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Therapeutic role of low-carbohydrate ketogenic diet in diabetes

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Abstract

Introduction: Changes in dietary habits influence the glycemic level. Preliminary studies using the low-carbohydrate ketogenic diet (LCKD) were found to be quite promising in controlling diabetes mellitus. Therefore, the objectives of this study are to investigate the therapeutic effects of LCKD in experimental diabetic rats following the administration of streptozotocin (STZ).

Materials and methods: Adult rats were divided into three groups: normal diet, LCKD, and high-carbohydrate diet. Each group was subdivided into normal, sham, and diabetic groups. Diabetes was induced by a single intraperitoneal injection of STZ (55 mg/kg). Specific diets were given to each group of animals for a period of 8 wk and then the animals were sacrificed. The rats were monitored daily for food and water intake, whereas body weight, urine output, and blood glucose levels were monitored weekly. The histology of the islets of Langerhans was studied by histochemical methods.

Results: The results showed that LCKD was effective in bringing blood glucose level close to normal ($P < 0.01$). Food and water intake and urine output were increased in all groups except the LCKD group ($P < 0.01$). The body weight was significantly reduced in all diabetic animals except in the LCKD group ($P < 0.01$). Histologic studies showed significant decrease in the islet size and number of β cells in all the diabetic groups.

Conclusion: This study indicates that LCKD has a significant beneficial effect in ameliorating the diabetic state and helping to stabilize hyperglycemia.

Keywords: Diabetes mellitus; Low-carbohydrate ketogenic diet; Streptozotocin; Biochemistry; Histology

Introduction

Diabetes mellitus (DM) is a serious universal health problem. The prevalence of this condition is rapidly increasing in the world. Similarly, in the Gulf region and especially in Kuwait, DM is spreading widely. Changes in lifestyle and dietary habits, in conjunction with genetic susceptibility, have resulted in a remarkable increase in the incidence and prevalence of diabetes in the world [1,2].

Type 1 diabetes, or insulin-dependent diabetes (IDDM), is caused by the autoimmune destruction of pancreatic β cells leading to insulin deficiency. Hence, the administration of insulin is essential for the metabolism and survival of these patients. Type 1 diabetes accounts for only 5–10% of all the diabetic cases [3]. Type 2 diabetes, on the other hand, is due to impaired insulin secretion and/or insulin resistance. This type of insulin-independent diabetes is much more widespread and accounts for almost 90–95% of the DM cases [4,5].

Recently several animal models have been used in the study of diabetes. Streptozotocin (STZ) is commonly used to alter pancreatic β-cell function leading to diabetes in experimental animals. STZ is a nitrosourea derivative [(2-deoxy-2-(3-(methyl-3-nitrosoureido)-D-glucopyranose)], isolated from *Streptomyces achromogenes*, which selectively destroys pancreatic β cells [6]. It causes DNA methylation,

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DNA damage, severe adenosine triphosphate depletion, and eventually necrosis of the β cells [7]. Hence, a high dose of STZ severely impairs insulin secretion, mimicking type 1 diabetes [7]. However, specific STZ doses cause a partial destruction of β-cell mass. Therefore, administration of calculated doses of STZ can be used to produce type 2 DM [8]. But it is quite difficult to judge the appropriate dosage to create stable type 2 DM without either gradual recovery or deterioration into type 1 DM. STZ reliably produces many of the signs such as increased intake of water and food, failure to gain weight, and increased blood glucose concentrations and several symptoms of chronic human diabetes, in particular, diastolic cardiac dysfunction, cataracts, and neuropathy.

Maintaining blood glucose levels within the normal range is of utmost importance in the management of diabetes. Diet is one factor that can have a great impact upon stabilizing blood glucose levels in diabetic patients. Recent studies have reintroduced the concept of using a ketogenic diet with low-carbohydrate content in a variety of disease states, such as epilepsy [9], obesity [10], cardiovascular diseases [11], and diabetes [12].

A low-carbohydrate ketogenic diet (LCKD) is a high-fat, low-protein, low-carbohydrate (<100 g/d) diet that has been employed as a treatment for intractable epilepsy and obesity [13]. It consists of long-chain saturated triglycerides in a 3:1–4:1 ratio of fats to carbohydrates + protein (by weight). LCKD mimics the physiologic state of fasting [13].

It is generally believed that a high-fat diet causes obesity. As fat has a higher caloric density than carbohydrate, it is assumed that consumption of a high-fat diet will be accompanied by a higher energy intake [14]. On the contrary, current studies quite evidently show that a ketogenic diet can cure obesity [10] and obesity-associated diseases [11,12,15]. This concept that fat can be eaten ad libitum and still induce weight loss in obese subjects is actually quite old [16].

