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Original Article

Effects of long-term carbohydrate restriction on oral glucose tolerance in spontaneously diabetic Goto-Kakizaki rats

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ABSTRACT

In recent years, a carbohydrate-restricted diet (very low-carbohydrate diet) has been recognized as effective for preventing and alleviating lifestyle-related diseases such as diabetes. The antidiabetic effects of a carbohydrate-restricted diet are based on suppressing postprandial hyperglycemia, leading to glycation and oxidative stress, risk factors for cardiovascular diseases. On the contrary, several studies have reported that carbohydrate-restricted diets reduce glucose tolerance. However, these studies have examined glucose tolerance after a relatively short duration of carbohydrate restriction of several weeks to several months. Dietary therapy for diabetes is long-term, lasting for years and even for the rest of the life. Therefore, the effects of carbohydrate restriction on glucose tolerance over a longer period must be investigated. In this study, we investigated the effect of long-term moderate and severe carbohydrate-restricted diets on glucose tolerance using Goto-Kakizaki rats, an insulin-deficient diabetic model. The results reconfirmed that early carbohydrate restriction worsens glucose tolerance. Long-term carbohydrate restriction appears to improve glucose tolerance; however, this may be more influenced by aging. Considering the enormous age-related weight loss and the high number of rat deaths in the severe carbohydrate-restricted group, severe long-term carbohydrate restriction should be carefully carried out.

KEYWORDS: carbohydrate restriction, long-term, high-protein diet, glucose tolerance, GK rats.

INTRODUCTION

According to the International Diabetes Federation, 463 million people worldwide had diabetes in 2019, which is higher than the estimated 382 million people in 2013 and 108 million in 1980 [1-3]. The number of patients is rapidly increasing and is projected to reach 578 million by 2030 and 700 million by 2045. Type 2 diabetes accounts for approximately 90% of the cases [1]. This type is common among middle-aged and older people and is a preventable lifestyle-related disease [4, 5]; therefore, a healthy and balanced diet, moderate exercise, proper weight control, and smoking cessation are effective in preventing and alleviating type 2 diabetes [6]. The World Health Organization estimates that diabetes caused 1.5 million deaths in 2012, making it the eighth leading cause of death [3]. However, an additional 2.2 million deaths worldwide were attributed to increased risks of hyperglycemia, cardiovascular disease, renal failure, and other related complications [3, 7], and these conditions often lead to early death [8].

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In recent years, a carbohydrate-restricted diet (very low-carbohydrate diet) has been recognized as an effective diet for preventing and alleviating lifestyle-related diseases such as diabetes [9]. This diet restricts carbohydrate consumption compared with the average diet. Therefore, carbohydrate-rich foods (including sugar, bread, and pasta) are restricted and replaced with foods high in fat and protein (meat, poultry, fish, shellfish, eggs, cheese, nuts, seeds, etc.) [10]. The antidiabetic effects of a carbohydrate-restricted diet are based on the suppression of postprandial hyperglycemia leading to glycation and oxidative stress, which are risk factors for developing cardiovascular disease [11, 12]. Carbohydrate restriction suppresses postprandial hyperglycemia because carbohydrates are the only nutrients that strongly increase the postprandial blood glucose concentration in type 1 diabetes [12, 13]. In 2013, the American Diabetes Association recommended a carbohydrate-restricted diet as the first option for diabetes treatment [14].

However, several studies have reported that carbohydrate-restricted diets reduce glucose tolerance [15-19]. These studies have examined glucose tolerance after a relatively short duration of carbohydrate restriction of several weeks to several months. Diet therapy for diabetes is long-term, lasting for years and even for the rest of the life. Therefore, the effects of carbohydrate restriction on glucose tolerance over a longer period must be investigated. The ability of Japanese people to secrete insulin is lower than that of Europeans and Americans. Even patients with mild obesity may not cope with the increased need for insulin due to insulin resistance, leading to diabetes [20]. This study investigated the effect of long-term moderate and severe carbohydrate-restricted diets on glucose tolerance using Goto-Kakizaki (GK) rats, an insulin-deficient diabetic model [21].

