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Effects of a Very Low Carbohydrate (Pork Rind-Based) Diet on Weight Gain, Serum Levels of Cholesterol, Triacylglycerol, Glucose, Ketone Bodies and Insulin and Body Composition in Adult Rats

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A study was carried out to determine the effect of a very low-carbohydrate diet on weight gain, body composition, and serum levels of cholesterol, triacylglycerol, glucose, ketone bodies and insulin. Twenty rats (mean initial weight, 212 g) were divided into two groups and each group was assigned a conventional high-carbohydrate diet (control) or a very low-carbohydrate diet containing 59.8% ground pork rind snack and fed the diet for four weeks. Average daily body weight gain was not different between the two groups during the first two weeks, but was significantly lower in rats fed the very low-carbohydrate diet than in those in the control group during third ($p<0.05$) and fourth weeks ($p<0.01$). Feed intake as well as energy intake was lower in rats fed the very low-carbohydrate diet than in those in the control group. The very low-carbohydrate diet reduced ($p<0.01$) serum triacylglycerol (34±3 vs 82±8 mg/100 mL) and insulin (3.90±0.53 vs 7.60±0.61 μIU/mL) levels, while increasing ($p<0.01$) ketone body level (368±25 vs 236±24 μmol/L), compared with the control. Serum glucose and total cholesterol levels were not different ($p>0.05$) between the two dietary treatments. Proximate analysis of carcasses showed that the very low-carbohydrate diet decreased ($p<0.01$) body fat (26.1±1.04 vs 30.5±0.86%), while increasing ($p<0.01$) body protein (63.1±0.94 vs 59.4±0.70%) contents. Results indicate that short-term feeding of a very low-carbohydrate diet is beneficial for alleviating risk factors known to involve cardiovascular diseases or artherosclerosis. However, more studies with model animals as well as humans are recommended to examine the long-term health benefits of low-carbohydrate diets.

Key words : Rats, Low-carbohydrate diets, Ketone bodies, Lipids, Insulin resistance

INTRODUCTION

Low-carbohydrate diets gained much attention after the publication in 1992 of cardiologist Robert Atkins' "New Diet Evolution",[1)] a New York Times bestseller for five years, but their long-term effects and safety remain unknown.[2-4)] A success story on the use of low-carbohydrate diets in reducing body weight was reported by Banting,[5)] who found that a 66-year-old, 100 kg man lost more than 20 kg in a year without feeling hungry while on a low-carbohydrate diet. He reported that the effects appeared within a week. Supporters of low-carbohydrate diets claim that diets low in carbohydrates but high in protein increase the metabolic rate in the adipose tissues without deleterious effects because of a lack of available glucose in the tissues, resulting in reduced body weight.[6)] In contrast, scientific societies including the American Dietetic Association and the American Heart Association oppose the use of low-carbohydrate diets,[2,7-9)] arguing that individuals on such diets are at risk of compromised vitamin and mineral intake, as well as potential cardiac, renal, bone, and liver abnormalities.[2)]

Most studies done on the effect of low-carbohydrate diets on risk factors of artherosclerosis were carried out with small number of patients and the findings are variable.[10-13)] Layman et al.[14,15)] indicated that diets with a low ratio of carbohydrate to protein (a low-carbohydrate diet) had positive effects on body composition, blood lipid level, blood glucose clearance, satiety and postprandial insulin response in women under weight control. More recently Pelkman et al.[16)] reported that a moderate-fat weight loss diet improved the cardiovascular disease risk profile in overweight and obese human subjects compared to a low-fat weight loss diet.

The low-carbohydrate, high-protein, high-fat diet (Atkins diet) has not been critically examined and most human studies have not been conducted under controlled environments but rather with freely moving individuals. A recent review paper[3)] indicated that insufficient data are available to date to accept or reject the use of low-carbohydrate diets.[3)] We assessed the effect of feeding a very low-carbohydrate diet containing high levels of protein and fat (as compared with a conventional high-carbohydrate diet) on weight gain, body composition and

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serum levels of lipids, glucose, ketone bodies and insulin in adult rats raised in controlled environments (diet, temperature, light, limited movement, etc.).

