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Effects of the Dietary Protein Level on Plasma Glucose, Lipids and Hormones in Streptozotocin-Diabetic Rats

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ABSTRACT

Atherosclerotic vascular disease is a major cause of the increased morbidity and mortality associated with diabetes mellitus. The prominent role of nutrition in hypercholesteolemia and atherosclerosis is generally accepted. Diet is a key element in the management of diabetes (type I -IDDM), yet the appropriate diet for patient with diabetes mellitus is not well known. Dietary protein has been shown to have a significant effect on plasma cholesterol levels in both experimental animals and humans.

The present experiment was designed to determine the effect of the dietary protein level(20% vs 60%) on plasma glucose concentration, lipids profile, insulin and glucagon levels from non-diabetic and streptozotocin-induced diabetic rats. Results showed that a high protein diet decreased triglyceride concentration in diabetic rats. Also diabetic rats fed a high protein diet were hypocholesterolemic than rats fed a control diet. There were no effects by level of protein on fasting blood glucose concentration and insulin/glucagon ratio.

Results from the present study suggest that a high protein diet may be beneficial to control plasma lipids in chemically-induced diabetic rats.

KEY WORDS : protein level · blood glucose · lipids · insulin · glucagon · streptozotocin-induced diabetic rats.

Introduction

Diabetes mellitus and its complications are now thought to be one of the main leading cause of death in Korea, trailing only cancer, cardiovascular disease and accidents. The number of diabetics in Korea is about 8 to 9 percent of the population, and the incidence is increasing yearly due to changes of life style and food intake[1-3]. Moreover, although the acute and often lethal symptoms of diabetes can be controlled with insulin therapy, the long term complications of the disease reduces life expectancy by as much as a third[4]. Of great importance to this proposed research is the higher prevalence of cardiovascular disease which accounts for about 75% of all deaths among those with diabetes. People with diabetes are frequently hypercholesterolemic, with more cholesterol in plasma low density and very low density lipopro-

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teins, and less cholesterol in high density lipoprotein[5]. Recent studies indicate that levels of high-density lipoprotein cholesterol(HDL) may be inversely related to the development of cardiovascualr disease. Conversely, levels of low-density lipoprotein (LDL) appear to be positively related to the risk for cardiovascular disease[6].

The prominent role of nutrition in hypercholesterolemia and atherosclerosis is generally accepted. Dietary protein has been shown to have a significant effect on plasma cholesterol levels in both experimental animals and humans[7-9]. Diet is a key element in the management of diabetes, yet the appropriate diet for diabetes mellitus is not well known. Animal models have been useful in examining the interaction between diet and diabetes[10].

It is well known that changes in the composition of diet influence blood glucose, insulin, and glucagon secretion. Blazquez et al have demonstrated the significant stimulatory effect of a high protein diet on plasma glucose, but they showed that plasma insulin in rats fed a high protein diet remained at the same level as in rats fed control diet[11]. In addition, Eisenstein et al have reported that feeding a high protein, carbohydrate-free diet to rats increases plasma glucagon but not blood glucose or plasma insulin[12].

Recently, investigators have begun testing the effects of more complex dietary components such as carbohydrate, protein and fat on modification of streptozotocin-induced diabetes. Siegel et al[13] demonstrated marked improvement in the diabetic state of rats fed a low carbohydrate : high protein diet (6% : 63% of calories) compared to rats fed a standard diet (68% as carbohydrate, 20% as protein, 12% as fat). Also Schmidt et al[14] compared to a control diet (68% as carbohydrate, 20% as protein, 12% as fat) and a low-carbohydrate-high-protein diet(6% carbohydrate, 63% protein, 31% fat). Rats fed the high protein diet showed a decrease in blood glucose. The pancreatic insulin content at death of rats fed the high protein diet were significantly increased. Another recent study[15] also showed that a high protein diet (protein 55%, carbohydrate 30%, fat 15%) enhances insulin secretion but not glucagon in non-diabetic rats.

Insulin is necessary for normal growth, development, and maintenance. In the diabetic, the anabolic actions of insulin on amino acid and protein metabolism are depressed, and the catabolic processes promoted by the catabolic hormones are enhanced. In diabetes mellitus, particularly, a decrease in rate of protein synthesis has been observed[16].

Also Eizirik and Migliorini[17] have reported that rats adapted to a high protein, carbohydrate-free diet, have a decreased susceptibility to the diabetogenic action of streptozotocin. Results indicated reduced mortality and decreased severity of diabetes as judged by several parameters including plasma glucose, serum and pancreatic insulin levels. Similar beneficial results[18] were noted in high protein diet of plant origin. Recent experiments have demonstrated that diets ars low in carbohydrate, moderately high in fat and high in protein may be beneficial in reducing hyperglycemia, polyuria, polydipsia, glycosuria and hypertriglyceridemia. Recent data have demonstrated that diabetic rats allowed a free choice tended to consume more protein and consumed significantly less carbohydrate than non-diabetic rats[10].

