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Relationship between Higher Protein Contents in the Diet and Adipose Tissue Fat Accumulation (Ⅱ)
-Effect of isocaloric low, medium and high protein diets on the cellular activities of rat liver-

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높은률의 단백질 함유 식이와 지방 세포의 지방축적과 상호 관계(Ⅱ)
-동 열량의 저, 중, 고 단백식이가 흰쥐의 간 세포활성에 미치는 영향-

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국 문 초 록

카제인 8%, 13%, 18% 수준의 동열량 함유 식이를 8%를 기준으로 하여 1일동일 열량 섭취하도록 급식시킨 이유쥐의 성장, 지방축적, 에너지 소비를 관찰하였다. 30일 급식시켰을 때 낮은 수준의 단백식이 (8%)군의 몸무게는 높은 수준(18%)의 단백섭취군 보다 43.01g 더 가벼운 것으로 나타났다 (최종 몸무게증가 ; 85.57±7.5g 대 128.58±11.64g, $p < 0.001$).

도살체당 지방축적량은 8% 단백식이군의 작은 몸의 크기에도 불구하고, 13%, 18% 단백식이군과 비교할 때 차이를 보이지 않았다. 이와 반대로, 체질소 함량은 13%와 18% 단백식 군들이 8%보다 훨씬 높았다.

총 실험기간(32일)동안 8%, 13%, 18%군의 에너지 소비는 각기 4,576.61kJ, 5,440.80kJ, 5,607.67kJ로 추정되었다. 낮은 수준의 단백 식이군이 섭취한 과다에너지는 손실된 것으로 보인다. 간세포의 malic enzyme 활성은 식이변화에 따라 변동이 없는 것으로 나타났다. 이러한 실험결과들로 부터, 낮은 수준의 단백식이의 성장지연은 성장에 필요한 열량부족에서 온 것이 아니라, 질소공급의 부족에서 초래된 것이며, 단백질 급여 제한은, 에너지 섭취를 같게한 고단백군들과 비교할 때, 지방이외 무게는 감소시키는 반면, 체지방 합성과 축적능력에는 영향을 미치지 않는다는 결론을 얻었다.

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ABSTRACT

The growth response, lipid deposition, fat free body mass and energy expenditure of weanling rats fed the equal amount of isocaloric diets containing 8%, 13% and 18% casein were investigated. After a period of 30 day feeding, the rats fed low level of protein diet were 43.01 g lighter than 18% protein group (weight gains of 85.57± 7.50 g vs. 128.58 ± 11.64 g, $p < 0.001$). Despite of the smaller body size, there were no significant differences in lipid deposition in grams per carcass. Whereas, nitrogen accumulation was significantly greater in 13% and 18% protein fed groups compared to 8%. The estimated energy expenditure were 4,576.61 kJ, 5,440.80 kJ and 5,607.67 kJ for 8%, 13% and 18% protein groups respectively. The part of excess energy consumed by the low protein group may have been dissipated.

The malic enzyme activity in the liver of rats was found to be unaltered by different dietary treatments.

From these observations, it was conluded that the retarded growth response in lower protein level may have been originated from the shortage ge of protein supply rather than that of the energy. The protein restriction appeared to be resulted in the lower fat free compartment without affecting the ability of rats to synthesize body lipid in a similar rate to the higher protein group when energy intakes were equalized.

INTRODUCTION

It has been well documented that protein deprivation of rats during early post weaning periods decreases the overall growth potential and the epididymal adiposity[1)2)3)], while the overnutrition with high protein diet, in a range of 15 to 25 percent, results in increase in total adipose mass during that time or later in life[4)5)]. However, the exact mode of lipogenesis at the different levels of protein with controlled energy intake has not been elucidated. The retarded growth response accompanied with low protein diet has been generally considered to be resulted from the shortage of energy as well as protein. However, Coward et al.[6)] have shown that the food consumption was not reduced in rats maintained on low protein : energy diets. Moreover, it has been suggested that the animal is facing the problem of how to deal with a surplus rather than the energe shortage as judged by the theoretical estimation of energy requirements for maintenance and growth of rats[7)].

The objectives of this study were to determine if the lower protein diet is in a short supply of energy for the better growth of rats by comparing the growth response of rats on a low protein : energy diet to that of higher protein : energy diets when the total energy intakes were controlled to the same level. The relationship between these dietary treatments, and lipid deposition and lipogenic activity in the liver, as indicated by *in vitro* examination of malic enzyme (EC 1.1.1.40) activity, was also investigated.