The extent of blood glucose level is determined by the amount and rate of glucose absorption from the gut and also by the rate of its utilization or storage when it enters the circulation [17]. In diabetes, as ingested carbohydrates are absorbed mainly as glucose, there is an immediate rise in the blood glucose level. The contents of an LCKD are mainly absorbed as triglycerides and proteins rather than glucose, so this would alleviate one of the major factors in diabetes.

Due to the above-mentioned reasons, this study is aimed at investigating the therapeutic effects of LCKD in diabetic rats as compared to normal and high-carbohydrate diets.

Materials and methods

Animals and experimental design

A total of 63 male Wistar rats, weighing 250–300 g, were used in this study. Animals were housed singly, under controlled environmental conditions of temperature 22.3 ± 0.3°C, 31.2 ± 0.8% humidity, and a 12-h light/dark cycle in the Animal Care Facility at Kuwait University. This study was approved by the Animal Protection Ethical Committee of Kuwait University.

The animals were randomly assigned to the three diet groups: (1) normal diet (ND) of regular commercial rat food [18]; (2) high-carbohydrate diet (HCD) of 70% carbohydrate, 10% fat, and 20% protein; and (3) LCKD of 60% fat, 10% carbohydrate, and 30% protein. Each group was further subdivided into three subgroups: control, sham, and diabetic rats (each group consisting of seven rats). All the groups had free access to water and food based on the type of diet. Each group of rats was fed with the specific type of diet for 8 wk.

Diabetes was induced first in rats by the intraperitoneal injection of STZ (S-0130, Sigma, Ronkonkoma, NY, USA), freshly prepared [19], at a concentration of 55 mg/kg in saline, and the animals in the sham control group were given only saline.

Before STZ injection, rats were caged singly in metabolic cages for 24 h to collect urine for analysis and for measuring the urine output. On the day of STZ injection, the level of blood glucose was measured from the rat tail using a glucometer (One touch ultra, Lifescan, Tokyo, Japan) [20]. After STZ injection and the development of diabetes, which was confirmed using Keto-Diabur test strips (Accu-chek, Roche, Selangor Daral Ehsan, Malaysia) [21], the animals were transferred into normal cages.

Daily measurements of food and water intake as well as weekly measurement of body weight were taken during the whole experiment. In addition, blood glucose level (diabetic ≥ 250 mg/dL) [20] and urine output were measured once a week.

Preparation of specimen

At the end of 8 wk, animals were anesthetized using ether, and blood was collected in vacutainer tubes (Vacutainer Brand, 5181548, BD Diagnostics, NJ, USA) by cardiac puncture. After the collection of blood samples, the animals were sacrificed, the abdomen opened with a midline incision, and the pancreas taken for histologic analysis by routine hematoxylin and eosin and Gomori's chrome alum hematoxylin-phloxine staining methods.

Gomori's chrome alum hematoxylin-phloxine stain was used to distinguish endocrine cells of pancreas and to highlight insulin-producing β cells from α and δ cells [22]. Briefly, the Gomori's chrome alum hematoxylin-phloxine staining method is as follows. Sections after their initial fixation in 10% formalin were treated with Bouin's fluid for 16–24 h. The slides were then washed in tap water to remove picric acid and then treated for 1 min with a mixture containing an equal amount of 0.3% potassium permanganate and 0.3% sulfuric acid. The tissues were decolorized with 2–5% solution of sodium bisulphate and washed well in running tap water. The slides were then stained with hematoxylin solution for 15 min until the β cells became deep blue. The slides were further

rinsed in water and differentiated in acid alcohol for about 1 min to remove the background staining. Again the slides were washed for 10 min in running tap water until the sections were clear blue. After that, the slides were stained in 0.5% aqueous phloxine for 5 min, rinsed in water, and then treated with 5% phosphotungstic acid for another min. The slides were washed in running tap water for 5 min so that sections became red and then differentiated in 95% alcohol. Finally, the slides were dehydrated, cleared and mounted with a cover slip using a mixture of distyrene (a polystyrene), a plasticizer (tricresyl phosphate), and xylene (DPX).

The tissue sections were examined using a light microscope (Zeiss, Hamburg, Germany) and images were captured with a Zeiss digital camera using Axiovision software (Zeiss, Germany).

Statistical analysis

The Student's *t* test and analysis of variance with Bonferroni correction were performed for the comparisons between animals fed the normal, high-carbohydrate, and low-carbohydrate ketogenic diets. A value of $P < 0.05$ was considered to be significant.

Results

There were no significant differences between the control group and the sham control for each diet group. Therefore, the control group and the sham control were combined to make the ''control'' group for each diet.