The primary purpose of the present experiment was designed to determine the effect of the dietary protein level (20% vs 60%) on plasma glucose concentration, lipids profile, and hormone levels from non-diabetic and streptozotocin-induced

diabetic rats.

Materials and methods

Twenty-five (Sprague-Dawley) male rats were obtained from K.L.E.C.(Korea Life Engineering Corporation, Seoul, Korea). The average body weight of the animals upon arrivals was 141± 11g. Animals were housed individually in stainless steel cages (10″×15″×7″) in a room with controlled temperature and exposed to an alternating twelve hour period of light and dark. All animals were fed a Samyang rat chow diet and water ad libitum for three weeks after arrival. Body weights were recorded weekly.

When the animals attained weights of from 190 to 210 grams, one-half of the animals with higher body weight were made chemically diabetic by an intramuscular injection of streptozotocin (Sigma Chemical Co., St. Louis, MO) at a dose of 50mg/Kg body weight in 0.25M citrate buffer, pH 4.5[19]. At the same time, 0.25M citrate buffer, pH 4.5, was injected intramuscularly into the non-diabetic rats.

Five days after streptozotocin treatment, and the appearance of glucose in the urine(Multistix, Ames Company Division Miles Laboratory. Inc., Elkhart, IN) was used to confirm the diabetic state. Blood sample were taken from the tail vein of unanesthetized rats using heparinized, micro-hematocrit capillary tubes, and the glucose concentration of the plasma was analyzed using GLUCOPAT (Kyoto Daiichi Kagaku Co., LTD JAPAN). Animals were diagnosed as being diabetic if they had a non-fasting blood glucose concentration which was greater than 300 mg/dl of total blood.

Rats were then randomly assigned to two experimental diet(high protein : 60% or control protein : 20%) groups of diabetic rats(6 rats per group) or two groups of non-diabetic rats(6 rats per group). The composition of diets are presented in Table 1. At the end of 14 days of experimental diets, rats were sacrificed after being treated with light anethesia(ethyl ether). Blood samples were taken from the inferior vena cava and were then centifuged for 30 minutes at 3000 rpm 4℃ to obtain plasma. The plasma was frozen at −20℃

Table 1. Composition of experimental diets

Ingredient	Dietary Groups	
	Casein 20	Casein 60
Casein[1)]	20.00	60.00
Dextrin[2)]	65.70	25.70
Corn-oil[3)]	5.00	5.00
Cellulose[4)]	3.80	3.80
Mineral Mixture[5)]	3.50	3.50
Vitamin Mixture[6)]	1.80	1.80
Choline[7)]	0.20	0.20
Total	100.00	100.00

" Gross Energy. Kcal/g : 3.878

1) Casein high protein. Supplied by U.S. CORNING Laboratory Service Company.
Teklad Test Diets, Madison, WI. Biological Test Material No. 160030.

2) Dextrin. Supplied by U.S. CORNING Laboratory Service Company.
Teklad Test Diets, Madison, WI. Biological Test Material No. 50704.

3) Corn-oil. 동방유량주식회사. 서울시 영등포구 양평동 4-2. 규격번호 : KSH 2102.

4) Cellulose. Supplied by U.S. CORNING Laboratory Service Company.
Teklad Test Diets, Madison, WI. Biological Test Material No. 160390.

5) Mineral Mixture. Supplied by U.S. CORNING Laboratory Service Company.
Teklad Test Diets, Madison, WI. Biological Test Material No. 170915.

6) Vitamin Mixture. Supplied by U.S. CORNING Laboratory Service Company.
Teklad Test Diets, Madison, WI. Biological Test Material No. 40077.

7) Cholin Bitartate. Supplied by U.S. CORNING Laboratory Service Company.
Teklad Test Diets. Madison, WI. Biological Test Material No. 30190.

for later determination of concentrations of total cholesterol, triglyceride, glucose, insulin, and glucagon. Blood glucose concentration and plasma lipids were determined by enzymatic methods. Plasma insulin and glucagon concentrations were determined by radioimmunoassay kits.

Statistical analysis ;

Data were analyzed by computer using the Stasistical Analysis System(SAS) ; general linear model (GLM) procedure allowing for unequal number of sample among the experimental groups. Values are expressed as mean± SM. Statistical analysis for the comparison for the two different diet groups (20% casein, 60% casein) and for the two conditions of animals (non-diabetic and diabetic) was performed using Two-Factor Analysis of Varience. Multiple range test was taken by the Duncan's.

