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

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ABSTRACT

The number of diabetics in Korea is about 3 to 5 percent of the population, and the incidence is increasing yearly due to changes of life style and food intake. Diet is a key element in the management of diabetes, yet the appropriate diet for diabetes remains controversial.

We have recently shown that a diet rich in protein of animal origin(casein) seems beneficial to controling plasma glucose and lipids in streptozotocin-induced diabetic rats. It therefore seemed desirable to find out whether the beneficial effect of high casein diet in experimental diabetes could also be reproduced with a vegetable source of protein(soy). The purpose of this study is to compare these results with the results of our previous study.

In the present study, non-diabetic and streptozotocin-induced diabetic rats were studied in order to examine the effects of altering the level(20% vs 60%) of dietary soy protein on blood glucose, lipids, and hormones. Results of the present study showed that a high soy protein diet decreased triglyceride concentration in diabetic rats. However, diabetic rats fed a high soy protein diet were not hypocholesterolemic compared to rats fed a control diet.

Moreover, diabetic rats fed a high soy protein diet had significantly increased plasma glucose concentration compared to rats fed a control diet. This study was not able to discern a specific effect of dietary protein level on insulin, glucagon, or insulin/glucagon ratio. Except for the hypotriglyceridemic effect, the results were not similar to the findings of our previous study which showed a beneficial effect on streptozotocin-induced diabetic rats fed a high casein diet.

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

Introduction

The number of diabetics in Korea is about 3 to 5 percent of the population, and the incidence is increasing yearly due to changes of life style and food intake[1-3]. It has been clearly shown that dietary protein can influence blood lipids and hormonal metabolism in animals. There is a significant literature suggesting a major difference in the effects of dietary animal and vegetable proteins on the metabolism of cholesterol. The chole-

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sterolemic effect of the dietary protein source has been established primary from a comparison of soy protein to casein in semipurified that diets of variable nutrient composition. Casein, when compared to soy protein, is hypercholesterolemic in numerous species including rabbits[4)5)], rats[6-8)], mice[9)], guinea pigs[10)], and pigs[11)].

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[12)]. 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[13)].

Investigators have begun testing the effects of more complex dietary components such as carbohydrate, protein and fat on streptozotocin-induced diabetes. Siegel et al[14)] demonstrated the 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[15)] compared a control diet(68% as carbohydrate, 20% as protein, 12% as fat) with 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[16)] also showed that a high protein diet(protein 55%, carbohydrate 30%, fat 15%) enhances insulin secretion but not glucagon in non-diabetic rats.

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.

An alternative approach to the study of diet and diabetes is the use of dietary self-selection. Since it has been observed that animals select diets on the basis of their nutritional requirements, the choice of diabetic animals may provide information useful for identifying their nutritional needs.

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[18)].

We have recently shown that a diet rich in protein of animal origin(casein, 60% of total diet composition) seems beneficial to controling plasma glucose and lipids in streptozotocin-induced diabetic rats[19)].

Dietary protein has been shown to have a significant effect on plasma cholesterol levels in both experimental animals[20)] and humans[21)]. A number of studies have demonstrated that dietary amino acid composition can influence the level of plasma cholesterol[22)].

It therefore seemed desirable to find out whether the beneficial effect of high casein diet in experimental diabetes could also be reproduced with a vegetable source of protein(soy). In the present, non-diabetic and streptpzotocin-induced diabetic rats were studied in order to examine the effects of altering the level(20% vs 60%) of dietary soy protein on blood glucose, lipids, and hormones.

The present experiment was designed to determine whether the type of protein can alter 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[23]. At the same time, 0.25M citrate buffer, pH4. 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, microhematocrit 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 300mg/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 at

Table 1. Composition of experimental diets

Ingredient	Dietary groups	
	Soybean 20%	Soybean 60%
Soybean[1)]	20.00	60.00
Dextrin[2)]	65.70	25.70
Corn-oil[3)]	5.00	5.00
Cellulose[4)]	3.80	3.80
Min. Mix[5)]	3.50	3.50
Vit. Mix[6)]	1.80	1.80
Choline[7)]	0.20	0.20
Total	100.00	100.00