Effect of different diets on blood glucose level

The non-fasting blood glucose level was measured weekly from the tail vein of rats using a glucometer. After the administration of STZ, the blood glucose level was significantly increased in all diabetic groups compared with their control at all time points, except baseline. But the increase in the blood glucose level of LCKD diabetic (LCKD-D) group was significantly lower ($P < 0.005$ and $P < 0.01$) than the other diabetic groups. From the 6th wk onward, the blood glucose level of the diabetic groups of LCKD was almost similar to the control group (Fig. 1). Weekly comparison (using analysis of variance with Bonferroni correction) of LCKD-D with ND-D and HCD-D showed that starting from week 4 of the experiment, there were significant differences in the level of blood glucose between LCKD-D and ND-D as well as LCKA and HCD-D.

Effect of different diets on body weight

With STZ administration, there was a significant decrease ($P < 0.01$) in the body weight in both ND-D and HCD-D groups. On the other hand, there was no significant difference in body weight between the control and LCKD diabetic group (Fig. 2).

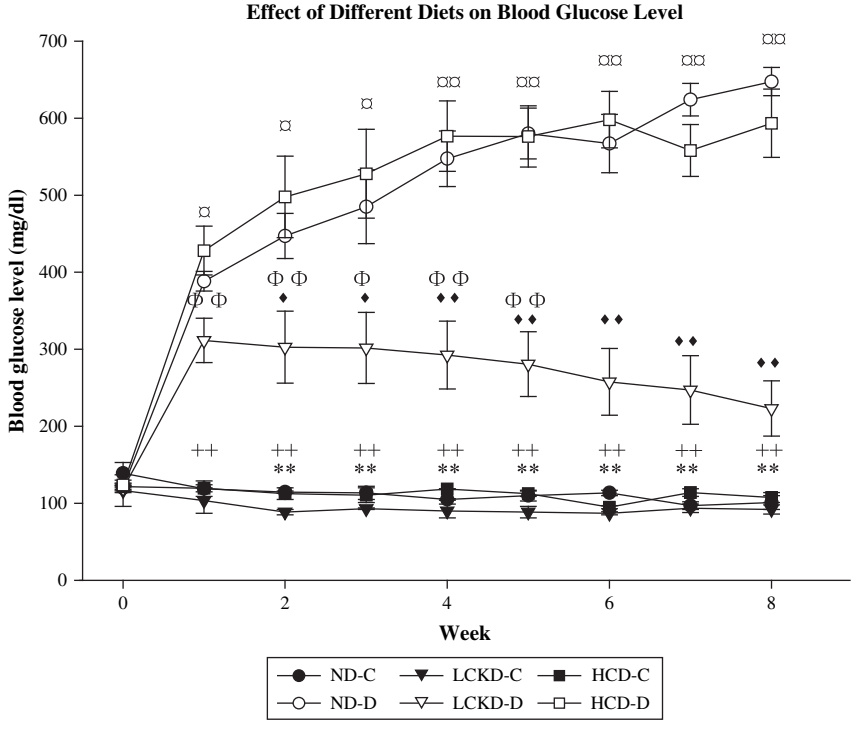

Fig. 1. The effect of different diets: normal diet (ND), high-carbohydrate diet (HCD), and low-carbohydrate ketogenic diet (LCKD) on blood glucose level (mg/dL) in control (C) and diabetic (D) rats. The values are mean ± SEM ($n = 42$). **$P < 0.01$, ND-C compared with ND-D; ++, $P < 0.01$, HCD-C compared with HCD-D; ¤, $P < 0.05$, ¤¤, $P < 0.01$, ND-D compared with LCKD-D; ◆, $P < 0.05$, ◆◆, $P < 0.01$, HCD-D compared with LCKD-D; Φ, $P < 0.05$, ΦΦ, $P < 0.01$, LCKD-C compared with LCKD-D.

Changes in food intake in different groups

After STZ administration, as expected, the diabetic groups of ND and HCD showed an increase in food consumption. On the other hand, the LCKD groups showed the least food intake ($P < 0.01$) as compared with other groups (Fig. 3).

Calorie intake of different groups

All the control groups and LCKD-D ingested almost the same number of calories throughout the experiment. However, the diabetic groups of ND and HCD showed a significant ($P < 0.001$) high-calorie intake (Fig. 4).

Changes in water intake in different groups

The ND-D and HCD-D groups showed a significant increase ($P < 0.01$) in water intake as compared with the LCKD-D group throughout the experimental period.

Effect of different diets on urine output

Excretion of urine was monitored weekly. After STZ administration, there was a significant increase ($P < 0.01$) in the urine output in ND-D and HCD-D compared with their control groups and with LCKD-D. At the end of the study, the increase in urine output in the ND-D and HCD-D groups reached up to 250 mL/d. Although there was a slight difference in urine output between the control and diabetic groups of LCKD during the first few weeks, the urine excretion in the LCKD-D groups decreased constantly throughout the entire study period.