Results and Discussion

This study determined effects of the dietary protein level(20% vs 60%) on the metabolism of plasma lipids, glucose and hormones in steptozotocin-diabetic rats.

Weight of both groups at the beginning of the experimental period was not statistically different (Table 2). In body weight gain during the experimental period, diabetic rats failed to gain weight which continued until the experimental period of 14 days ended. The effects of protein levels on weight gain were not significantly different within non-diabetic and diabetic rats. From data shown in Table 3, it is evident that diabetic rats were hyperphagic, consuming an average amount 27 g of diet/day.

In food efficiency ratio(FER) and protein efficiency ratio(PER), there was significant difference between the mean FER and PER in the two dietary groups among non-diabetic rats. Among non-diabetic rats, rats fed a casein-20 diet had *significantly higher* FER and PER than rats fed a casein-60 diet. On the other hand, in diabetic rats, effects of protein levels were not seen both in FER and PER.

Effects of protein levels on plasma glucose and

Table 2. Body weight of Sprague-Dawley rats at begining and sacrifice of non-diabetic and diabetic rats.

Diet / Condition	Non-diabetes	Diabetes
	Mean± SE (g)	*Mean± SE* (g)
Weight at beginning		
Casein 20	198.75± 7.46[a]	203.08± 5.33[a]
Casein 60	203.00± 5.91[a]	201.17± 4.57[a]
Weight at sacrifice		
Casein 20	225.92± 10.67[a]	173.33± 12.07[b]
Casein 60	221.50± 18.15[a]	182.50± 9.09[b]
Weight gain		
Casein 20	27.17± 4.84[a]	−29.75± 11.75[b]
Casein 60	18.50± 14.39[a]	−18.67± 6.99[b]

*Means in the same row or column not sharing a common superscript are significantly different at $p<0.05$.

Table 3. Daily food intake, gross weight gain efficiency and protein efficiency ratio of non-diabetic and diabetic rats fed the experimental diets

Diet / Condition	Non-diabetes	Diabetes
	Mean± SE	Mean± SE
Food intake(mg/day)		
Casein 20	19.13± 1.02[a]	26.66± 1.34[b]
Casein 60	20.32± 1.33[a]	26.87± 1.02[b]
Food efficiency ratio		
Casein 20	1.46± 0.28[a]	−1.19± 0.47[c]
Casein 60	0.73± 0.78[b]	−0.64± 1.13[c]
Protein efficiency ratio		
Casein 20	7.27± 1.43[a]	−5.94± 2.36[c]
Casein 60	1.22± 1.31[b]	−1.08± 0.43[c]

*Means in the same row or column not sharing a common superscript are significantly different at $P<0.05$.

lipids concentrations are shown in Table 3. Within non-diabetic groups and diabetic groups, there were no significant effects of protein levels on plasma glucose concentration.

Hypertriglyceridemia occurs frequently in diabetes mellitus[20]. In the present study, diabetic rats showed significantly increased plasma triglyceride concentration compared to non-diabetic rats. It is consistent with the findings reported by Bar On[20]. Diabetic rats fed casein-20 diet had significantly increased plasma triglyceride concentration compared to non-diabetic rats. However plasma triglyceride concentration of rats fed a casein-60 diet was not statistically different between non-diabetic and diabetic rats.

Within non-diabetic groups, there was no significant difference in triglyceride concentration between casein-20 and casein-60 protein fed animals. Within diabetic groups, the plasma triglyceride concentration of rats fed a casein-20 diet was significantly higher than that of those fed a casein-60 diets. Therefore, these finding support the earlier study of the hypolipidemic effect of high protein diet in chemically-induced diabetic rats[18].

Studies both in man and experimental animals have shown that the concentration of serum cholesterol can be greatly affected by the proportion of dietary protein. In comparison of plasma cholesterol levels in diabetic patients and matched controls, in general, cholesterol concentrations are significantly elevated in diabetics than in those of non-diabetic patients[21-23]. Diabetic animal models when fed control protein level have also exhibited increased total plasma cholesterol concentrations[20].

In effects of protein levels on total plasma cholesterol concentration, the comparison between groups fed experimental diets whithin non-diabetic rats showed that there was no significant difference between casein-20 and casein-60 diets. The comparison between animals fed experimental diets whithin diabetic groups showed that those fed a casein-20 diet had significantly greater total plasma cholesterol concentration than those fed a casein-60 diet.