*Gross Energy, Kcal/g : 3.878

1) Soy Assay Protein, Supplied by U.S. CORNING Laboratory Services Company, TEKLAD TEST DIETS, Madison, Wisconsin.
Biological test Material No. 160480
2) Dextrin, Supplied by U.S. CORNING Laboratory Services Company, TEKLAD TEST DIETS, Madison, Wisconsin.
Biological test Material No. 50740
3) Corn-oil, Dong-Bang Yuo-Ryang Co. Yangpyung-Dong 4-2, Youngdongpo-Gu Seoul : KSH 2102
4) Cellulose, Supplied by U.S. CORNING Laboratory Services Company, TEKLAD TEST DIETS, Madison, Wisconsin.
Biological test Material No. 160390
5) Mineral mixture, Supplied by U.S. CORNING Laboratory Services Company, TEKLAD TEST DIETS, Madison, Wisconsin.
Biological test Material No. 170915
6) Vitamin mixture, Supplied by U.S. CORNING Laboratory Services Company, TEKLAD TEST DIETS, Madison, Wisconsin.
Biological test Material No. 40077
7) Choline Bitartate, Supplied by U.S. CORNING Laboratory Services Company, TEKLAD TEST DIETS, Madison, Wisconsin.
Biological test Material No. 30190

4℃ to obtain plasma. The plasma was frozen at −20℃ 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 (Abott, North Chicago, USA).

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± SD. 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-way Analysis of Varience. Multiple range test was taken by the Duncan's.

Results and Discussion

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

Weight of both groups at the beginning of the experimental period was not statistically different (Table 2). During the experimental period of 14

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

Condition / Diet	Non-diabetes Mean± SD (g)	Diabetes Mean± SD (g)
Weight at beginning		
Soybean 20	198.42 ± 8.39^{a}	206.30 ± 4.33^{a}
Soybean 60	196.20 ± 5.05^{a}	202.20 ± 4.49^{a}
Weight at sacrifice		
Soybean 20	224.67 ± 14.82^{a}	198.70 ± 3.40^{a}
Soybean 60	236.20 ± 4.49^{b}	195.20 ± 16.65^{a}
Weight gain		
Soybean 20	26.25 ± 6.86^{a}	−7.60 ± 9.26^{c}
Soybean 20	40.00 ± 5.00^{b}	−7.00 ± 14.18^{c}

*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

Condition / Diet	Non-diabetes Mean± SD	Diabetes Mean± SD
Food efficiency ratio		
Soybean 20	1.33 ± 0.27^{a}	−0.24 ± 0.49^{c}
Soybean 60	2.59 ± 0.35^{b}	−0.19 ± 0.68^{c}
Protein efficiency ratio		
Soybean 20	6.67 ± 1.36^{a}	−0.64 ± 2.3^{b}
Soybean 60	4.32 ± 0.59^{a}	−0.32 ± 1.13^{b}

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

days, the diabetic rats failed to gain weight. In non-diabetic rats, rats fed a high protein diet gained a significantly higher body weight compared to rats fed a control diet. This is not consistent with our previous study in which the protein levels did not have a significant effect on weight gain with non-diabetic rats. In the current study, protein levels did not have a significant effect on weight gain with diabetic rats. This is same as the results of our previous study[19].

Food intake for non-diabetic and diabetic rats is presented in Table 3. Diabetic rats normally consume more energy than the non-diabetic rats fed the same diet as the former lose energy substrates in their urine and hence are less efficient than their controls. This was seen in the our previous study but not in the present experiment. In the current study, when feeding intakes were ad libitum, differences between diabetic and non-diabetic rats were not pronounced, even though diabetic rats ate more food than non-diabetic rats. In the our previous study, in marked contrast, for the animals fed casein protein, diabetic rats were hyperphagic, consuming an average amount 27g of diet per day. With both non-diabetic and diabetic rats, there were no significant effects of protein level on food intake[19].

There was no significant difference between the mean food efficiency ratio(FER) and protein efficiency ratio(PER) in the two dietary groups among diabetic rats. The results of the present study are in agreement with our previous study. Among non-diabetic rats, rats fed a soy-60 diet had significantly higher FER. On the other hand, effects of protein levels on PER were not seen in non-diabetic rats. This is not consistent with our previous study that rats fed a casein-20 diet had significantly higher PER than rats fed a casein-60 diet.

