A Thesis

For The Degree of Master of Agriculture

Effect of feeding very low carbohydrate (horsemeat- or beef- based) diets and re-stricted feeding on weight gain, feed and energy efficiency, and serum levels of cholesterol, triacylglycerol, glucose, insulin and ketone bodies in adult rats

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요 약

이 연구는 체중증가, 사료효율, 혈청 내 콜레스테롤, 중성지방, 글루코스, 케톤체, 인슐린에 미치는 저탄수화물 사료와 제한급여 (무제한 급여량의 70%)의 영향을 측정하기 위해 수행되었다. 실험1과 실험2에서 실험쥐 (Sprague-Dawley rat) 각 40마리 (평균체중 238 g)와 30마리 (평균체중 251g)를 10마리씩 나누고 각각 4주와 26일 동안 사료를 급여하였다. 실험 1에서, 두 그룹의 쥐에게는 보통사료(AIN-93G), 다른 두 그룹의 쥐에게는 10% 전분과 53% 냉동 건조된 분말 말고기가 포함된 저탄수화물 사료 (LC)를 급여하였다. 두 그룹 중 하나를 무제한 급여하였고 다른 하나를 무제한 급여 그룹과 짝을 지어 무제한 급여 그룹의 섭취량과 대사체중에 기초하여 70%에 해당하는 양을 급여하였다. 실험 2에서는 세 그룹에 각각 대조구 (AIN-93G) 또는 10% 전분과 53% 냉동 건조된 분말 말고기 또는 쇠고기가 함유된 사료를 급여하였다. 실험 2에서 평균 일일 체중 증가가 급여기간 초기에 저탄수화물 사료가 급여된 쥐에서 보다 대조구 사료가 급여된 쥐에서 낮은 반면 실험 1에서는 두 사료 사이에 차이가 없었다. 실험 1에서 제한급여 그룹이 무제한 급여 그룹보다 체중감소가 더 컸다. 사료 섭취량은 두 실험 모두 저탄수화물 그룹보다 대조구 diet 그룹에서 높았지만 (P<0.01) 실험 1에서 에너지 섭취량은 두 diet 간에 차이가 없었다. 실험 1에서 저탄수화물그룹이 대조구 그룹보다 혈청 중성지방이 감소하였고 (P<0.01) 대조구 그룹에서 무제한 급여 그룹보다 제한 급여 그룹에서 혈청 중성지방, 글루코스, 인슐린 수준이 현저하게 감소하였다 (P<0.05). 혈청 LDL 콜레스테롤 수준은 제한급여 (실험1) 또는 저탄수화물 그룹(실험2)에서 증가하였다. 체조성은 diet간에 차이는 없었지만 제한급여에서 지방 양은 감소하면서 단백질 양은 증가하였다. 혈청 케톤체 수준은 실험 2에서 AIN-93G diet 그룹보다 LC diet 그룹에서 높았다. 실험 2에서 모든 측정치에서 소고기와 말고기 그룹간 차이가 없었다. 결과적으로 LC diet 섭취는 심혈관 질환이나 동맥경화에 관계되는 혈청 중성지방 수준 같은 질병요인을 완화시키는데 유익하고, 제한급여는 혈청 중성지방 수준을 현저하게 감소시킨다는 것을 알아내었다. 그러나 앞으로 인간과 모델 동물을 사용하여 저탄수화물 식이

의 장기간 섭취가 건강에 어떤 영향을 미치는지 연구를 계속할 필요가 있다.

Ⅰ. INTRODUCTION

Low-carbohydrate diets (LC) have been used to improve multiple chronic health conditions including diseases, hyperlipidemia, artherosclerosis and for many years. The popularity of such diets have increased since the publication of books such as "Dr. Atkins' New Diet Revolution" (Atkins, 1992). Despite low-carbohydrate being increasingly employed benefits or adverse health consequences of long-term uses of LC are puzzling and not well understood (Crow, 2005).

Most studies have shown a positive effect of low-carbohydrate on risk factors of hyperlipidemia and artherosclerosis (Westman et al, 2002; Sondike et al, 2002). Layman et al. (2003) reported that diets with a reduced ratio of dietary carbohydrate to protein (a low-carbohydrate diet) had positive effects on body composition, blood lipid level, blood glucose clearance, postprandial insulin response in adult women under weight control. Volek et al. (2003) reported that a very low-carbohydrate diet improved postprandial lipemic responses, and serum levels of cholesterol and triacylglycerol compared with a low-fat diet in normal weight, normolipidemic women. Yancy et al. (2004) also reported that a low-carbohydrate, ketogenic diet improved blood lipid level compared a low-fat diet in obesity and hyperlipedemia.