MATERIALS AND METHODS

1. Animals and Diets

Twenty male Sprague Dawley rats (Daehan Biolink, Eumsung, Korea) were individually housed in suspended wire cages in a room maintained at 23 ℃ with a 12-h light (0700 to 1900) and 12-h dark (1900 to 0700) cycle. After five-day adaptation to the experimental conditions, 20 rats (mean weight, 212 g) were blocked by weight and divided into two groups, and each group was assigned a conventional high-carbohydrate diet (control) or a very low-carbohydrate diet containing 59.8% ground pork rind snack (KBF, Inc., Munsan Korea) (Table 1)

Table 1. Composition of diets

Ingredient	Control[1)]	Low carb[2)]
Casein (vit-free)	20	20
L-Methionine	0.3	0.3
Corn starch	54.7	
Sucrose	10	
Corn oil	10	10
Pork rind[3)]		59.8
Vitamin mix[4)]	1.0	1.0
Mineral mix[4)]	3.5	3.5
Choline Cl	0.5	0.5
Total	100	100

1) Analyzed values (%) for control and very low-carbohydrate (low-carb) diets: crude protein 18.3 and 42.9, ether extract 10.3 and 30.2, ash 2.3 and 3.8, NFE 58 and 6.6, crude fiber 0.3 and 10.9, moisture10.9 and 5.6, respectively.
2) Low-carbohydrate diet.
3) Ground pork rind snack was prepared with fat-removed pork rind toasted in corn oil (KBF, Inc., Munsan, Korea). Analyzed composition was 43.7, 52.0, 1.7 and 5.1% for crude protein, ether extract, ash and moisture, respectively.
4) *AIN 93 mix*[28)], Halan Teklad, Madison, WI, USA.

and fed the diet for four weeks. Dietary fat and carbohydrate provided approximately 23% and 58%, and 58% and 6% of the daily energy intake for the control and rats fed the very low-carbohydrate diet, respectively. Animals had free access to diet and water throughout the experimental period. Feed consumption and body weight were recorded every two days during the four-week period. At the end of the four-week feeding period, rats were sacrificed after a 12-hour fast and blood samples were collected and centrifuged to collect sera.

2. Analysis of Cholesterol, Triacylglycerol, Glucose, Ketone Bodies and Insulin in the Serum

Total cholesterol, triacylglycerol and glucose concentrations in serum were determined using their respective assay kits (SICDIAL kits, Shin-Yang Chemical, Seoul, Korea) and an autoanalyzer (TBA200FR, Toshiba, Tokyo, Japan). Total ketone body was the sum of acetoacetate and β-hydroxybutyrate, the concentrations of which were determined using a commercial assay kit (Kainos, Tokyo, Japan) and autoanalyzer (JCA-BM1250, Jeol, Tokyo, Japan). Insulin concentration was determined using a radioimmunoassay kit (Diagnostic Products Corp., Los Angeles, CA) and a γ-counter (COBRA II, Packard, Meridan, MD).

3. Proximate Analysis of Feed and Carcass Samples

Carcass including emptied GI tract, skin and hair was cut into small pieces and dried in an air-forced drying oven at 80 ℃ for 24 hours. Dried carcass was frozen in liquid nitrogen and ground in a blender (model #852-28, Sunbeam, Inc., Boca Raton, Florida, USA). Representative samples were taken from the ground carcass and used for proximate analysis. Crude protein, crude fat, crude fiber and ash contents in feed and carcass were analyzed using Kjeldahl (model #1030, Foss Tecator, Hoganas, Sweden), Soxtec (#2050), Fibertec (#1017) systems and an electric muffle furnace (HY8000, Dae-Han, Inc., Seoul, Korea), respectively, on the basis of AOAC.[17)]

4. Statistical Analysis

The student t-test was used to compare means of two dietary treatments.[18)]

RESULTS

Average daily gain was not different between the two dietary treatments during the first two weeks, but it decreased in rats fed the very low-carbohydrate diet compared to those in the control group during the third

Table 2. Effect of feeding a very low carbohydrate diet on average daily gain, feed intake and gain/feed ratio in rats[1)]