Effect of different diets on urine glucose and other analysis

The glycosuria was negative throughout the experiment in LCKD control (LCKD-C) and LCKD-D groups. In the LCKD-D group, trace to 250 mg/dL glucose was present during the first 3 wk. For the diabetic ND and HCD groups, there was a significant increase in the level of glucose in the urine (above 1000 mg/dL). Compared with LCKD-C, the control group of HCD showed trace to 250 mg/dL glycosuria during the whole experiment. Although urine did not show the presence of ketones in the normal state, the urine levels of ketones and proteins in the experimental rats were consistent with the diabetic state of the animals in different diet groups.

Histologic assessment of islets of Langerhans

Hematoxylin and eosin staining showed the presence of several round to elongated, normal islets of Langerhans in the control groups (Fig. 5). On the other hand, in all the diabetic groups, the islet morphology was altered with vacuoles

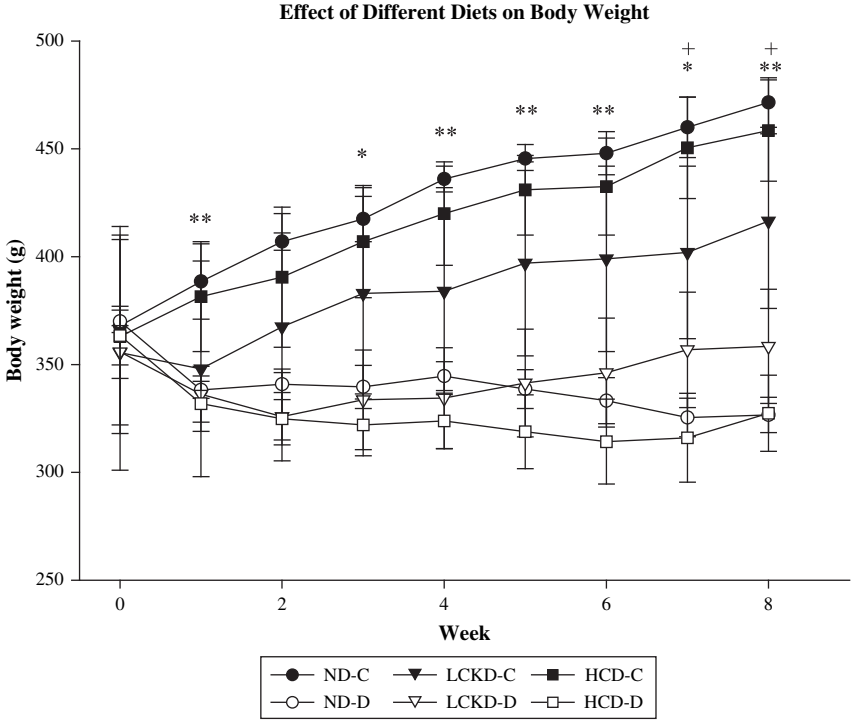

Fig. 2. Effect of different diets: normal diet (ND), high-carbohydrate diet (HCD), and low-carbohydrate ketogenic diet (LCKD) on body weight (g) in control (C) and diabetic rats (D). The values are mean ± SEM ($n = 42$). *$P < 0.05$, **$P < 0.01$, ND-C compared with ND-D; +, $P < 0.05$, ++, $P < 0.01$, HCD-C compared with HCD-D.

