The effects of dietary caloric restriction on antioxidant status and lipid peroxidation in mild and severe streptozotocin-induced diabetic rats

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Abstract

Background: Dietary caloric restriction (CR) without malnutrition is effective in the control of diabetes mellitus by stabilizing glucose homeostasis and enhancing glycemic control. Mild and severe streptozotocin-induced diabetic and non-diabetic rats were subjected to caloric restriction and ad libitum feeding to evaluate their effects on oxidative stress and lipid profile in the plasma of experimental animals. Methods: Mild and severe diabetes were induced in Male Wistar rats by intraperitoneal injection of 35 and 65 mg/kg streptozotocin respectively. The experimental animals were subjected to 40% caloric restriction and ad libitum feeding for 9 weeks. Results: CR was effective in significantly reducing body weight, blood glucose, HbA1C and TG concentrations (all \( p < 0.001 \)) in mild diabetic rats and non-significantly improving the plasma HDL-cholesterol concentrations. However, CR did not produce any significant effect on the antioxidant enzyme activities and MDA concentrations in all the groups nor in any of the parameters measured in non-diabetic rats except their overall weight change. There were significant (\( p < 0.001 \)) decreases in body weight and non-significant fluctuating results in HbA1C and HDL-cholesterol in severe diabetic animals. Conclusions: These results demonstrate that caloric restriction is most effective in mild than in non-diabetic or severe diabetic animals.

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1. Introduction

Dietary caloric intake has been linked to risks for the development of certain chronic diet-related diseases especially non-insulin dependent diabetes mellitus (NIDDM) and obesity [1,2]. Impaired insulin secretion and insulin resistance characterize NIDDM, which accounts for 90% of all diabetic cases, with hyperglycemia as the dominant feature [3]. The characteristic hyperglycemia and lipoprotein abnormalities of NIDDM are hypothesized to be responsible for the damage to cell membranes [4], which in turn results in elevated production of reactive oxygen species [5]. These species are found to play a role in the pathogenesis of NIDDM [6]. Therefore prevention or delay in the development of NIDDM or the associated complications is crucial in promoting good quality...
of life and reducing the socioeconomic cost and high mortality rates associated with NIDDM. Caloric restriction has been shown to produce anti-aging actions in animals [7,8] and effect significant improvements in both glycemic control and insulin sensitivity during weight loss in obese NIDDM patients [9,10] and in preventing NIDDM in diabetes-prone rats [1]. Some studies showed that a very low caloric diet was effective in promoting weight loss as well as lowering blood glucose, systolic blood pressure, fasting plasma insulin and total cholesterol [11]. Others showed that imposition of severe gastric restriction by gastric bypass surgery on severely overweight subjects with impaired glucose tolerance resulted in a 30-fold reduction in the rate of type 2 diabetes [12]. However, there has not been any comparative study on the biochemical effects of dietary caloric restriction on mild and severe streptozotocin-induced diabetic rats. We investigated the effects of dietary caloric restriction on overall weight changes, blood glucose concentrations, glycated hemoglobin, lipid profile and antioxidant enzymes.

2. Materials and methods

2.1. Animals

Thirty-six 3-month-old male Wistar rats (Harlan Sprague-Dawley, Indianapolis, IN) weighing 375–399 g were used in this study. The animals were housed singly in metabolic cages in the Animal Care Facility at Florida A&M University under controlled environmental conditions of temperature (68–72°F) and relative humidity (50 ± 5%) and a 12-h light/dark cycle. Upon arrival, the animals were allowed to acclimatize for 7 days. Deionized distilled water was provided ad libitum, and feeding was maintained on regular commercial rat feed (19.92% protein, 5.67% fat, 4.37% ash, 53.66% nitrogen free extract, 2.90% linoleic acid (Harlan Teklad, Madison, WI) except as indicated in the experimental plan for animals undergoing caloric restriction.