Low-carbohydrate diets show low glycemic index (GI) that is an indicator of positive effects on adiposity, glucose homoeostasis and plasma lipids (Pawlak et al, 2004), and helps cardiovascular disease prevention (Brand-Miller, 2005). Reducing intake of carbohydrates and increasing consumption of fats and protein are thought to increase satiety, facilitate weight loss, and improve cardiovascular risk factors (Hu, 2005).

However, low-carbohydrate diets are low in fiber, folate, some minerals, some vitamins (Freedman et al. 2001; Stern et al. 2004; Brehm et al. 2003), and bring about a greater risk of being nutritionally inadequate as they recommend restriction of food choices (Crow, 2005). Because low-carbohydrate diets derive large proportions of calories from protein and fat, there has been considerable concern for their potentially detrimental impact on cardiovascular risk (Blackbern et al. 2001).

Restricted energy intake had positive effects on body composition and lipid concentration (Fransworth et al. 2003; Noakes et al. 2005). Sun et al. (1989) reported that restricted feeding tended to show higher insulin sensitivity compared to the ad libitum intake when they fed SD rats a medium fat-medium carbohydrate diet and epididymal adipose tissue culture for fatty acid synthesis with various insulin levels added.

This study was designed to evaluate the effect of feeding a very low-carbohydrate diet and restricted feeding on weight gain, body composition and serum levels of lipids, glucose, ketone bodies and insulin in adult rats raised in controlled environments (diet, temperature, light, limited movement, etc.)

I . LITERATURE REVIEW

Very low carbohydrate diet

Investigations reported over the past several years indicate that low-carbohydrate diets promote a greater degree of weight loss in the short term than does the conventional high-carbohydrate, low-fat diet (Sondike et al. 2003; Hu. 2005; Brehm et al, 2006). The short-term efficacy of low-carbohydrate diets has been demonstrated for some lipid parameters of cardiovascular risk and measures of glucose and insulin sensitivity (Volek et al. 2003; Sharman et al. 2002; Layman et al. 2003; Layman et al. 2003).

Westman et al. (2002) reported that a very low-carbohydrate diet program led to sustained weight loss during a 6-month period. Brehm et al. (2006) also reported that a very low- carbohydrate diet was effective for weight loss over a 6-month period in obese women. Moreover, Foster et al. (2003) reported that a low-carbohydrate, high-fat diet reduced body weight in 63 obese patients more efficiently than a high-carbohydrate, low-fat diet during a 3- to 6-month period, but the difference became insignificant over a one-year period. Similarly, in a study done with 132 obese adults, the difference in weight loss between subjects fed a low-carbohydrate diet and a conventional diet became insignificant over a one-year period (Stern et al. 2004). Many intervention studies have tested the hypothesis that low-glycemic index diets will improve not only glucose control but also lipid metabolism, and lowering daylong glycemia, with or without weight loss, may improve cardiovascular disease risk. (Brand-miller. 2005).

Volek et al. (2003) reported that a very low-carbohydrate diet significantly decreased fasting and postprandial triacylglycerol, increased HDL-cholesterol, and decreased the total cholesterol/HDL-C ratio in normal weight, normolipidemic women. Layman et al. (2003) found that a low-carbohydrate diet produced greater improvements in body composition with an increased ratio of fat/muscle loss. Further, these diets produced positive changes in blood lipid with reduction of triacylglycerol levels and the ratio of TAG/HDL-C. Yancy. (2004) reported that a low-carbohydrate, ketogenic diet led to greater weight loss, reduction in serum triacylglycerol level, and increased HDL cholesterol level compared with a low-fat diet.

Layman et al. (2003) found that a diet with increased protein and reduced levels of carbohydrate stabilizes blood glucose during nonabsorptive periods and reduces postprandial insulin response. Klaus. (2005) also reported that a high-protein, high-fat diet improved glucose homeostasis and delayed the development of adiposity in mice. Increasing dietary protein was effective in type-2 diabetes (Gannon et al. 2003). In addition, Sharman et al. (2002) showed that a low-carbohydarte diet decreased fasting serum triacylglycerol (-33%), insulin (-34%), while increasing HDL-C (11.5%) compared with a high-carbohydrate diet in normal-weight men. Pawlak et al. (2004) showed that a low-glycemic index had positive effect on body composition and plasma lipid and glucose homeostasis in an animal model, compared to a high-glycemic index. Seo et al. (2004) reported that a very low-carbohydrate diet had positive effect of weight loss, serum cholesterol, triacylglycerol, glucose, ketone bodies and insulin and body composition in adult rats.

In contrast, Axen et al. (2003) 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 also may promote the development of disease in previously lower risk, nonobese individuals. In addition, higher-fat diets make it more difficult to control energy intake (Bray et al. 2004). One study evaluating adherence to a weight-loss low-carbohydrate diet fed over 6 months found adverse effects: 63% reported bad breath, 51% headache, 10% hair loss, one women increased menstrual bleeding, although a low-carbohydrate diet program had positive effect on weight loss (Westman et al. 2002).