Item	Control	Low-carb	P value
Initial body weight, g	212±3	212± 2	
Final body weight, g	365±3	354±8	
Average daily gain, g			
1st wk	8.6±0.23	9.1±0.33	0.2680
2nd wk	5.8±0.20	5.5±0.34	0.4760
3rd wk	4.0±0.25	3.2±0.24	0.0351
4th wk	3.5±0.13	2.8±0.20	0.0083
Overall	5.5±0.14	5.0±0.25	0.1674
Average daily feed intake, g	19.4±0.2	15.9±0.4	0.0001
Daily energy intake,[2)] kcal	77.3±0.9	74.6±2.1	0.0195
Gain/Feed, g/g	0.28±0.005	0.32±0.008	0.0020
Gain/energy intake, g/kcal	0.070±0.001	0.067±0.002	0.1257

1) Values are means ± SE of 10 rats.
2) Energy intake was calculated by multiplying protein, fat and nitrogen-free extract intake by 4, 9 and 4 kcal/g, respectively.

Table 3. Effects of feeding a very low-carbohydrate diet on serum cholesterol, triacylglycerol, glucose, insulin and ketone body levels in rats[1)]

Item	Control	Low-carb	P value
Total cholesterol, mg/100 mL	122±4	115±6	0.3412
Triacylglycerol, mg/100 mL	82±8	34±3	0.0001
Glucose, mg/100 mL	145±2	146±20	0.7515
Insulin, μIU/mL	7.6±0.61	3.9±0.53	0.0003
Total ketone body, μmol/L	236±24	368±25	0.0014
Acetoacetic acid, μmol/L	41±5	55±5	0.0607
β-(OH) butyric acid, μmol/L	195±22	313±21	0.0012

1) Values are means±SE of 10 rats.

($p<0.05$) and fourth week ($p<0.01$). Energy intake (74.6 ± 2.1 vs 77.3 ± 0.9 kcal/day) as well as feed intake (15.9 ± 0.4 vs 19.4 ± 0.2 g/day) was lower in rats fed the very low-carbohydrate diet compared to those in the control group (Table 2).

Serum triacylglycerol level was much lower ($p<0.01$) in rats fed the very low-carbohydrate diet (33.9 ± 3.1 vs 82.0 ± 7.6 mg/mL), whereas serum cholesterol or glucose level was not influenced by diet (Table 3). Serum level of ketone bodies (368 ± 25 vs 236 ± 24 μmol/L) was higher ($p<0.01$) and serum insulin level (3.9 ± 0.5 vs 7.6 ± 0.6 μIU/mL) was lower ($p<0.01$) in rats fed the very low-carbohydrate diet than those in the control group. The very low-carbohydrate diet decreased ($p<0.01$) body fat (26.1 ± 1.0 vs 30.5 ± 0.9%) while increasing ($p<0.01$) body protein content (63.1 ± 0.9 vs 59.4 ± 0.7%) (Table 4).

Table 4. Effects of feeding a very low-carbohydrate diet on body composition in rats[1)] (% on a dry matter basis)

Item	Control	Low-carb	P value
Crude protein	59.4±0.70	63.1±0.94	0.0082
Crude fat	30.5±0.86	26.1±1.04	0.0077
Crude ash	10.7±0.34	11.2±0.28	0.2172

1) Values are means±SE of 10 rats.

DISCUSSION

Our study clearly demonstrated that short-term feeding of a very low-carbohydrate diet reduced serum lipid level and body fat deposition in adult rats and thus may be beneficial for preventing cardiovascular diseases or artherosclerosis. Although our very low-carbohydrate diet contained a rather high level of protein (43%) and fat (30%) and these two nutrients provided approximately 92% of the daily energy intake, we found no deleterious effect of the diet on growth and health. The decreased weight gain in rats fed the very low-carbohydrate diet compared to those in the control group appeared to be due to decreased energy intake because the ratio of weight gain to energy intake was the same for both groups (Table 2).