2.2. Induction of diabetes

After acclimatization, the 36 rats were subjected to a 16-h fast. The fasting blood glucose concentrations were measured from the tail vein using a blood glucose monitoring system (LifeScan, Milpitis, CA). Diabetes was induced in 24 rats with streptozotocin (STZ) (Sigma, St Louis, MO) at a dose of 35 mg/kg (12 animals) and 65 mg/kg (12 animals) intraperitoneally to obtain mild and severe diabetic rats respectively. Some investigators showed that an injection of 24–100 mg/kg streptozotocin produced a dose-dependent hyperglycemia [13,14]. The STZ was freshly dissolved in citrate buffer (0.01 mol/l, pH 4.5) and kept on ice prior to use. Control rats received only the buffer. One week after injection of streptozotocin, diabetes was confirmed in STZ-treated rats with fasting blood glucose concentrations between 180 and 250 mg/dl for mild diabetic rats and >350 mg/dl for severe diabetic rats.

2.3. Experimental plan

The animals were divided into six groups of six each. Group 1 was non-diabetics (NAL) on a 100% feed diet. Group 2 was non-diabetics (NCR) on a 60% diet. Group 3 was mild diabetics (MDAL) on 100% diet. Group 4 was mild diabetics (MDCR) on 60% feed diet. Group 5 was severe diabetics (SDAL) on 100% diet. Group 6 was severe diabetics (SDCR) on 60% diet. After 1 week of induction of diabetes, dietary caloric restriction was accomplished by 10% reduction in the average daily food intake every 5 days until a 40% reduction was achieved. Daily measurements of the rats’ feed, body weight and blood glucose concentrations were taken. After 9 weeks of study, blood was collected from the hearts of the animals by cardiac puncture prior to euthanasia, in EDTA vacutainer tubes and centrifuged at 4000 x g at 4 °C for 15 min, and stored at −80 °C until analysis.

2.4. Biochemical assays

The biochemical reagents and enzymes were purchased from Sigma. Blood was used for assaying blood glucose and glycated hemoglobin while plasma were used for triglyceride, HDL cholesterol and protein concentrations using assay kits (Sigma), superoxide dismutase [15], catalase [16] and glutathione peroxidase [17]; and lipid peroxidation using malondialdehyde concentrations [18]. The malondialdehyde
concentrations were converted to µg using formula weight. All assays were carried out in triplicates using a Beckman-Coulter DU 400 spectrophotometer (Fullerton, CA). The body weights of the experimental animals were taken daily to ensure good health in accordance with Animal Care and Use Committee (ACUC) guidelines. For experimental purposes, only the weights before caloric restriction and those on day 64 were used for analyses.

2.5. Statistical analysis

The results were analyzed for statistical significance by one-way ANOVA test and Tukey’s multi-comparison post-test using GraphPad Prism computer software package version 3.0 (San Diego, CA, USA). All data was expressed as mean ± SEM, n = 6. Differences between groups were considered significant at p < 0.001.

3. Results

3.1. Effects of caloric restriction on blood glucose, glycated hemoglobin and body weight

The blood glucose concentrations (Fig. 1a) and body weights (Fig. 1c) of the non-diabetic and mild diabetic rats were significantly (p < 0.001) reduced by caloric restriction compared to those fed ad libitum. However, caloric restriction did not produce any significant effects on blood glucose concentrations of severe diabetic rats. There was a statistically significant (p < 0.001) increase and decrease in the body weights of non-diabetic rats fed ad libitum and severe diabetic rats, respectively. The glycated hemoglobin concentrations of the mild diabetic rats fed ad libitum was significantly (p < 0.001) higher than those under caloric restriction (Fig. 1b). Caloric restriction produced non-significant decreases in the glycated hemoglobin of non-diabetic and severe diabetic rats.

3.2. Effects of caloric restriction on antioxidant enzyme activities

Caloric restriction did not produce any statistically significant differences in the SOD (Fig. 2a) and CAT (Fig. 2b) activities in the experimental rats. However, non-significant increases were obtained in the SOD activities of all rats under caloric restriction and in severe diabetic rats for catalase, but there were non-significant decreases in CAT activity in the non-diabetic and mild diabetic rats. The glutathione peroxidase activity in non-diabetic rats was non-significantly higher than in mild diabetic rats and significantly (p < 0.001) higher than in severe diabetic rats (Fig. 2c). Like the SOD activity, caloric restriction produced non-significant increases in the GPx activities of all experimental rats.