Restricted feeding

Restricted feeding exerts a variety of beneficial effects public health and may prolong life in both humans and animal (Eiam-ong and Sabatini 1999).

Restricted feeding diet was decreased weight than ad libitum intake of diet (Eiam-ong and Sabatini 1999; Blouet et al. 2006; Kolaja et al. 1996). Blouet et al. (2006) reported that restricted feeding diet (90% of ad libitum) had positive effect on fat deposition compared to ad libitum, when a normal-protein, high-carbohydrate diet was fed. Masoro et al. (1992) reported that plasma glucose and insulin concentrations in food-restricted rats were 15 and 50% lower, respectively, than in those with free access to food. Also, Eiam-ong and Sabatini (1999) reported that plasma glucose, cholesterol and blood urea nitrogen were 12, 9 and 11% lower, respectively, in food restricted rats (60% of ad libitum) than in rats with ad libitum intake rats. In addition, food restriction prevents renal membrane lipid depositon and decreases plasma cholesterol.

Consumption of a reduced energy diet for 4 mo reduced body weight, blood pressure and directly measured muscle sympathetic nerve activity in obese women (Andersson et al. 1991). Overton et al. (1997) also, reported that eight weeks of food restriction to 60% of ad libitum intake beginning at 5 wk of age attenuated the development of hypertension by ~20 mmHg in spontaneously hypertensive male rats.

Food restriction prolongs the mean and maximal life spans of rats and mice (Lane and Dickie. 1957; Ross. 1972; Matsuzaki et al. 2000). Lane and Dickie (1957) found that the mean life span of the obese animals with ad libitum intake was 457 days, 290 days less than the mean of 747 days of the thin non-obese controls and 338 days less than the mean of 795 days of the obese mice on restricted food intake. Matsuzaki et al. (2000) found that a food restriction diet is suppressed implanted tumor growth in mice and survival time was prolonged in these mice.

Ⅲ. MATERIALS AND METHOD

Exp. 1 Effect of feeding AIN-93G or a low carbohydrate diet and restricted feeding on weight gain, feed efficiency, body composition and serum levels of cholesterol, triacylglycerol, glucose and insulin in adult rat.

1. Animals and diets

Forty male Sprague-Dawley rats were individually housed in suspended wire cages in a room controlled for temperature (23±2℃) under a 12-h light:dark cycle (light on, 0800-2000 h). After one week adaptation to the experimental conditions, 40 rats (mean weight, 238g) were blocked by body weight and divided into four groups of 10 rats each. Each group was assigned to ; 1) ad libitum-AIN-93G 2) 70% ad libitum-AIN-93G 3) ad libitum-very low-carbohydrate (LC) 4) 70% ad libitum-very low-carbohydrate. Rats with ad lib intake and rats with 70% of the ad lib intake were pair-fed on the basis of metabolic body size ($wt^{0.75}$) of the previous day. Rats had free access to water the feeding period. Body weight and feed consumption were recorded everyday. The composition of the experimental diets is shown in Table 1. At the end of the four-week feeding period, rats were killed after a 17-hour fast and blood samples were collected and centrifuged (3000×g, 15 min) to collect sera, and serum samples were stored at -70℃ until used.

Table 1. Composition of diets

Ingredient	AIN-93G(Control)[1]	Low Carb[2]
Casein (vit-free)	20	20
L-Cysteine	0.3	0.3
Starch	39.7485	10
Sucrose	10	-
Dextrin	13.2	-
Soybean oil	7	7
Horsemeat or beef[3]	-	52.9485
Powdered cellulose	5	5
Vitamin mix	1.0	1.0
Mineral mix	3.5	3.5
Choline bitartrate	0.25	0.25
t-Butylhydroquinone	0.0015	0.0015
Total	100	100

[1]AIN 93 mix, Hanlive, Yeo-Joo, Korea

[2]Low-carbohydrate diet.

[3]Horsemeat and beef were prepared with loin eye purchased from meat market, ground and lyophylized. Analyzed composition of horsemeat and beef was 60.0 and 58.1, 31.1 and 34.6, 5.7 and 4.3, and 0.65 and 1.60% for crude protein, ether extract, ash and moisture, respectively.

2. Analysis of Cholesterol, Triacylglycerol, Glucose and insulin in the Serum

Total cholesterol, triacylglcerol and glucose concentrations in serum were determined using their respective assay kits (SECDIAL kits, Eiken, Tokyo, Japan) and an autoanalyzer (200FR, Toshiba, Tokyo, Japan). HDL cholesterol and LDL cholesterol concentrations were determined using their respective assay kits (HDL; L-Type HDL-C, Wako, Osaka, Japan. LDL; LDL regent, Basel, Swiss) and autoanalyzer (HDL; 200FR, Toshiba, Tokyo, Japan. LDL; Olympus, Toshiba, Hitachi, Tokyo, Japan). Insulin concentration was determined using a RIA(radioimmunoassay) kit (Diagnostic Products Corp., Los Angeles, USA) and γ-counter (Cobra II, Packard, Meridan, USA).

