pH 4.5 before use. Forty-eight hours after injection of streptozotocin, diabetes was confirmed by testing glucosuria of the rats using glucose urine strips (Glkostest, Roche Germany) and by observing polydipsia and polyuria. The researchers indicated polydipsia by observing the rapid emptying of the water cans and polyuria by observing wetting of cage bottoms; however, this was not quantitatively measured.

Experimental design

Five isocaloric, isonitrogenous experimental diet mixtures were prepared according to the American Institute of Nutrition-93G (AIN-93G) (Reeves, 1997). The animals were randomly divided into five groups, eight animals each, having the same average body weights. Group 1 was fed with casein diet (control), group 2 with 57% raw whole lentil (RWL), group 3 with 52% cooked whole lentil (CWL), group 4 with 51% raw dehulled lentil (RDL) and group 5 with 47% cooked dehulled lentil (CDL). Modifications in lentil content in the four lentil diets were made according to macronutrient content analyses. The details of the diet composition are listed in Table 1. Food given to each group provided the same amount of protein, fat, carbohydrates, vitamins, minerals, fibre and calories. All animals were fed with experimental diets for six weeks with free access to water and food. Animal weights and food intake were monitored once a week, whereas water intake was monitored daily.

At the end of the experiment, the rats were starved overnight, anesthetised with chloroform and weighed. Then 10 ml whole blood was collected from the right ventricle of the heart, centrifuged at 3000 rpm for 15 min to obtain serum. Serum was stored at -18 ºC for analyses. Livers were removed, cleaned and weighed.

Biochemical tests

Serum glucose and lipid concentrations were analysed in the Medical Laboratories of Khalidi Medical Center (Amman, Jordan). COBAS INTEGRA 400/700/800 system, an automated clinical chemistry analyser, was used for the analysis. Before performing the analyses tests, calibration of the analyser for glucose, triglyceride, total cholesterol (TC), LDL and HDL was done according to the manufacturer’s instructions.

Statistical analysis

Statistical analysis was performed using Statistical Analysis System (SAS, 1998) software package Version 7. The values were analysed by one-way analysis of variance (ANOVA) followed by Least Significant Difference (LSD). All of the results were
expressed as mean ± standard error of mean (SEM) for eight rats in each group. P< 0.05 was considered significant.

RESULTS

Proximate composition of lentils

Table 2 shows proximate composition of raw whole lentil, cooked whole lentil, raw dehulled lentil and cooked dehulled lentil. Moisture content was 8.1% and 7.6% for raw whole lentil and raw dehulled lentil respectively. In cooked whole lentil and cooked dehulled lentil, the moisture contents after drying were 4.2% and 4.7% respectively. All other analyses were carried out on a dry-matter basis.

Ash content was 3.1%, 2.6%, 2.9% and 2.8% for raw whole lentil, raw dehulled lentil, cooked whole lentil and cooked dehulled lentil respectively. Crude fibre content in dehulled lentils was less than that in whole lentils; crude fibre in cooked lentils was also less than in raw lentils. The content of crude fibre was 7.5%, 4.6%, 3.9% and 1.8% in raw whole lentil, cooked whole lentil, raw dehulled lentil and cooked dehulled lentil respectively.

Contrary to popular belief, the cooking and dehulling process increased the proportion of crude protein content namely, 20.8%, 22.9%, 23.2% and 25.2% for raw whole lentil, cooked whole lentil, raw dehulled lentil and cooked dehulled lentil respectively. Crude fat content was 0.75%, 1.4%, 1.7% and 1.8% for raw whole lentil, cooked whole lentil, raw dehulled lentil and cooked dehulled lentil respectively.
Effect of Dehulling and Cooking of Lentils on Serum Glucose & Lipoprotein Levels in Diabetic Rats

Nitrogen-free extract contents were similar: 67.8%, 68.1%, 68.7% and 68.5% for raw whole lentil, cooked whole lentil, and raw dehulled lentil and cooked dehulled lentil respectively.

Body weight

The initial and final body weights of rats fed five experimental diets for six weeks are presented in Table 3. Initial body weights were essentially similar (P>0.05) in all rats of experimental groups. In addition, rats of all experimental groups fed different diets exhibited similar (P>0.05) final body weights. Weight change of the rats throughout the experiment is also presented in Table 3. There were no significant differences (P>0.05) in body weight change.
for rats fed the five experimental diets after six weeks of feeding.