3.3. Effects of caloric restriction on malondialdehyde, HDL-cholesterol and triglyceride concentrations

No statistically significant differences in the concentrations of malondialdehyde (Fig. 3a) and HDL-cholesterol (Fig. 3b) were observed in all the experimental animals under caloric restriction. However, caloric restriction produced non-significant reductions in MDA concentrations in non-diabetic and severe diabetic rats but not in mild diabetic rats. The treatment was found to have improved effects on HDL cholesterol concentrations in mild and severe diabetic rats. The triglyceride concentrations in mild diabetic rats under caloric restriction were significantly (p < 0.001) reduced compared to all the other groups. There were no significant differences in TG concentrations in the non-diabetic and severe diabetic groups. However, there was a non-significant increase in TG concentrations in the diabetic rats fed ad libitum.

4. Discussion

This study was undertaken because there were no published reports on the effects of dietary caloric restriction (without malnutrition) on mild and severe STZ-induced diabetes mellitus in rats. Previous studies [14,19–21] categorized STZ-induced diabetes in rats broadly into type 2 (<50 mg STZ/kg body weight) and type 1 (>50 kg STZ/body weight) diabetics. Some studies showed that 35 mg/kg injection of STZ produced type 2 diabetes [19] and 65 mg/kg body weight gave rise to type 1 diabetes [22] in rats. Therefore the STZ-treatments used in this study, 35 and 65 mg/kg body weight represent type 2 and type 1 diabetes, respectively.
Some studies demonstrated the preventative effect of caloric restriction on hepatic glucose output [10,23]. Other [11] showed that very low calorie diet reduced body weight, glycated hemoglobin (HbA1c), blood pressure, total triglyceride and cholesterol concentrations in Type 2 diabetes. Lane et al. [24] differentiated between caloric restriction studies per se from low or very low calorie diet; caloric restriction typically involving modification of total calorie content rather than changes in relative proportion of fat, protein or carbohydrate as found in studies using low or very low calorie diet. We investigated whether caloric restriction could ameliorate the effects of diabetes during the 9-week study. Our results indicate that all the diabetic animals used were hyperglycemic.

Fig. 1. Effects of dietary caloric restriction on blood glucose concentrations (panel a), glycated hemoglobin (panel b) and body weight on day 1 and day 64 (panel c) in non-diabetic, mild and severe streptozotocin-induced diabetic rats. Values are mean ± SEM; n = 6. *p < 0.001 as compared to non-diabetic rats fed ad libitum. †p < 0.001 as compared to mild diabetic rats fed ad libitum. ‡p < 0.001 as compared to non-diabetic rats fed ad libitum-final weight. §p < 0.001 as compared to mild diabetic rats with caloric restriction-final weight. ¶p < 0.001 as compared to severe diabetic rats with caloric restriction-final weight. ·p < 0.001 as compared to severe diabetic rats fed ad libitum-final weight.
trations in non-diabetic and mild diabetic rats but not in severe diabetic rats was probably due to the selective modulation of caloric restriction on type 2 but not on type 1 diabetes in the experimental animals.

During diabetes, the excess glucose present in the blood reacts with hemoglobin to form glycated hemoglobin. Glycated hemoglobin (HbA1c) is therefore used as an index in the management of diabetes and for early detection of change in blood glucose control [25,26]. In our study, the concentration of HbA1c was higher in diabetic rats when compared with non-diabetic rats, which further confirmed hyperglycemia in these diabetic rats. Dietary caloric restriction was significantly effective in lowering the concentrations of HbA1c in mild diabetic rats. This again confirms the selective effectiveness of caloric restriction on type 2 rather than type 1 diabetic rats.

Caloric restriction was found to significantly reduce the weight of mild diabetic rats when compared with those feeding ad libitum. This suggests that
Caloric restriction may be a viable strategy for the overall control of type 2 diabetes. Reductions in body weights observed in severe diabetic rats feeding ad libitum and under caloric restriction might not be due to caloric restriction and therefore, there could have been other factors responsible for this.