3. Proximate Analysis of Feed and Carcass Samples

Carcass including empited 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. 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 (AOAC, 1996).

4. Statistical Analysis

Data are reported as mean(±SE), and statistical analyses were performed by using a two-way ANOVA for SAS program. Duncan's multiple range test was used to mean compare values of individual treatments. *P* value of <0.05 was used for statistical significance.

Exp. 2 Effect of very low carbohydrate (horsemeat- or beef-based) diet on weight gain, feed efficiency and serum levels of cholesterol, triacyglycerol, glucose, ketone bodies and insulin in adult rat.

1. Animals and diets

Thirty male Sprague-Dawley rats were used and after adaptation to the experimental conditions during 3 day, 30 rats (mean weight, 251g) divided into three groups. Treatment group were blocked depending on feed composition; 1) AIN-93G 2) very low-carbohydrate (beef-based), 3) very low -carbohydrate (horsemeat-based). Other experimental resource and feed composition equal to exp 1. At the end of the 26 day feeding period, rats were sacrificed after a 12-hour fast and blood samples were collected and centrifuged (3000×g, 15 min) to collect sera.

2. Analysis of Cholesterol, Triacylglycerol, Glucose, ketone body and insulin in the Serum

Total cholesterol, HDL cholesterol, LDL cholesterol, triacylglcerol and glucose and insulin concentrations in serum were determined by using the same methods used in exp 1. 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-BM12, Kinase, Tokyo, Japan).

3. Statistical Analysis

Data are reported as mean(±SE), and statistical analyses were performed by using a one-way ANOVA for SAS program. *P* value of <0.05 was used for statistical significance.

Ⅳ. RESULT

Exp. 1 Effect of feeding AIN-93G or low carbohydrate diets and restricted feeding on weight gain, feed efficiency, body composition and serum levels of cholesterol, triacylglycerol, glucose and insulin in adult rat.

Average daily gain was not different between the two dietary treatments, but it different between the two intake levels within each dietary treatment ($P<0.01$)(Table 2). Average daily gain by week is shown in Fig 1. Feed intake was different between the two dietary treatments and between the two intake levels within each dietary treatment ($P<0.01$). The significant main effects of diet and level without a significant interaction indicate that the LC and level effects were independent and additive ($P<0.01$). Energy intake as well as feed intake was not different between the two dietary treatments, but it was different between the two intake levels within each dietary treatment ($P<0.01$). Feed efficiency was different between the two dietary treatments and between the two intake levels within each dietary treatment ($P<0.01$), but energy efficiency was different only between the two intake levels ($P<0.01$).

Table 2. Effect of feeding a very low carbohydrate (horsemeat-based) diet and restricted feeding on average daily gain, feed and energy efficiency in adult rats[1]

	AIN-93G		Low Carb		P value		
Item	Ad lib	70% Ad lib[2]	Ad lib	70% Ad lib	Diet	Lev[3]	Diet × Lev[4]
Initial body weight, g	239±2.3	239±2.2	238±2.2	237±2.1			
Final body weight, g	410±5.9	308±3.9	409±9.9	312±5.2			
Average daily gain, g	6.1±0.2[a]	2.5±0.1[b]	6.1±0.3[a]	2.7±0.1[b]	0.6778	<.0001	0.6778
Average daily feed intake, g	22.8±0.4[a]	14.9±0.2[c]	17.3±0.5[b]	12.0±0.3[d]	<.0001	<.0001	0.0018
Daily energy intake, kcal	84.7±1.5[a]	55.3±0.9[b]	83.7±2.6[a]	57.8±1.4[b]	0.6645	<.0001	0.2976
Gain/Feed, g/g	0.27±0.0[b]	0.17±0.0[d]	0.35±0.0[a]	0.22±0.0[c]	<.0001	<.0001	0.0980
Gain/energy intake, g/kcal	0.072±0.006[a]	0.045±0.002[b]	0.073±0.002[a]	0.046±0.002[b]	0.6415	<.0001	0.9175

[1]Values are means ±SE of 10 rats.

[2]Rats were pair-fed at 70% level of intake (calculated on the basis of metabolic body size, $g^{0.75}$)their counterparts fed ad lib.

[3]Level of intake.

[4]Interaction between diet × intake level.

[5]Means in the same row not sharing the same superscript letters differ (P<0.05).

[6]Energy intake was calculated by multiplying protein, fat and nitrogen-free extract intake by 4, 9 and 4 kcal/g, respectively.