**Serum glucose, triglycerides and lipoprotein cholesterol**

Table 4 shows serum glucose, TC, LDL, HDL and triglycerides in mg/dl of rats fed the five experimental diets for six weeks. Serum glucose level was lowest in the cooked dehulled lentil group as compared with other groups. The glucose for this group (387.9 ± 53.3 mg/dl) was significantly lower (P<0.05) than that for control, raw dehulled lentil and raw whole lentil groups, (529.0 ± 11.7, 542.1 ± 45.0, 538.6 ± 45.0 mg/dl respectively), but not significantly lower than cooked whole lentil (397.6 ± 65.3 mg/dl).

No significant differences (P>0.05) were detected in total serum cholesterol level among the five experimental groups. Similarly, there were no significant differences (P>0.05) in LDL concentration among the five experimental groups. On the other hand, there were significant differences (P<0.01) in HDL concentration between the five experimental groups. HDL concentration was greater in the cooked whole lentil group in comparison with the other groups. The HDL for this group (66.3 ± 1.9 mg/dl) was significantly higher (P<0.01) than that in the control, raw whole lentil group and raw dehulled lentil group (54.9 ± 3.5, 50.8 ± 4.2, 54.0 ± 3.4 mg/dl respectively), but not significantly higher than cooked dehulled lentil groups (65.7 ± 4.0 mg/dl). No significant differences (P>0.05) were detected in triglycerides level among the five experimental groups.

**DISCUSSION**

**Proximate composition of lentils**

Although proximate composition of lentil has been widely documented in the literature, the data are not always comparable due to differences in genotypes, environments and methods of analysis (Wang & Daun, 2006). Furthermore, the cooking and dehulling process may cause changes in the composition and hence the nutritional value of lentils. This will lead to variations in the chemical composition of whole and dehulled lentils regarding moisture, protein, fat carbohydrate, minerals and vitamins (Wang et al., 2009). Most studies have been undertaken on the chemical composition of raw lentils; there is little information available on the impact of processing on physiological characteristics of lentils (Wang et al., 2009).
The crude protein content for raw whole lentil in the present study is similar to that reported by Costa et al. (2006) while it was slightly lower than what was reported by USDA (2009). It is evident that the cooking and dehulling process increases the proportion of crude protein. This is in agreement with Wang and his colleagues who found that dehulling increased the protein level (Wang et al., 2009). Moreover, Pirman and Stibilj observed an increase in the content of the essential and non-essential amino acids of lentils (with the exception of threonine) as a result of cooking (Pirman & Stibilj, 2003). The increase in crude protein in cooked lentils may be attributed to the loss of soluble solids during cooking, which would increase the concentration of protein in cooked seeds (Wang et al., 2009). In addition, seed coats are reported to contain little protein, meaning that dehulled seeds would consequently contain more protein (Wang et al., 2009). On the contrary, raw and whole lentils contained more crude fibre than cooked and dehulled lentils; the content was similar to that reported by Costa et al. (2006). Since seed coats contain most of the fibre, it is concluded that dehulled seeds would contain proportionately less fibre. Furthermore, Wang and his colleagues reported that softening of soluble fibres occurred with the cooking process, reducing its content, whereas the insoluble fibre may be increased due to the formation of protein-fibre complexes during cooking (Wang et al., 2009).

The crude fat content for raw whole lentil was similar to that reported by USDA (2009). Pirman and Stibilj (2003) reported that fat content of lentil was low like in many other pulses.

The ash content for raw whole lentil was similar to that reported by Costa et al. (2006). The decrease in ash content of cooked and dehulled lentils might result from diffusion of certain minerals into the cooking water (Wang et al., 2009).

**Induction of diabetes**

Streptozotocin-induced diabetes is a chemical model of experimental DM that develops hyperglycaemia and is widely used nowadays in many studies on diabetes (Tchamadeu et al., 2010).

Previous studies categorised streptozotocin-induced diabetes in rats broadly into type 2 diabetes if the streptozotocin dose was <50 mg/kg body weight and type 1 diabetes if it was >50 mg/kg body weight (Ugochukwu, Bagayoko & Antwi, 2004). The intra-peritoneal administration of streptozotocin in the present study was 35 mg/kg body weight where insulin is markedly depleted but not absent. In this model, diabetes arises from irreversible destruction of the β-cells of the pancreas, causing reduction in insulin secretion (Eidi & Eidi, 2009). The observed significant increase in the level of blood glucose in streptozotocin-induced diabetic rats could be due to the partial destruction of pancreatic β-cell. The main cause of streptozotocin-induced a-cell death is alkylation of DNA by the nitrosourea moiety of this compound (Szkudelski, 2001).