MATERIALS AND METHODS

All animal procedures were approved by the Animal Care and Use Committee of Kagawa University (approval number: 14030).

Animals and diets

Forty-eight male GK rats (5-week-old) were obtained from Japan SLC (Shizuoka, Japan) and they were individually caged at 22 ± 1 °C, with light from 08:00 to 20:00. They were fed MF, a commercial rodent diet (Oriental Yeast Co., Ltd., Tokyo, Japan), and had access to water *ad libitum* until they were six weeks old. Then, rats were randomized into three groups of 16 rats with equal mean body weight. The composition of the experimental diets is shown in Table 1. Control (CO), moderate carbohydrate-restricted (MCR), and severe carbohydrate-restricted (SCR) diets contained 55.0, 23.3, and 13.0% energy of carbohydrates out of the total dietary energy, respectively (Table 1). Soy protein provided by J-Oil Mills, Inc. (Kanagawa, Japan) was used as the protein source for the experimental diets. The vitamin and mineral mixtures were purchased from Oriental Yeast Co. Ltd. (Tokyo, Japan).

Experimental design

Three groups of rats were given experimental diets (Table 1) with free access to water for 20 months. Body weights and food intake were recorded every 2 days. After 4, 8, 12, and 16 months of the feeding period, oral glucose tolerance tests (OGTTs) were performed on 6-8 rats randomly selected per group. Rats were weighed after 12 h of fasting. Subsequently, a 2 g/kg body weight of glucose was administered orally through a gavage needle using a freshly prepared 50% glucose solution. Blood samples were obtained from the tail vein using a capillary tube at baseline (0) and 30, 60, 90, and 120 min after glucose administration.

Plasma analyses

Plasma glucose and insulin concentrations were determined using various kits (Glucose CII-test, LBIS Rat Insulin ELISA Kit, FUJIFILM Wako Chemicals, Osaka, Japan).

Data analysis

All statistical analyses were performed with Bell Curve for Excel (SSRI, Tokyo, Japan). Data were expressed as the mean ± SE. Data were analyzed by the one-way analysis of variance (ANOVA) and the Tukey-Kramer test. Statistical significance was established at $p < 0.05$.

RESULTS AND DISCUSSION

Body weight and energy intake

Body weight increased rapidly in approximately 4 months after the initiation of the test diet

Table 1. Composition of experimental diets.

	CO	MCR	SCR
Ingredients (g/kg)			
Soy protein	280.0	365.0	550.0
DL-Methionine	3.0	3.0	3.0
Corn starch	519.9	237.9	59.6
Soybean oil	50.0	50.0	50.0
Beef tallow	50.0	250.0	250.0
Mineral mixture[1]	35.0	35.0	35.0
Vitamin mixture[1]	10.0	10.0	10.0
Cellulose	50.0	47.0	40.3
Chorine chloride	2.0	2.0	2.0
Butylhydroxytoluene	0.1	0.1	0.1
Total	1000.0	1000.0	1000.0
Nutritional composition (g/kg)			
Carbohydrate	509.1	279.8	156.7
Fat	100.1	299.8	299.9
Protein	190.4	247.3	371.2
Fiber	60.1	60.1	60.1
Energy ratio (%)			
Carbohydrate	55.0	23.3	13.0
Fat	24.4	56.1	56.1
Protein	20.6	20.6	30.9
Energy (kcal/g)[2]	3.70	4.81	4.81

[1]Based on the AIN-76 mixture. [2]Carbohydrate, fat, and protein provide energy at 4, 9, and 4 kcal/g, respectively. CO, MCR, and SCR are abbreviations for the control, moderate carbohydrate-restricted, and severe carbohydrate-restricted diets.

ingestion, reaching a peak at 16 months, and then decreased (Figure 1). No significant differences in body weight were observed between the three groups after 4, 8, and 12 months of feeding. At 20 months, body weight was significantly lower in the SCR group than in the MCR group. No significant differences in energy intake were found between the three groups throughout the experimental period (Figure 1). Increasing the percentage of fat weight in the experimental diets increased the energy per gram; however, the energy intake was approximately the same. Many similar results have already been reported [22]. During the experimental period, 2 of 16 rats in both the CO and MCR groups died, and 5 of 16 rats in the SCR group died. The death rate was higher in the SCR group than in the other groups, and the rats weighed less at 20 months. Several studies have reported the adverse effects of the long-term intake of carbohydrate-restricted diets. Wu *et al*. [23] reported that the survival rate of senescence-accelerated prone mice (SAM)