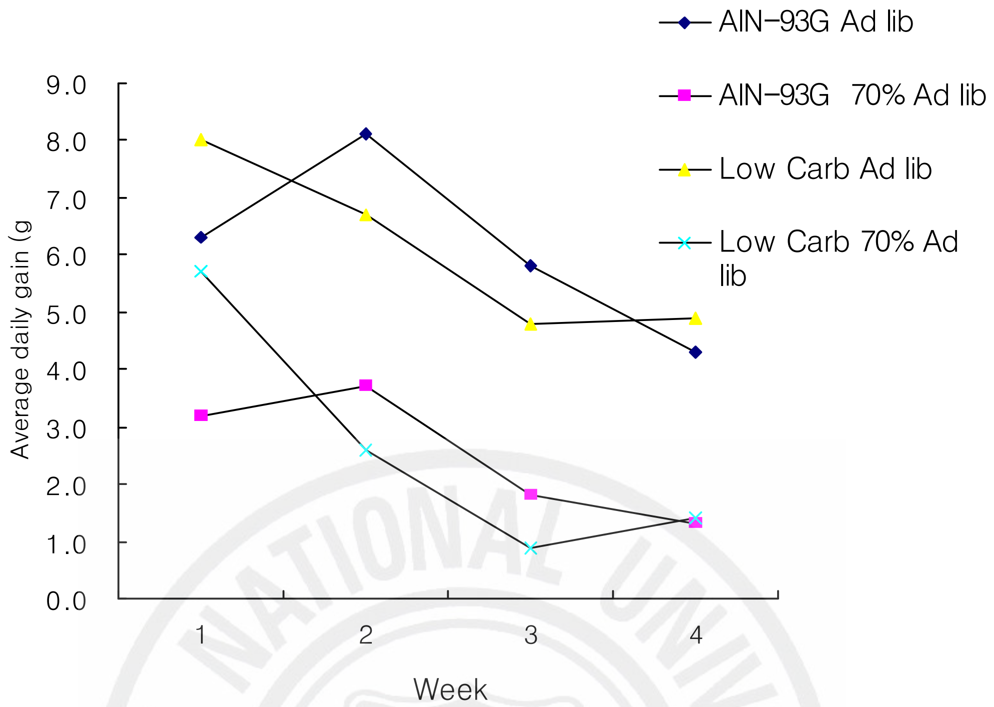

Fig. 1. Effect of feeding the very low carbohydrate diet and restricted feeding on daily body weight gain in adult rats.

Results of serum analysis are shown in Table 3. Total cholesterol and HDL-C levels were lower in rats fed the LC diet than in those fed AIN-93G diet ($P<0.05$), and LDL-C level was not different between the two dietary treatments, but it was lower in ad libitum group compared with that found in restricted feeding group ($P<0.01$). Serum triacylglycerol level was significantly lower in LC diet group than in AIN-93G diet group and was much lower in restricted feeding group than in ad libitum group ($P<0.01$). Moreover, an interaction was found between diet and intake level. Triacylglycerol level was lower in rats fed the restricted feeding group in AIN-93G diet group than in those fed LC diet group ($P<0.01$). Serum glucose level was higher in LC group than in AIN-93G group and was lower in restricted feeding group than in ad libitum group ($P<0.01$), and an interaction was noted between diet and intake level ($P<0.5$). Serum insulin level was lower in LC group or in restricted feeding group compared with that found in AIN-93G group or ad libitum group ($P<0.01$), respectively. An interaction was found between diet and intake level ($P<0.05$).

The body composition was not different between AIN-93G diet group and LC diet group, but body fat was lower in restricted feeding group than in fed ad libitum, whereas body protein and ash content were higher in restricted feeding group than in rats fed ad libitum ($P<0.01$).

Table 3. Effect of feeding a very low carbohydrate (horsemeat-based) diet and restricted feeding on serum levels of cholesterol, triacylglycerol, glucose and insulin in adult rats[1]

	AIN-93G		Low Carb		P-value		
Item	Ad lib	70% Ad lib[2]	Ad lib	70% Ad lib	Diet	Lev[3]	Diet × In[4]
Total cholesterol, mg/100mL	91±5.7[a]	90±3.1[a]	74±3.9[b]	85±3.3[ab]	0.0101	0.2548	0.1559
HDLcholesterol, mg/100mL	57±3.3[a]	55±2.8[a]	46±2.6[b]	52±1.8[ab]	0.0170	0.4563	0.1521
LDL cholesterol, mg/100mL	9±0.6[b]	18±0.9[a]	12±0.7[b]	18±1.2[a]	0.1656	<.0001	0.2893
Triacylglycerol, mg/100mL	157±21.3[a]	55±6.1[b]	75±5.6[b]	36±3.3[c]	0.0001	<.0001	0.0093
Glucose, mg/100mL	123±2.6[b]	113±2.1[c]	138±2.4[a]	118±2.5[bc]	0.0002	<.0001	0.0437
Insulin, μIU/mL	3.1±0.4[a]	1.4±0.1[b]	1.5±0.2[b]	1.3±0.1[b]	0.0008	0.0002	0.0026

[1]Values are means ±SE of 10 rats.