This present study does not support hypercholesterolemia in diabetes when high protein level

Table 4. Plasma glucose and lipids concentration in rats fed experimental diets

Diet	Condition	Non-diabetes	Diabetes
		Mean± SE	Mean± SE
		(g)	(g)
Plasma glucose(mg/day)			
Casein 20		153.17± 11.52[a]	377.80± 66.50[b]
Casein 60		153.00± 26.60[a]	404.60± 55.12[b]
Plasma triglyceride(mg/dl)			
Casein 20		54.40± 3.39[a]	153.62± 36.57[b]
Casein 60		49.96± 10.69[a]	70.04± 8.75[a]
Plasma cholesterol(mg/dl)			
Casein 20		56.32± 3.23[a]	62.31± 10.33[b]
Casein 60		48.54± 5.33[a]	45.09± 4.27[a]
Cholesterol/Body weight(kg)			
Casein 20		256.40± 19.52[a]	351.67± 77.60[b]
Casein 60		235.78± 52.08[a]	269.36± 35.13[a]

*Means in the same row or column not sharing a common superscript are significantly different at $P<0.05$.

Table 5. Effect of the level of protein on the concentration of plasma insulin and glucagon in non-diabetic and diabetic rats

Diet	Condition	Non-diabetes	Diabetes
		Mean± SE	Mean± SE
		(g)	(g)
Plasma insulin(μ unit/ml)			
Casein 20		18.78± 1.99[a]	14.40± 1.06[a]
Casein 60		15.70± 0.77[a]	7.47± 1.10[b]
Plasma glucagon(pg/ml)			
Casein 20		126.29± 6.73[a]	104.68± 6.77[b]
Casein 60		141.06± 35.95[a]	86.43± 8.68[b]

*Means in the same row or column not sharing a common superscript are significantly different at $P<0.05$.

was used. Still unclear is why, in chemically-induced diabetic rats, diets containing high proteins give lower levels of plasma cholesterol than diets containing control proteins.

The effects of dietary proteins on the concentrations of plasma insulin and glucagon in Sprague-Dawley rats are shown in Table 5. No significant differences due to the level of protein were observed among non-diabetic rats. The results of the present study are not consistent with the previous study which showed that a high protein diet (casein 55%) enhanced insulin secretion in non-diabetic rats[15]. The comparison of experimental diets within diabetic rats showed that those fed a casein-20 diet had a significantly greater plasma insulin concentration than those fed a casein 60 diet. Eventhough, the mean plasma insulin level was lower in rats fed a casein-60 diet, there was no difference in insulin/glucagon ratios among diabetic rats.

In effects of protein levels on concentration of plasma glucagon, significant differences between groups of non-diabetic and diabetic rats in plasma glucagon concentration were found in both those fed casein-20 and casein-60 diets. Diabetic rats fed both casein-20 and casein-60 diets showed significantly decreased plasma glucagon concentration when compared to non-diabetic rats. Among non-diabetic rats fed experimental diets, there were no significant levels of protein effects on plasma glucagon concentrations. Also there were no significant difference in plasma glucagon levels between diabetic animals fed casein-20 and casein-60 diets. The results of the present study are not consistent with the earlier study which showed that a high protein diet increased plasma glucagon concentration[12].

In summary, results from the present study suggest that a high protein diet may be beneficial to maintain the weight, make efficient energy use and control plasma lipids in diabetes. Metabolic errors of chemically-induced diabetic rats fed high protein are more controlled than that of those fed a control diet. However, further studies have to be done in order to elucidate the mechanism underlying these effects of a high protein diet in chemically-induced diabetic rats. Also effects of different proteins on plasma glucose, lipids, and hormones in chemically-induced diabetic rats should be studied.

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= 국문초록 =

식이단백질의 양이 당뇨쥐의 혈당, 혈중지질 그리고 호르몬에 미치는 영향

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죽상동맥경화성 질환은 당뇨병환자에게 주된 합병증 내지 사망원인이 되고 있다. 고콜레스테롤혈증과 죽상동맥경화증에 식이는 중요한 역할을 한다. 당뇨병관리에 식이는 중요한 인자이나 아직도 적당한 식이요법은 알려져 있지 않다. 식이단백질은 실험동물과 사람에 있어 혈장 콜레스테롤 수준에 상당한 효과를 미치는 것으로 나타났다. 이 논문은 식이단백질의 양이 (casein 20% VS 60%) 당뇨쥐와 비당뇨쥐에 있어서 혈당, 혈중지질 그리고 호르몬에 미치는 영향을 실험하였다. 실험결과 당뇨쥐에 있어서 고단백질 식이군에 혈중 지질의 농도가 낮았다. 또한 대조 단백질 식이군보다 고단백질 식이군에서 혈중 콜레스테롤농도도 낮게 나타났다. 그리고 식이단백질의 양에 의한 혈당과 호르몬에 미치는 영향에서는 차이는 없었다. 이 연구결과 고단백식이는 화학적으로 유발시킨 당뇨쥐에 있어서 혈중 지질을 조절하는데 유익한 것으로 보여졌다.