Effects of protein levels on plasma glucose and lipids concentrations are shown in Table 4. Diabetic groups showed significant increased plasma glucose concentrations when compared to non-diabetic groups. In addition, within non-diabetic groups, there were no significant effects of protein levels on plasma glucose concentration. However within diabetic groups, plasma glucose concentrations of rats fed a soy-60 diet were significantly higher than glucose concentration in rats fed a soy-20 diet. The animals fed a soy-60 diet had

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

Condition / Diet	Non-diabetes Mean± SD	Diabetes Mean± SD
Plasma glucose (mg/dl)		
Soybean 20	126.50 ± 21.84[a]	179.25 ± 9.85[a]
Soybean 60	122.80 ± 7.20[a]	354.80 ± 102.30[b]
Plasma triglyceride (mg/dl)		
Soybean 20	56.61 ± 9.27[a]	110.31 ± 16.44[b]
Soybean 60	50.66 ± 4.71[a]	57.66 ± 7.16[a]
Plasma cholesterol (mg/dl)		
Soybean 20	57.06 ± 3.48[a]	60.97 ± 6.62[a]
Soybean 60	47.48 ± 4.12[a]	58.90 ± 9.81[a]
Cholesterol/Body weight (Kg)		
Soybean 20	260.34 ± 24.80[a]	279.27 ± 38.29[a]
Soybean 60	202.33 ± 20.56[a]	266.23 ± 43.87[a]

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

two fold greater plasma glucose levels than those fed a soy-20 diet(179mg/dl vs 354mg/dl).

Hypertriglyceridemia occurs frequently in diabetes mellitus[14]. In the present study, diabetic rats fed a soy-20 diet had significantly increased plasma triglyceride concentration compared to non-diabetic rats. However, plasma triglyceride concentration of rats fed a soy-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 animals fed soy-20 and soy-60 protein diet.

Within diabetic groups, the plasma triglyceride concentration of rats fed a soy-20 diet was significantly higher than that of those fed a soy-60 diets. Therefore, these finding support the earlier study [20] and our previous research[19] of the hypolipidemic effect of high protein diet in chemically-induced diabetic rats.

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 diabetic compared to those of non-diabetic patients[24][25]. Diabetic animal models, when fed a control protein level(casein 20%), have also exhibited increased total plasma cholesterol concentrations[14][15].

The comparison between groups fed experimental diets with both non-diabetic and diabetic rats showed that there was no significant difference between soy-20 and soy-60 diets on total plasma cholesterol concentration. This present study does not support a hypocholesterolemic effect in streptozotocin-induced diabetic rats when soy instead of casein was used as a high protein source.

Of interest is why diets containing high soy proteins give higher levels of plasma cholesterol than diets containing high casein proteins in chemically-induced diabetic rats. The cholesterolemic effect of the dietary protein source has been established primarily from a comparison of soy protein to casein in semipurified diets of variable nutrient composition. With appropriate dietary regimens, casein has been reported to be hypercholesterolemic and atherogenic. In contrast, an equivalent level of soy protein in the diet has been reported to be hypocholesterolemic, and, when included with casein or other animal proteins, can inhibit the hypercholesterolemic response to the animal protein.

The effects of dietary proteins on the concentrations of plasma insulin and glucagon in chemically-induced diabetic rats are shown in Table 5. Significant differences due to the level of pro-

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

Condition	Non-diabetes	Diabetes
Diet	Mean± SD	Mean± SD
Plasma insulin (μunit/dl)		
Soybean 20	23.90 ± 2.26[a]	13.46 ± 1.46[b]
Soybean 60	15.05 ± 1.81[b]	16.10 ± 2.37[b]
Plasma glucagon (pg/dl)		
Soybean 20	136.84 ± 14.42[a]	82.71 ± 10.37[b]
Soybean 60	69.06 ± 10.43[b]	104.12 ± 13.22[b]

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

tein were observed with the non-diabetic rats. The concentration of plasma insulin was significantly higher in rats fed a soy-20 diet than for those fed a soy-60 diet. 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[17]. Also this data is not consistent with our previous study which showed that there was no significant effect of levels of protein diets on plasma insulin concentrations. It is reasonable to consider that the different effects of dietary proteins on plasma insulin level are due to, in part, the differences in the amino acid profiles of the proteins. Studies both in man and experimental animals have shown that the concentration of serum insulin level can be greatly affected by the nature and the proportion of dietary protein[16)26].