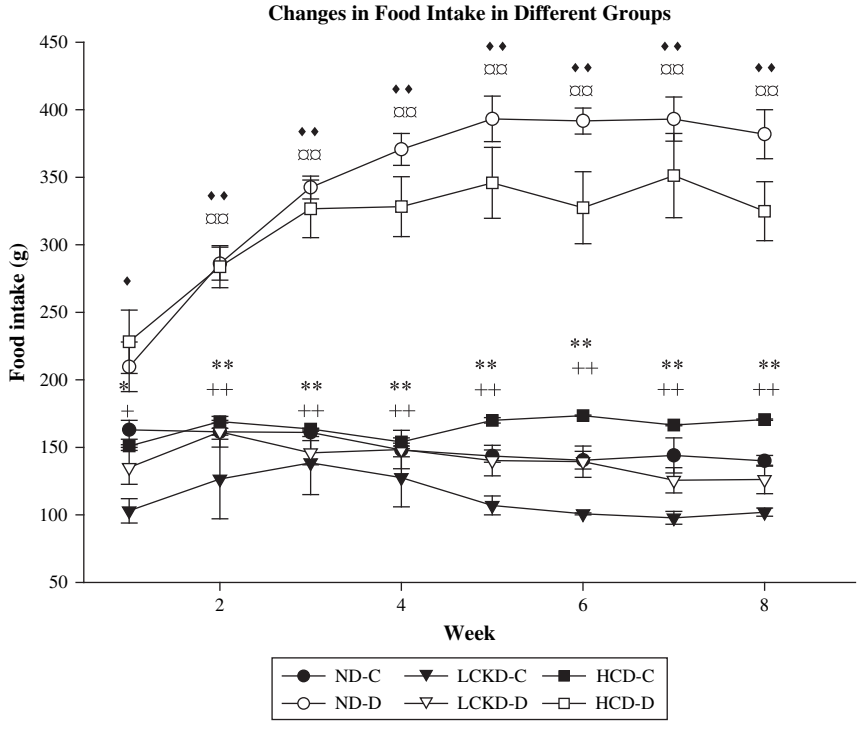

Fig. 3. The effect of different diets: normal diet (ND), high-carbohydrate diet (HCD), and low-carbohydrate ketogenic diet (LCKD) on food intake (g/wk) in control (C) and diabetic rats (D). The values are mean ± SEM (n = 42). ¤¤, $P < 0.01$, ND-C compared with ND-D; ◆, $P < 0.05$, ◆ ◆, $P < 0.01$, HCD-C compared with HCD-D; *$P < 0.05$, **$P < 0.01$, ND-D compared with LCKD-D; +, $P < 0.05$, ++, $P < 0.01$, HCD-D compared with LCKD-D.

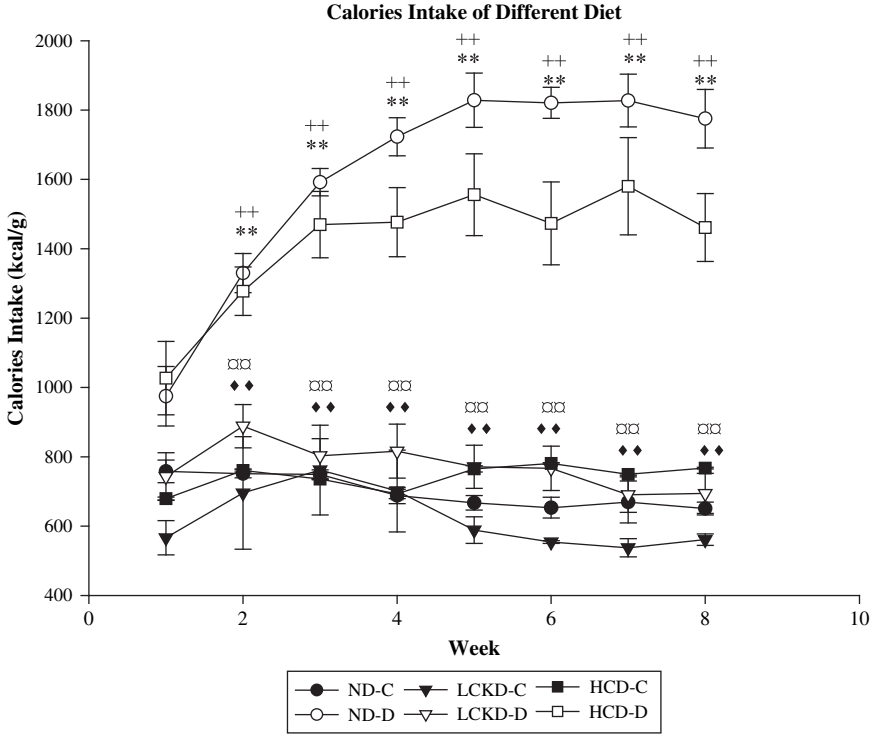

Fig. 4. The calorie intake of different diets: normal diet (ND), high-carbohydrate diet (HCD), and low-carbohydrate ketogenic diet (LCKD) in control (C) and diabetic rats (D). The values are mean ± SEM ($n = 42$). **$P < 0.001$, ND-C compared with ND-D; +, $P < 0.05$, ++, $P < 0.01$, HCD-C compared with HCD-D; ¤¤, $P < 0.001$, ND-D compared with LCKD-D; ◆ ◆, $P < 0.001$ HCD-D compared with LCKD-D.