[2]Rats were pair-fed at 70% level of intake (calculated on the basis of metabolic body size, $g^{0.75}$)their counterparts fed ad lib.

[3]Level of intake.

[4]Interaction between diet × intake level.

[5]Means in the same row not sharing the same superscript letters differ (P<0.05).

Table 4. Effect of feeding very low-carbohydrate (horsemeat-based) diet and restricted feeding on body composition in adult rats[1] (% on a dry matter basis)

	AIN-93G		Low Carb		P-value		
Item	Ad lib	70% Ad lib	Ad lib	70% Ad lib	Diet	Intake	Diet × In
Crude protein	47.3±2.18[bc]	54.8±3.23[a]	49.2±1.86[c]	56.8±1.74[ab]	0.4074	0.0025	0.9966
Crude fat	39.4±2.07[a]	29.5±2.70[b]	38.3±1.39[a]	29.0±1.35[b]	0.6985	<.0001	0.8972
Crude ash	9.8±0.52[c]	11.7±0.73[ab]	9.0±0.58[bc]	11.3±0.46[a]	0.3509	0.0013	0.8645

[1]Values are means ±SE of 10 rats.

Exp. 2 Effect of very low carbohydrate (horsemeat- or beef-based) diet on weight gain, feed efficiency and serum levels of cholesterol, triacyglycerol, glucose, ketone bodies, insulin in adult rats.

Average daily gain was higher in LC group than in AIN-93G group ($P<0.01$), but was not different between beef and horsemeat group (Table 5). Average daily gain by week is shown in Figure 2. Average feed intake and energy intake were higher in LC group than in AIN-93G group ($P<0.01$), but was not different between beef group and horsemeat group. There was no significant diet effect on feed efficiency and energy efficiency.

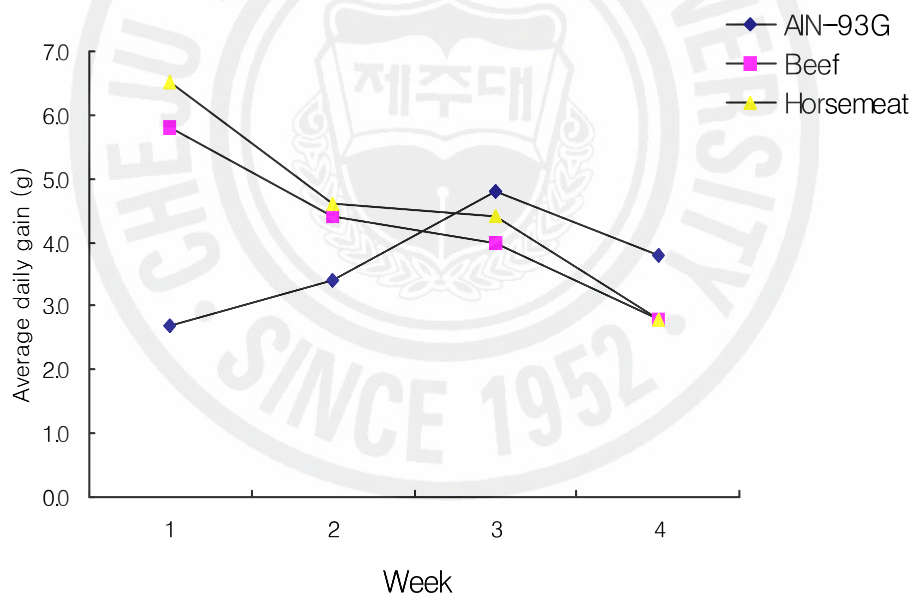

Fig. 2. Effect of feeding the very low carbohydrate diet (horsemeat- or beef-based) on daily body weight gain in adult rats.

Table 5. Effect of feeding a very low carbohydrate (horsemeat- or beef-based) diet on average daily gain, feed intake and feed and energy efficiency in adult rats[1]

Item	AIN-93G[2] (control)	Low Carb		P value
		Beef[3]	Horsemeat[4]	
Initial body weight, g	250.9±7.0	251.0±4.2	251.1±4.2	
Final body weight, g	350.1±21.0	367.9±14.9	376.4±16.9	
Average daily gain, g	3.8±0.6[b]	4.5±0.6[a]	4.8±0.6[a]	0.0032
Average daily feed intake, g	19.8±1.46[a]	15.93±9.3[b]	16.61±0.89[b]	<.0001
Daily energy intake, kcal	70.8±1.7[b]	76.9±1.3[a]	80.2±1.4[a]	0.0004
Gain/Feed, g/g	0.15±0.22	0.17±0.27	0.17±0.25	0.9758
Gain/energy intake, g/kcal	0.054±0.002	0.058±0.002	0.060±0.002	0.2302

[1]Values are means ±SE of 10 rats.