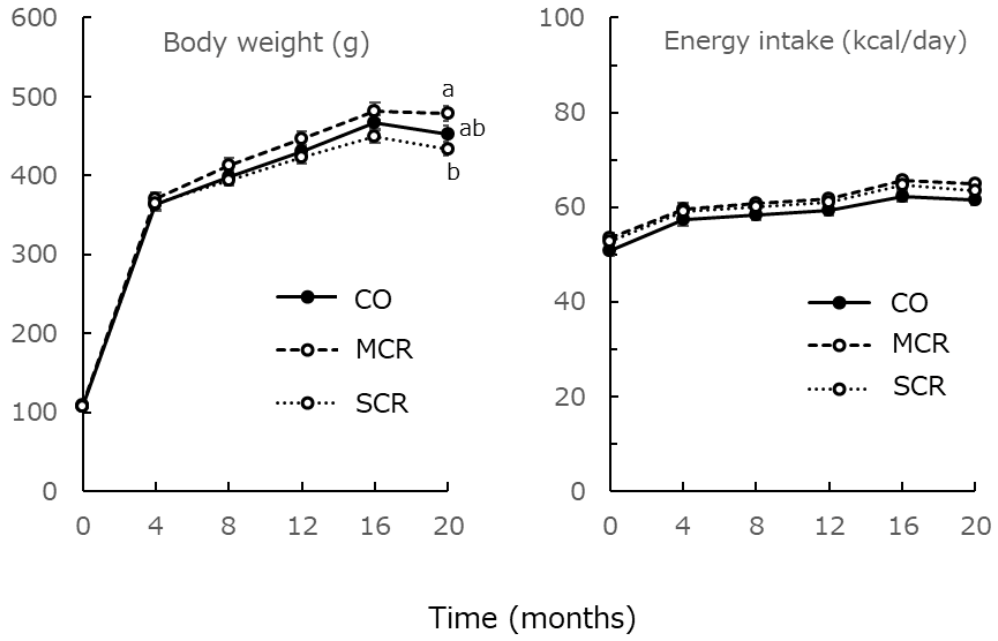

Figure 1. Body weight and energy intake in rats fed control (CO), moderate carbohydrate-restricted (MCR), and severe carbohydrate-restricted (SCR) diets. Data are presented as the means ± SE for 16 rats. The superscripts indicate statistical differences between values ($p < 0.05$).

decreased with a long-term carbohydrate-restricted diet. They suggested that the activation of the mammalian target of rapamycin (mTOR) is responsible for the accelerated aging in SAM. Solon-Biet *et al*. [24] reported that a high-protein/low-carbohydrate diet activates the mTOR and shortens lifespan in mice. A high-protein diet increases blood levels of leucine, which strongly activates mTOR [25]. Clinically, a few studies have highlighted concerns about long-term carbohydrate-restricted diet intake [26-28]. A meta-analysis of the effects of low-carbohydrate diets on mortality and cardiovascular disease found that low-carbohydrate diets increased overall mortality [29]. A low-carbohydrate diet will inevitably lead to increased protein intake. A study that followed 62,582 men and women for up to 17.8 years and investigated their association with cancer incidence found that animal protein intake was associated with airway cancer, and saturated fatty acid intake with colorectal cancer [30]. A cohort study showed an increased risk of death when the carbohydrate energy intake was < 40% or > 70%. The safety of long-term carbohydrate restriction may need further investigation.

Plasma glucose and insulin concentrations after oral glucose administration in each rat group

During the feeding period, the plasma glucose levels in the three groups of rats after OGTT increased rapidly after administration, peaking at 60-90 min (Table 2). Even 120 min after administration, the plasma glucose levels of rats were maintained at approximately 1.5-2.0 times the fasting levels. On the contrary, the plasma insulin level after OGTT increased moderately, and the longer the feeding period, the slower the insulin response to glucose administration (Table 3). These results are characteristic of GK rats with poor insulin secretory capacity and have been previously reported [31].