In diabetic rats, even though the mean plasma insulin concentration was higher in rats fed a soy-60 diet, the effects of soy protein level were not seen statistically in insulin concentration. Even though, the mean plasma insulin level was lower in rats fed a soy-60 diet, there was no difference in insulin/glucagon ratios among non-diabetic rats. The latter findings are in agreement with the reports of our previous study[19].

Significant differences in plasma glucagon concentration between groups of non-diabetic and diabetic rats were found in those fed both soy-20 and soy-60 diets. Non-diabetic rats fed a soy-20 diet showed significantly increased plasma glucagon concentration when compared to that of rats fed a soy-60 diet.

The results of the present study are not consistent with the earlier study which showed that a high protein diet increased plasma glucagon concentration[15].

Also the results of the present study are not consistent with our previous study which showed that no significant differences due to the level of casein protein were observed among non-diabetic rats. In diabetic rats, there were no significant difference in plasma glucagon concentration between animals fed a soy-20 and a soy-60 diet. Diabetic rats fed a soy-20 diet showed significantly decreased plasma glucagon concentration when compared to non-diabetic rats. This was not found in our previous study. However, there were no difference in insulin/glucagon ratios in the two dietary groups among diabetic rats. This is consistent with our previous results that there was no effect by level of casein protein on insulin/glucagon ratio.

In summary, metabolic errors of chemically-induced diabetic rats fed high soy protein are less controlled than that of those fed a control(20% soy) diet. A point of the importance is the fact that ad libitum-fed rats eating the high soy diet not only had increased blood glucose level but had an alteration in plasma glucose in favor of increased plasma cholesterol concentration compared with rats fed a high casein diet. These data showed that the beneficial effects of high protein diet in streptozotocin-induced diabetic rats is not maintained when casein is replaced by a vegetable protein source(soy protein isolate).

The mechanism underlying the differential effects of dietary protein(casein vs soy) on plasma glucose and cholesterol in streptozotocin-induced diabetic rat should be elucidated.

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=국문초록=

식이 콩단백질의 양이 당뇨쥐의 혈당, 혈중지질 그리고 호르몬에 미치는 영향

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한국에서 당뇨병환자의 수는 총인구의 3～5%에 이르며 식품섭취와 생활습관의 변화로 그 발생률은 해마다 증가되고 있다. 식이는 당뇨병조절에 아주 중요한 요소이지만 아직 적절한 식이는 알려지지 않고 있다. 우리는 최근에 고단백질의 식이가(casein 60%) 화학적으로 유도된 당뇨쥐에 있어서 혈당, 혈중지질을 조절하는데 유익한 것으로 사료된다고 보고 하였다. 그래서 이 유익한 효과가 단백질 급원을 casein대신에 식물성 단백질인 콩단백질(soy protein isolate)로 하여도 나타나는지 알아보는 것이 바람직하다고 생각한다. 이 연구의 목적은 우리의 전번 연구와 비교하기 위한 것이다. 이 논문은 콩단백질의 량이(soybean 20% VS 60%) 당뇨쥐와 비당뇨쥐에 있어서 혈당, 혈중지질, 인슐린 그리고 글루카곤 호르몬에 미치는 영향을 실험 하였다.

실험결과 당뇨쥐에 있어서 고콩단백질 식이군에서 중성지질의 농도가 낮았다. 그러나 고콩단백질을 먹인 당뇨쥐에서 control식이를 먹인 것과 비교했을 때 hypocholesteroemic한 효과를 볼 수 없었다. 더욱이 고콩단백질 식이를 먹인 당뇨쥐는 control식이를 먹인 당뇨쥐보다 혈당 농도가 더 높게 나타났다. 이 연구에서 단백질의 량이 당뇨쥐에 미치는 인슐린과 글루카곤 혹은 인슐린 글루카곤의 비율에 미치는 효과는 없었다. 고단백질식이가 혈중 중성지질을 낮추는 효과외엔 전번 연구에서 고단백질(casein) 식이를 streptozotocin으로 유도한 당뇨쥐에게 섭취시켰을 때 나타났던 유익한 효과를 볼 수 없었다.