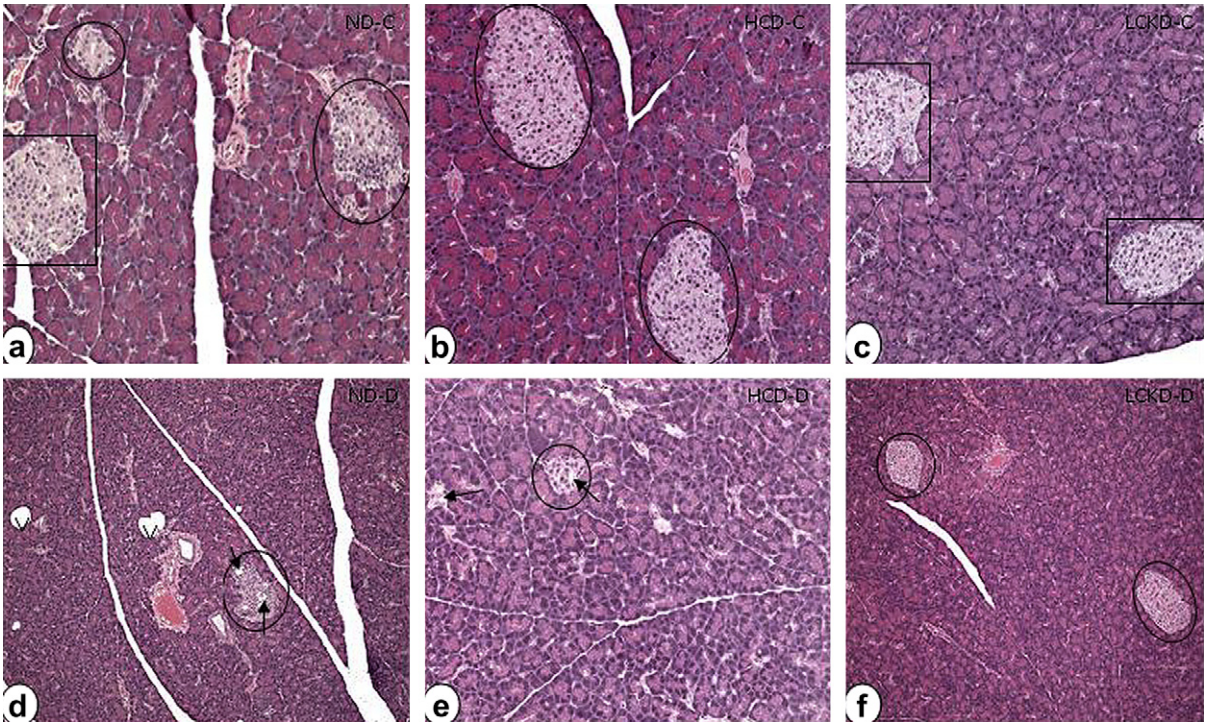

Fig. 5. Sections of the pancreas from control and diabetes rats of postfed experiment stained with hematoxylin and eosin. Circles show islets of Langerhans. Arrows show vacuoles. a, b, c and e: Magnification 20×; d and f: magnification 10×.

and only a few islets were present in tissue sections (Fig. 5). Furthermore, Gomori's chrome alum hematoxylin-phloxine staining showed the presence of only very few necrotic β cells in the islets. However, α and δ cells of the islets were not affected by STZ (Fig. 6). Macrophage infiltration and the presence of vacuoles were observed among lytic β cells. Statistical analysis showed a significant decrease in the number of β cells (Fig. 7) in all diabetic groups as compared with the respective control groups ($P < 0.01$).

Discussion

The data presented in this study clearly indicate the beneficial effects of LCKD in improving the diabetic status in terms of body weight, blood glucose, urine output, and food and water intake. In this study, after STZ injection, the rats were randomly assigned to the three diet groups to ensure that the results were due to the dietary effects rather than any other factors. Also, it is important to emphasize that this experiment was carried out without the usage of any hypoglycemic medication.

Effect of diets on blood glucose levels

After STZ administration, there was a significant increase in the blood glucose levels of all the diabetic groups as compared with their controls. However, the blood glucose level of the LCKD-D group was significantly lower ($P < 0.005$ and $P < 0.01$) than the other groups. As shown in Figure 2, there was a decrease in the blood glucose level in response to the LCKD diet from week 1, which reached almost normal levels (<200 mg/dL) at week 6. On the other hand, the rats assigned to the other two diets showed continuous increase in the blood glucose levels, reaching approximately 650 mg/dL. Therefore, the data presented in this study suggest that even short-term use of the LCKD has significant beneficial effects in STZ-treated diabetic rats. Several studies in which LCKD was administered in parallel with insulin and hypoglycemic medication, either for short or long periods, have shown the therapeutic effect of LCKD in improving the glycemic level as well as reducing the need for such medications [23–26]. This improvement in the glycemic level was achieved with a low-carbohydrate, high-protein diet [27,28] as well as with a low-carbohydrate, high-fat diet [29,30]. These studies suggest that reducing the amount of dietary carbohydrate is important in regulating diabetes. Moreover, our results on the metabolic improvement were similar when a 6% carbohydrate diet was given 11 days after the induction of diabetes [31], as well as with a carbohydrate-free diet given after 6 wk for spontaneously diabetic BB Wistar rats [32]. In 1990, Henry and his colleagues [33] showed that the improvement in the blood glucose levels is also due to the direct effect of the ketone bodies on the hepatic glucose output. Similarly, Müller and his colleagues [34] found that infusion of ketone bodies caused a decrease in hepatic glucose production, blood glucose, and glucose utilization.