Four months after feeding, plasma glucose levels 30 min after glucose administration were higher in the carbohydrate restriction groups, which was significantly higher in the MCR group than in the CO group (Table 2). However, no significant differences between groups were found in plasma glucose levels at other time points or in the area under the curve (AUC) values (Table 2). Conversely, plasma insulin concentrations 60-120 min after

Table 2. Plasma glucose concentrations (mg/dL) after oral glucose administration in each group of rats.

Groups	Time after administration (min)					AUC (mg/dLxh)
	0	30	60	90	120	
4 (months)						
CO	150.9 ± 2.7	229.7 ± 10.9[b]	325.3 ± 23.3	294.7 ± 14.6	275.0 ± 11.2	493.6 ± 23.7
MCR	167.5 ± 12.4	283.1 ± 13.5[a]	353.5 ± 10.9	344.7 ± 11.3	308.2 ± 22.4	567.6 ± 19.3
SCR	158.6 ± 4.1	253.7 ± 12.5[ab]	246.2 ± 28.0	300.7 ± 12.6	304.5 ± 17.2	526.4 ± 20.3
8 (months)						
CO	139.8 ± 2.5	245.3 ± 11.5	283.1 ± 11.3	306.6 ± 14.4	227.4 ± 14.8	474.3 ± 17.7
HF	136.8 ± 5.1	263.9 ± 10.8	298.6 ± 25.9	309.2 ± 23.0	264.8 ± 29.7	502.0 ± 37.9
LC	138.3 ± 4.7	248.3 ± 10.5	272.8 ± 13.3	297.5 ± 11.7	255.0 ± 30.0	473.0 ± 20.3
12 (months)						
CO	148.7 ± 7.1	247.9 ± 18.2	261.7 ± 14.8	300.6 ± 12.6	269.9 ± 14.5	472.5 ± 19.6
MCR	135.5 ± 4.1	240.9 ± 3.9	251.9 ± 6.8	276.9 ± 11.0	253.8 ± 6.6	448.3 ± 10.1
SCR	144.9 ± 4.7	238.2 ± 11.9	276.9 ± 15.1	288.0 ± 15.3	248.1 ± 16.6	463.6 ± 21.7
16 (months)						
CO	145.0 ± 5.9	271.7 ± 10.6	301.7 ± 12.9[a]	289.3 ± 11.6	279.3 ± 11.5[a]	501.2 ± 16.4[a]
MCR	141.4 ± 3.9	241.2 ± 13.3	272.4 ± 11.6[ab]	279.3 ± 11.5	141.4 ± 3.9[b]	444.8 ± 17.9[b]
SCR	139.2 ± 2.6	258.6 ± 16.7	258.6 ± 11.5[b]	247.4 ± 16.4	139.2 ± 2.6[b]	429.0 ± 14.3[b]

Data are the means ± SE for 6-8 rats. Superscripts indicate statistical differences between values ($p < 0.05$).
CO, MCR, and SCR are abbreviations for the control, moderate carbohydrate-restricted, and severe carbohydrate-restricted groups.

Table 3. Plasma insulin concentrations (ng/mL) after oral glucose administration in each group of rats.