Oxidative stress, which results from an imbalance in the oxidant/antioxidant system favoring the former, is implicated in diabetes [27]. This is thought to be due to either overproduction of oxidants or a decrease in antioxidant defenses, resulting in an irreparable oxidative damage to proteins, lipids and DNA overtime. Caloric restriction has been consistently found to increase life span of experimental animals [7,8] and this has been attributed to decreased mitochondrial respiration [28] and resultant decrease in free radical production. Thus, superoxide dismutase (scavenges superoxide anions), catalase (detoxifies H$_2$O$_2$) and

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Fig. 3. Effects of dietary caloric restriction on malondialdehyde (panel a), HDL-cholesterol (panel b) and triglyceride (panel c) concentrations in non-diabetic, mild and severe streptozotocin-induced diabetic rats. Values are mean ± SEM; $n=6$. *$p<0.001$ as compared to mild diabetic rats with caloric restriction.
GPx (removes H₂O₂ and lipid peroxides) are considered primary antioxidant enzymes since they are involved in direct elimination of these reactive oxygen species. From our results, caloric restriction does not have any significant effects on the activities of SOD and catalase in the plasma of non-diabetic, mild and severe diabetic animals. From our previous studies [29], we have observed similar trends in both activities in the kidneys of STZ-induced diabetic rats. Several studies found no differences in SOD [29–31] and catalase activities between diabetic and non-diabetic controls. Other studies reported decreases in the activities of SOD [4,6,32] and catalase [33,34] while others found increased activities of SOD [35,36] and catalase [4,29,30,32] in diabetic patients. These apparently contradictory results could be due to tissue-specificity, variation in severity, duration and treatment of the disease [37] or other experimental conditions. The results obtained for catalase might be due to its generally high concentration and its interaction with GPx, which shares a common substrate.

Like the SOD and catalase activities, some workers have found increases [29] and decreases [32,38] in glutathione peroxidase activity in diabetes. Our results showed a decrease in glutathione peroxidase activity in diabetic rats when compared to non-diabetic rats. Though, caloric restriction neither increased GPx activities nor reduced catalase activities significantly in mild diabetic rats, an oscillatory effect was observed between these enzymes. This may suggest that caloric restriction shifts the balance in the generation of ROS and its antioxidant defense systems [27].

Several workers [29,30,33,36] have reported increased levels of lipid peroxidation in diabetes. Increased lipid peroxidation suggests an increase in reactive oxygen species [29], which could be due to increased glucose concentration [39,40]. In this study, lipid peroxidation was evaluated by measuring the plasma concentrations of MDA. Caloric restriction did not significantly reduce the MDA concentrations in non-diabetic rats and severe diabetic rats. The exact mechanism by which increased blood glucose concentrations lead to lipid peroxidation in plasma and erythrocytes of diabetic patients is not known. However, in vitro studies suggest that enolization of glucose under physiological conditions results in reduction of molecular oxygen and production of oxygen free radicals and α-ketoaldehydes, which ultimately cause peroxidative breakdown of phospholipids in erythrocyte membranes and accumulation of malondialdehyde, a terminal compound of lipid peroxidation [31].

Lipid profile, which is altered in the diabetic plasma [30,41], appears to contribute significantly to the development of premature atherosclerosis and includes an increase in triglyceride and total cholesterol concentrations or decrease in HDL-cholesterol concentrations. Our results showed no differences in plasma HDL-cholesterol in non-diabetic rats fed ad libitum and those under caloric restriction. However, caloric restriction was found to improve the concentrations in mild and severe diabetic rats. Such improvements were also obtained in diabetic patients fed with very low calorie diet [11].

Caloric restriction was found to significantly reduce the triglyceride concentrations in mild diabetic rats and had no significant effect on severe diabetic rats or non-diabetic rats. Therefore caloric restriction can be effectively used to reduce triglyceride concentrations in type 2 diabetes.

In conclusion, caloric restriction had significantly improved effects on body weight, glucose, glycated hemoglobin and triglyceride concentrations and non-significant effects on GPx activity, MDA and HDL-cholesterol concentrations in mild diabetic rats during our 9-week study. These results suggest that increased lipid peroxidation and reduced antioxidant status in mild diabetic rats feeding ad libitum are substantially improved by dietary caloric restriction. This may represent ways in which caloric restriction can contribute to the prevention of diabetic complications in type 2 diabetes. However, caloric restriction had no sustained effects on severe (type 1) diabetic rats probably due to the etiology of the disease. Therefore, the effectiveness of caloric restriction might depend on the duration of treatment and the type of diabetes.

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