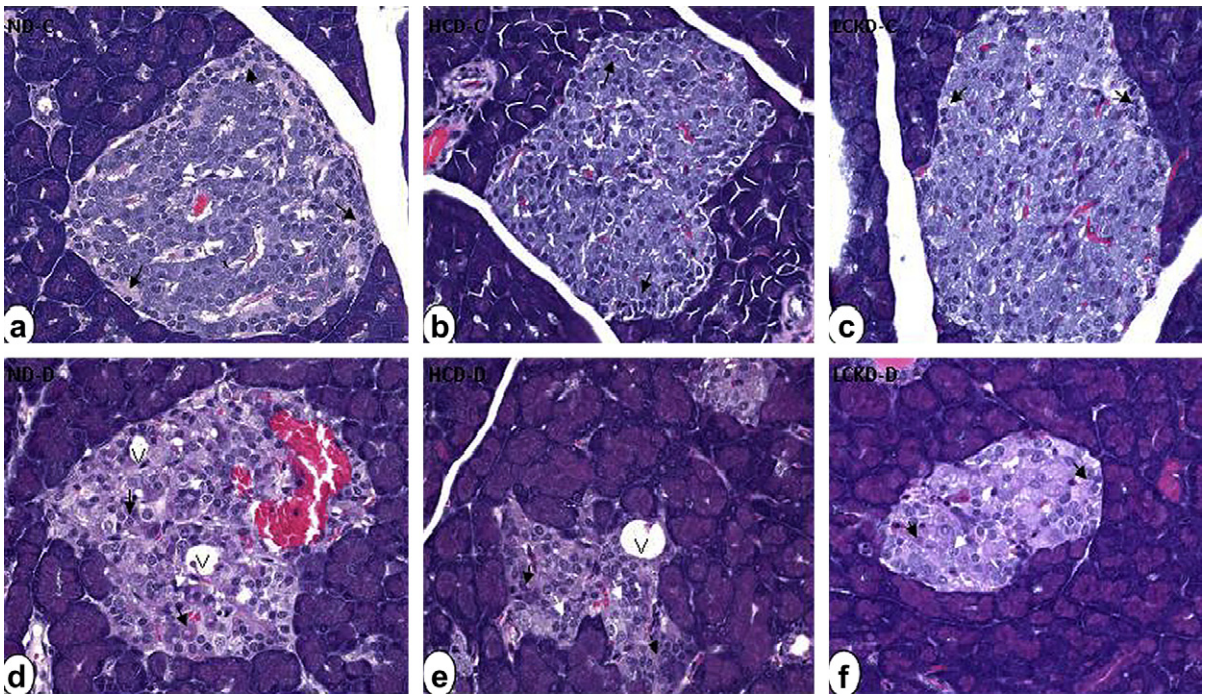

Fig. 6. Sections of the pancreas for control and diabetes rats of postfed experiment stained with Gomori's chrome alum hematoxylin-phloxine stain. Black arrows show α cells. White arrows show β cells. V, vacuoles. β cells with blue and located interior, α cells with red found at periphery, and δ cells pink to red are located among α cells. Magnification 40×.

Effect of diets on body weight and food intake

As expected with the diabetic condition, there was a significant ($P < 0.01$) reduction in the body weight and an increase in the food and caloric intake of the diabetic groups except for the LCKD group, confirming the beneficial effect of LCKD on the diabetic status. These results are similar to the studies of other investigators [36].

Effects of diets on water intake and urine output

As polydipsia and polyuria are conditions that are concomitant with the diabetic state, water intake and urine volume were markedly increased in the diabetic groups of ND and HCD. On the other hand, water intake in the LCKD-D group was within the normal range, whereas urine volume during the first 2 wk was considerably above the normal range due to glucose excretion. Gradually urine volume in the LCKD-D group returned to the normal level.

These results showing the general improvement in weight gain and polyuria condition were similar to the studies in which a low-carbohydrate diet [35–37] and carbohydrate-free diet [38] were used.