**Body weight**

As shown in Table 3, there were no significant differences (P>0.05) in initial body weight between all experimental groups. After six weeks of experimental period, differences in final body weight and weight change of rats fed different diets were also not significant(P>0.05). The diabetes induced by streptozotocin is associated with polydipsia and loss in body weight (Yang et al., 2008). In the present study, all experimental groups showed a decrease in body weight. These results are in accordance with results previously reported after streptozotocin treatment of diabetic rats (Ozsoy-Sacan et al., 2006).

Weight loss during diabetes is mainly related to urinary glucose excretion because
cells become unable to use glucose. Another factor could be the osmotic diuresis resulting in hyperosmotic dehydration (Rebsomen et al., 2006). In addition, decreased body weight observed in diabetic rats is due to excessive breakdown of tissue proteins (Ozsoy-Sacan et al., 2006).

### Serum glucose, triglycerides and lipoprotein cholesterol

The hypoglycemic effect of lentil has been demonstrated in experimentally induced diabetic rats, healthy volunteers, and insulin-dependent and non–insulin-dependent diabetic patients (Wolever, 1994; Shams et al., 2008).

In the present study, the administration of lentils significantly decreased serum blood glucose (P<0.05) and increased HDL cholesterol in diabetic rats (P<0.01). However, the changes had no significant influence on other lipid profiles including triglyceride, TC and LDL cholesterol. This may be due to an increase in adipose tissue lipolysis in the absence of insulin, and to a decrease in lipoprotein lipase activity (Eidi & Eidi, 2009). The results of the present study are in agreement with those of Shams et al. (2008) who found that addition of 50g cooked lentils to diabetic patient’s diet led to a significant decrease (P<0.05) in fasting blood glucose. It also led to a decrease in TC but not in LDL, HDL and triglycerides.

The effect of fibre in glycemic and lipidic control has been documented. Low glycemic index of diets that included lentils has been reported (Foster-Powell, Holt & Brand-Miller, 2002). Numerous studies have shown that low GI, high fibre diets improve glycated proteins levels (both haemoglobin A1c (HbA1c) and fructosamine), which are markers of glycemic control (Kendall, Esfahani & Jenkins, 2010). In addition, many studies have shown that dietary fibre, particularly the soluble type, improves glucose control and significantly reduces post-prandial plasma glucose levels, and the mean daily blood glucose profile (Giacco, Clemente & Riccardi, 2002).

Little information is known about the effect of cooking and dehulling methods because of the scarcity of studies dealing with the effects of such methods on serum glucose and lipoprotein levels. In the present investigation, it was found that the cooked lentils reduced the level of blood glucose more than raw lentils. Moreover, cooking of lentils improved the level of HDL cholesterol more than the raw lentils. It could be that cooked lentil was effective in reducing the glycemic response despite the reduction in soluble dietary fibre. Part of the starch, after heating and subsequent cooling, can be converted into an indigestible fraction called resistant starch (RS), which evolves from recrystallised forms of amylose and amyllopectin (Kim et al., 2003).

Lentils are rich in phytate; many studies have shown that phytate reduces the rate of starch digestibility in vitro and glycemic response to legumes in vivo (Lee et al., 2006). These effects appear to be related to reducing starch digestibility by the interactions of phytate with the starch, by inducing hormonal changes (Lee et al., 2006), or modulation of insulin secretion (Kumar et al., 2010). The real mechanism of action of phytate on insulin secretion is not fully understood; it appears that phytate may have an effect on calcium channel activity because it specifically inhibits serine threonine protein phosphatase activity. This, in turn, opens intracellular calcium channels, driving insulin release (Kumar et al., 2010).

In conclusion, cooked lentils (whole or dehulled) were found to be more effective in reducing high blood glucose level in the diabetic rats than raw lentils. In addition, it may improve HDL cholesterol level in diabetic rats. These results suggest that incorporation of lentils in human meals may help in protection and management of diabetes and CHD.
ACKNOWLEDGMENT

This research was financially supported by the Deanship of Scientific Research, University of Jordan, Amman, Jordan. The authors are indebted to Ihsan Al-Omari for her help in the animal experimentation.

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