[2]AIN-93G containing 63% carbohydrate.

[3]Low-carbohydrate containing 10% carbohydrate and 53% lyophilized ground beef.

[4]Low-carbohydrate containing 10% carbohydrate 53% lyophilized ground horsemeat.

[5]Means in the same row not sharing the same superscript letters differ (P<0.05).

Results of serum analysis are presented in Table 6. Total cholesterol and HDL-C levels were not different among the three dietary treatments. LDL-C level was different between the LC diet group and AIN-93G diet group ($P<0.01$), but was not different between the beef and the horsemeat group. Serum triacylglycerol level was significantly lower in LC group than in AIN-93G group ($P=0.0615$), but was not different between the beef and the horsemeat group. Glucose and insulin levels were not different among the three dietary treatments. Serum levels of ketone bodies were higher in LC group than in AIN-93G group ($P<0.05$), but was not different between the beef and horsemeat groups.

Table 6. Effect of feeding a very low carbohydrate (horsemeat- or beef-based) diet on serum cholesterol, triacylglycerol, glucose, insulin and ketone body levels in adult rats[1]

Item	AIN 93G[2]	Low Carb		P-value
	(control)	Beef[3]	Horsemeat[4]	
Total cholesterol, mg/100mL	104.80±5.5	118.30±3.1	118.30±6.1	0.1256
HDL cholesterol, mg/100mL	63.06±3.5	69.18±2.4	66.42±2.6	0.3342
LDL cholesterol, mg/100mL	15.80±1.0[b]	23.50±0.8[a]	26.50±2.3[a]	0.0001
Triacylglycerol, mg/100mL	111.40±9.6[a]	77.30±9.0[b]	79.50±13.4[b]	0.0615
Glucose,	154.90±3.3	170.60±6.4	167.70±5.5	0.0979
Insulin,μIU/mL	9.04±0.8	9.99±1.3	8.55±0.8	0.6075
Total ketone body, μmol/L	141.00±27.5[b]	367.50±17.5[a]	340.10±32.1[a]	<.0001
5-Hydroxy Butyric, Acid μmol/L	105.10±8.2[b]	301.40±5.7[a]	270.30±10.4[a]	<.0001
Acetoacetic Acid, μmol/L	35.90±21.2[b]	67.00±12.2[a]	62.80±25.2[a]	0.0278

[1]Values are means ±SE of 10 rats.

[2]AIN-93G containing 63% carbohydrate.

[3]Low-carbohydrate containing 10% carbohydrate and 53% lyophilized ground beef.

[4]Low-carbohydrate containing 10% carbohydrate 53% lyophilized ground horsemeat.

[5]Means in the same row not sharing the same superscript letters differ (P<0.05).

V. DISCUSSION

The results of this study show that a very low-carbohydrate diet and restricted feeding improved disease risk factor such as serum triacylglycerol level known to involve cardiovascular diseases or artherosclerosis, favorably affecting the serum triacylglycerol level. Although our very low-carbohydrate diet contained a rather high level of protein and fat, we found no deleterious effect of the diet on growth and health.

Based on the data in exp 1, restricted feeding either the AIN-93G or the low-carbohydrate diet reduced serum triacylglycerol level (157 vs 55 mg/100 mL in rats fed AIN-93G and 75 vs 36 in rats fed low-carb diet). Furthermore body fat content (39 vs 29%), daily weight gain (6.1 vs 2.5g) and daily energy intake (84 vs 55 kcal) were markedly reduced by restricted feeding, although they were similar in the two diet groups. Considering these factors directly related to human health, especially cardiovascular disease or artherosclerosis, moderate restriction of diet (or energy) intake is considered more efficient way than dietary regime in preventing cardiovascular disease.

Similar results to ours have been reported in many recent studies. During active weight loss, serum triacylglycerol level decreased more HDL-C level increased more with low-carbohydrate, compared with a low-fat diet (Yancy et al, 2004). Sharman et al. (2004) reported that a low-carbohydrate diet was more effective at improving characteristics of the metabolic syndrome as determined by decreased fasting serum triacylglycerol, the TAG/HDL-C ratio, postprandial lipemia, and improved LDL subclass distribution whereas low-fat diet was more effective at lowering serum LDL-C level in overweight men during six week. These studies found that a very low-carbohydrate markedly reduced serum triacylglycerol level. Layman et al. (2003) also reported that a high protein diet produced positive changes in blood lipids with reduction of triacylglycerol levels and the ratio of TAG/HDL-C and greater improvements in body composition with an increased ratio of fat/muscle loss.