Contrary to our findings and other recent studies mentioned above, a few studies [39–42] showed that the low-carbohydrate, high-fat diet had worsened the diabetic state. Although the studies of Chisholm and O'Dea [42] were similar to our studies and the composition of LCKD and HCD used were similar, the exact reason why their results contradict the findings presented in this study is not understood. However, in a recent study evaluating the response of two lower-carbohydrate diets that were rich in slowly digested carbohydrate and monounsaturated and omega-3 fatty acids

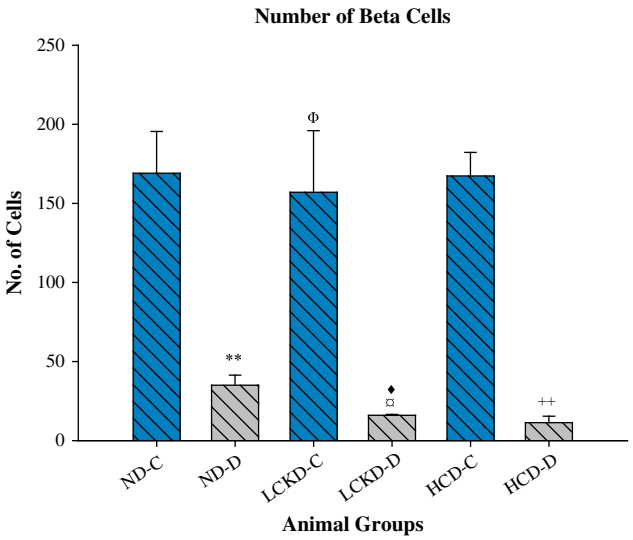

Fig. 7. The effect of different diet: normal diet (ND), high-carbohydrate diet (HCD), and low-carbohydrate ketogenic diet (LCKD) on number of β cells in control (C), and diabetic rats (D). The values are mean ± SEM ($n = 42$). **$P < 0.01$, ND-C compared with ND-D; ++, $P < 0.01$, HCD-C compared with HCD-D; ¤, $P < 0.05$, ND-D compared with LCKD-D; ◆, $P < 0.05$, HCD-D compared with LCKD-D; Φ, $P < 0.05$, LCKD-C compared with LCKD-D.

and a standard control diet in subjects with type 2 DM, it was shown that both diabetic-specific diets produced significantly lower blood glucose and insulin responses and higher levels of glucagonlike peptide-1 as compared with the standard diet [43].

Urine glucose and other analysis

Urine analysis showed that the glucose level in the ND-D and HCD-D was above 1000 mg/dL throughout the experimental period. However, in the LCKD-D group, high-level glucose was present only during the first 3 wk. Thereafter, the level of glucose in the urine of the LCKD-D group became almost normal. These results further suggest the beneficial effects of LCKD in the regulation of diabetes. Levels of ketones and proteins in the urine of experimental rats were consistent with the diabetic state of the animals.

Histologic assessment of the islets of Langerhans

Histologic studies showed a decrease in the number of islets as well as the number of β cells in all diabetic groups as compared with their respective controls. The histologic changes observed in this study correspond to the ultrastructural changes observed in the islets of Langerhans of mice in response to STZ [44]. Electron microscopic observation revealed early chromatin aggregation and cytoplasmic vesiculation in the central β cells during the first 2 h of STZ treatment. In addition, nuclear shrinkage and pyknosis with swelling of mitochondria and endoplasmic reticulum were observed [44]. Lysis of β cells occurred after 12 h of treatment. However, other cell types of the islets of Langerhans did not show any ultrastructural alteration. Macrophage infiltration and the presence of clear and large phagocytic vacuoles were observed among lytic β cells after 24 h of STZ administration. No features of apoptosis were observed, and the pancreatic tissue remained unaffected from the effect of STZ [44].

As shown by the metabolic parameters studied, during 8 wk of this experiment, diabetic rats on ND and HCD maintained a high glucose level throughout the experiment; in contrast, LCKD maintained the blood glucose level at near normal levels. These results are similar to the studies of other investigators [45,46] who used a low-carbohydrate diet in the treatment of diabetes. Recently various studies have been carried out on glycated hemoglobin (HbA_{1c}), which is considered as an index of blood glucose control and the degree of oxidative stress in diabetes [47,48]. It has been shown that administration of LCKD decreases the level of glycated hemoglobin in diabetic patients [12,23], suggesting a reduction in the generation of reactive oxygen species and an improvement in the oxidative status. All these studies suggest that LCKD may play a beneficial role in the amelioration of oxidative stress in diabetic patients. In support of this view, Falk and his collaborators [49] have shown that ketone bodies function as antiinflammatory agents through the reduction of reactive oxygen species and increase of glutathione peroxidase activity. Furthermore, according to Freeman and associates [50], the anticonvulsant role of a ketogenic diet could be due to the antioxidant mechanisms activated by fatty acids and ketones.

In conclusion, the histologic and biochemical data presented in this study support the view that the LCKD has a significant beneficial effect on ameliorating the diabetic state and helping to stabilize hyperglycemia and could result in improved β-cell function. Although the underlying mechanism of this protective effect is not understood, it is possible that, as mentioned above, the LCKD (fatty acids and ketone bodies) may have a significant role in reducing oxidative stress in STZ-induced diabetes in rats. Therefore, the LCKD may be effective in diabetes management by improving glycemia and reducing the need for medication in patients with diabetes.

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