Groups	Time after administration (min)					AUC (ng/mLxh)
	0	30	60	90	120	
4 (months)						
CO	2.50 ± 0.34	3.52 ± 0.45	3.37 ± 0.30^{a}	1.78 ± 0.19^{b}	4.23 ± 0.46^{a}	5.39 ± 0.31
MCR	2.96 ± 0.20	3.47 ± 0.45	2.45 ± 0.57^{ab}	1.49 ± 0.34^{b}	2.45 ± 0.47^{b}	4.24 ± 0.67
SCR	2.72 ± 0.21	3.47 ± 0.50	1.71 ± 0.30^{b}	4.15 ± 0.48^{a}	3.75 ± 0.37^{ab}	4.86 ± 0.46
8 (months)						
CO	3.86 ± 0.34	5.29 ± 0.34	5.19 ± 0.29	4.74 ± 0.32	4.52 ± 0.19^{a}	8.74 ± 0.31
MCR	3.85 ± 0.31	5.19 ± 0.56	5.17 ± 0.30	4.88 ± 0.43	4.24 ± 0.19^{a}	8.69 ± 0.62
SCR	3.71 ± 0.28	4.31 ± 0.36	4.31 ± 0.36	4.46 ± 0.28	3.38 ± 0.24^{b}	7.60 ± 0.61
12 (months)						
CO	4.17 ± 0.25	4.52 ± 0.19^{b}	4.79 ± 0.15	4.66 ± 0.28	4.66 ± 0.46	8.15 ± 0.28
MCR	4.21 ± 0.29	5.27 ± 0.25^{a}	5.11 ± 0.14	5.15 ± 0.30	5.29 ± 0.36	9.09 ± 0.29
SCR	4.00 ± 0.29	4.59 ± 0.25^{b}	4.82 ± 0.21	4.85 ± 0.39	3.92 ± 0.58	7.80 ± 0.64
16 (months)						
CO	5.21 ± 0.23	5.56 ± 0.44^{b}	5.68 ± 0.57	5.42 ± 0.41	5.40 ± 0.47	9.67 ± 0.67
MCR	5.32 ± 0.29	6.78 ± 0.93^{a}	6.21 ± 0.46	5.72 ± 0.36	5.72 ± 0.36	10.93 ± 0.96
SCR	5.68 ± 0.57	4.76 ± 0.44^{b}	5.44 ± 0.24	5.32 ± 0.13	5.09 ± 0.18	9.03 ± 0.47

Data are the means ± SE for 6-8 rats. Superscripts indicate statistical differences between values ($p < 0.05$).
CO, MCR, and SCR are abbreviations for the control, moderate carbohydrate-restricted, and severe carbohydrate-restricted groups.

administration did not stabilize and did not show a constant trend (Table 3). Plasma insulin levels 60 min after administration were significantly lower in the SCR group than in the CO group, and 120 min after administration was significantly lower in the MCR group than in the CO group (Table 3). At 8 and 12 months after feeding, no significant differences were found between the groups in terms of plasma glucose and insulin levels after OGTT, except for plasma insulin level 120 min after administration at 8 months (Tables 2 and 3). Plasma insulin levels 120 min after administration were significantly lower in the SCR than in the CO and MCR groups (Table 3). At 16 months after feeding, plasma glucose concentrations 60 and 120 min after OGTT were lower in the carbohydrate-restricted groups than in the CO group, which was surprisingly the opposite of the results after 4 months of feeding (Table 2). Furthermore, the AUC values were significantly lower in the MCR and SCR groups than in the CO group (Table 2). Plasma insulin levels 30 min after administration were significantly higher in the MCR group than in the CO and SCR groups (Table 3). However, no significant differences between the groups were found in plasma insulin levels at other time points or in AUC values (Table 3).

Several studies have reported that carbohydrate-restricted diets reduce glucose tolerance [15-19]. After 4 months of feeding the results of the present study support the findings of these previous studies. However, no adverse effect of carbohydrate restriction was observed in the OGTT after 8 and 12 months of feeding. The cause of this result is unknown. Moreover, regarding the OGTT results after 16 months of feeding, it is doubtful whether this was an improvement effect of severe carbohydrate restriction, partly because of a tendency to lose weight. Age-related frailty causes hypoglycemia [32]. After 20 months of feeding, the body weight of the SCR group was lower than that of the other groups, suggesting that long-term severe carbohydrate restriction could promote frailty.

CONCLUSION

The results reconfirmed that early carbohydrate restriction worsens glucose tolerance. Long-term carbohydrate restriction appears to improve glucose tolerance; however, this may be more influenced by aging. Considering the enormous age-related weight loss and the high number of rat deaths in the severe carbohydrate-restricted group, severe long-term carbohydrate restriction should be carefully carried out. When implementing long-term carbohydrate restriction, adopting a moderate carbohydrate-restricted diet would be better.

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CONFLICT OF INTEREST STATEMENT

The authors declare no competing or financial interests.

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