Interestingly, fasting serum glucose level was increased in rats fed very low-carbohydrate diet (significantly only in exp 1) but decreased in rats fed restricted feeding, while insulin level was decreased in rats fed very low-carbohydrate diet or restricted feeding (only in exp 1). Glucose level was measured after 17-hour fast and should have been much higher at a fed state in rats fed the AIN-93G containing 53% readily digestible carbohydrate. Such as the one used in the present study, these findings are consistent with the hypothesis about the role of carbohydrates in postprandial hypoglycemia and hunger and the effect of the glycemic index on blood glucose and insulin (Roberts. 2000; Cryer. 1999). This hypoglycemic effect occurs about two hours after a meal (Roberts, 2000). If insulin sensitivity is lower in the high-carbohydrate, reduced peripheral sensitivity should produce higher fasting serum glucose (Layman et al. 2003).

As expected, fasting serum ketone body levels increased with the very low-carbohydrate, compared to the control. Because serum samples were taken 12 hours after the last feeding, serum ketone body levels may have been lower in a fed state (Nelson et al. 2000). 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. (Foster et al. 2003). When fed low- calorie diets, using the fats stored in adipose tissue as their major energy source, levels of ketone bodies in the blood and urine must be monitored to avoid the dangers of acidosis and ketosis (ketoacidosis) (Nelson et al. 2000). Sharman et al. (2002) 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 β-hydroxybutyric acid (250%) after six-week feeding compared to the baseline levels.

Volek et al.(2003) found that a very low- carbohydrate diet had positive effects on blood lipid level, postprandial lipemic in normal weight, normolipidemic women. Brehm. (2003) found that a very low carbohydrate diet had positive effects on body weight, body composition, blood lipid, fasting glucose and insulin sensitivity compared to a calorie restricted low fat diet. Most recently, Pelkman et al. (2004) showed 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. (2004) did not include a high-fat diet group in their study, which could have shown a more pronounced effect on serum lipids.

In contrast to our findings, Axen et al.(2003) suggested that in the absence of weight loss, a high-fat, low-carbohydrate dietnot 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, nonobese individuals. In addition, dietary fat in one of the factors involved in the current epidemic of obesity and higher-fat diets make it more difficult to control energy intake (Bray et al. 2004).

In conclusion, some differences exist among studies, our result suggest that a very-low carbohydrate diet or restricted feeding was more effective various risk factors (blood lipid level, circulating insulin level and body fat, etc) associated with cardiovascular diseases or artherosclerosis and thus may have health benefits.

Ⅵ. ABSTRACT

Studies were carried out to determine the effect of very low carhohydrate diets and restricted feeding (70% ad lib intake) on weight gain, feed efficiency and serum levels of cholesterol, triacylglycerol, glucose, ketone bodies and insulin. Forty rats (mean initial weight 238g) or 30 rats (251g) were divided into groups of 10 rats each and fed for four weeks or 26 days in exp. 1 or 2, respectively. In exp. 1 two groups were assigned to a conventional diet (AIN-93G) or a very low carbohydrate (LC) diet containing 10% starch and 53% lyophilized ground horsemeat, and one of the two groups was fed ad lib and the other pair-fed 70% on the basis of metabolic body size of the ad lib intake. In exp. 2 each of the three groups was assigned to a control (AIN-93G) or diets containing 10% starch and 53% beef or horsemeat. Average daily body weight gain was not different between the two diets in exp. 1, whereas in exp. 2 was lower in rats fed the AIN-93G than in those fed the very low carbohydrate diets at beginning, but was reversed toward the end of the feeding period. Rats with restricted intake gained much less weight ($P<0.01$) than those had ad lib intake in exp. 1. Feed intake was higher in rats fed AIN-93G diet than those fed LC diet ($P<0.01$) in both experiments, whereas energy intake was not different between the two diets in exp. 1. LC diet reduced ($P<0.01$) serum triacylglycerol compared with AIN-93G diet and restricted feeding also reduced serum triacylglycerol, glucose and insulin levels especially in rats fed AIN-93G diet ($P<0.05$ for diet × feeding level) compared with ad lib intake in exp. 1. Serum LDL cholesterol level was increased by restricted feeding (exp. 1) or by LC diet (exp. 2), compared with ad lib intake or AIN-93G, respectively. Body composition was not affected by diet but protein content was increased with a concomitant decrease in fat content by restricted feeding. Serum ketone body level was higher in rats fed LC diet than in those fed AIN-93G diet in exp. 2. No differences in all the parameters determined were found between the horsemeat- and beef-based diets in exp. 2. Results indicate that feeding LC diet is beneficial for alleviating risk factors, such as serum triacylglycerol level known to involve cardiovascular diseases or artherosclerosis and restricted feeding with still gaining weight is highly effective in reducing serum triacylglycerol level. However, more studies with model animals as well as humans are required to examine long-term health benefits of low carbohydrate diets.

Key words : rats, low carbohydrate, restricted feeding, serum lipids and glucose and insulin

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