Similar results to ours have been reported in many recent studies. Feeding a high-fat (low-carbohydrate) diet was more efficient for weight control and for reducing serum triacylglycerol level in obese patients over a six-month period than a low-fat (high-carbohydrate) diet.[4)] Foster et al.[19)] reported that a low-carbohydrate, high-fat diet reduced body weight in obese patients more efficiently than a high-carbohydrate, low-fat diet during a three to six-month period, but the difference became insignificant over a one-year period. These two studies found no difference in serum cholesterol, but the latter found that HDL-cholesterol level increased with the low-carbohydrate diet compared to the high-carbohydrate diet. Hays et al.[13)] also found that a high-saturated fat, no-starch diet fed to patients with atherosclerotic cardiovascular disease resulted in weight loss and reduced body fat percentage and serum levels of glucose, insulin and triacylglycerol after six weeks over a baseline, and further weight loss with a lipid-neutral effect persisted for up to 52 weeks.

Interestingly, fasting serum glucose level was not different between those in the two dietary groups, while insulin levels were lower in rats fed the very low-carbohydrate diet (Table 3). Glucose level was measured after a 12-hour fast and should have been much higher in a fed state in the control rats fed the high-carbohydrate (58% NFE) diet. Such diets as the one used in the present study, which are high in refined grains or sugars, produce a rapid increase in postprandial levels of blood glucose and evoke an equally intensive response of insulin that leads to a hypoglycemic period and in turn increase hunger.[20-22)] This hypoglycemic effect occurs about two hours after a meal.[21)]

As expected, fasting serum ketone body levels increased ($p<0.01$) with the very low-carbohydrate diet, compared to the control (368 ± 25 vs 236 ± 24 μmol/L). Because serum samples were taken 12 hours after the last feeding, serum ketone body levels may have been lower in a fed state.[23)] Urinary ketone body excretion was markedly higher in obese human subjects fed a low-carbohydrate, high-protein, high-fat diet (Atkins diet) than those fed the high-carbohydrate diet until 12 weeks after the initiation of feeding of the trial diets, but the differences became insignificant after 12 weeks.[19)] Increases in blood ketone body level suppress not only feed intake[6)] but also the efficiency of dietary energy use and thus possibly result in reduced weight gain. Increased blood ketone body levels may be a concern in terms of unintended ketosis that can be kept in check by monitoring urinary ketone body levels.[13)] In addition to increased ketone body production, dietary lipid itself increases satiety by slowing gastric emptying[24)] and

increasing blood-free fatty acid levels that reduce hunger.[25] Sharman et al.[26] indicated that feeding a ketogenic (very low-carbohydrate) diet favorably affected biomarkers for cardiovascular disease in normal-weight men, showing that the ketogenic diet decreased fasting serum triacylglycerol (-33%) and insulin (-34%) while increasing ß-hydroxybutyric acid (250%) after six-week feeding compared to the baseline levels.

Layman et al.[14,15] found that a diet with a low ratio of carbohydrate to protein (a low-carbohydrate diet) had positive effects on body composition, blood lipid level, blood glucose clearance, satiety and postprandial insulin response in women under weight control. More recently, Pelkman et al.[16] reported that a moderate-fat weight loss diet (fat providing 33% of energy) improved the cardiovascular disease risk profile (serum triacylglycerol level and total and non-HDL- to HDL-cholesterol ratio), compared to a low-fat weight loss diet (fat providing 18% of energy) over a six-week feeding period in overweight and obese human subjects. Unfortunately, Pelkman et al.[16] did not include a high-fat diet group in their study, which could have shown a more pronounced effect on serum lipids. (Note that fat in the very low-carbohydrate diet used in the present study provided 58% energy.)

In contrast to our findings and most published data, Axen et al.[27] suggested that in the absence of weight loss, a high-fat, low-carbohydrate diet not only may be ineffective in decreasing risk factors for cardiovascular disease and type 2 diabetes but may promote the development of disease in previously lower risk, non-obese individuals. They used 33% hydrogenated oil containing 17% trans fatty acids and 25% protein for the low-carbohydrate diet and powdered Purina 5001 for a control.[26] Although some differences exist among studies, our rat study along with most studies done with humans suggests that short-term feeding of low-carbohydrate diets alleviates various risk factors (blood lipid level, circulating insulin level and body fat deposition, etc.) associated with cardiovascular diseases or artherosclerosis and thus may have health benefits.

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