MATERIALS AND METHODS

Twenty-one-day-old weanling, male, Fischer rats (obtained from the Seoul National University Small Animal Laboratory) were used in the present study. By random block design rats were allocated into three groups with eleven rats each. The mean body weight of rats in each group was 49.31 ± 3.7 g. The first group was fed *ad libitum* a diet containig 8% casein (Table 1). The second group was pair-fed with the first group on an isocaloric diet containing 13% casein. The third group received the 18% isocaloric casein diet in amounts equal to those eaten by the first group. After 32 days, the rats were killed by cervical dislocation. Portions of the liver were promptly removed and homogenized in cold 0.32 M sucrose-3.0mM $MgCl_2$ and centrifuged at 100,000 × g for 45 minutes at 4℃. The clear cytosol was taken for the measurement of malic enzyme. Ma-

Table 1. Composition of diets

Ingredient	8% protein	13% protein	18% protein
	g/100 g diet.		
Casein	8.9[a]	14.5[a]	20.0[a]
Corn starch	51.6	48.3	45.0
Sucrose	25.0	22.7	20.5
Corn oil	5.0	5.0	5.0
Cellulose	1.0	1.0	1.0
Mineral mix[1]	5.5	5.5	5.5
Vitamin mix[2]	3.0	3.0	3.0

1. Mineral mixture(g) $CaH_2PO_4 \cdot 2H_2O$ 29.587; $CaCO_3$ 4.86; $MgSO_4 \cdot 4H_2O$ 6.195; NaCl 1.204; $NaHCO_3$ 1.203; $MnSO_4 \cdot 4H_2O$ 0.1883; $CuSO_4 \cdot 5H_2O$ 0.0255; ferric ammonium citrate 0.1947; $ZnCl_2$ 0.036; KI 0.02745; K_2HPO_4 11.95.
2. Amount per kg of diet: retinol acetate 14,881 IU; cholecalciferol 1,654IU; α-tocopheryl acetate 82.7mg; ascorbic acid 744.3mg; inositol 82.7 mg; menadione 41.35mg; p-amino-benzoic acid 82.7mg; niacin 74.43mg; riboflavin 16.54mg; pyridoxine hydrochloride 16.54mg; thiamine hydrochloride 16.54mg; calcium pantothenate 49.62mg; biotin 0.3308 mg; folic acid 1.4886mg; vitamin B-12 0.0223mg.

a. The amount of casein was adjusted to have the actual protein content per 100 g diet of 8%, 13% and 18% respectively (protein=N×6.25).

lic enzyme was assayed by the method of Hsu and Lardy[8]. The results are expressed as units per milligram of cytosol protein. One unit is defined as that amount of enzyme required to catalyze the reduction of 1nmol NAD/min using an extinction coefficient for NAD of $E_{340}^{m\,mol}=6.3$. Proteins were assayed by the method of Lowry et al.[9].

For the carcass analysis, the gut was removed, washed thoroughly and then replaced in the carcass. The carcasses were frozen at -40℃ and kept until analyzed. Frozen carcasses were thawed at the room temperature, weighed and softened in an autoclave at 100℃ for 45 minutes prior to homogenization. The homogenate was used to determine carcass fat by the method of Bligh and Dyer[10]. Nitrogen content of carcass was assayed by the Kjeldahl method[11].

Diets were supplied at 900 hours daily. Spilled food was collected and weighed, and food consumption was calculated every day. The mode of pair feeding was the method of presenting the animal on a 13 or 18% casein diet with the amount of food eaten by its control on the previous day. Animals were weighed once a week. Body length (nose to tail) and tail length were measured in the beginning of the experiment and a prior to killing.

In order to obtain the carcass energy content, fat was assumed to represent 39.3 kJ/g and protein 18.8 kJ/g[12]. Maintenance requirement of energy was assumed to be $586 \times W^{0.75}$ kJ/day, and the cost of energy deposition to be 10.8 kJ/kJ depesited[7].

Statistical evaluation was carried out by student's t-test.

RESULTS

Body weight gain, food intake, weight gain per g N and changes of body and tail lengths are shown in Table 2. Body weight gain was sig

Table 2. Weight gain, total food intake, N intake, weight gain per g N intake and changes in body demension[1]

Group	Weight gain	Total food intake	N Intaintake	Weight gain per g N intake	Change in body length	Change in tail length
	g	g	g	g	cm	cm
8%	85.57 ± 7.50	285.06 ± 19.08	3.64 ± 0.24	23.50 ± 2.06	10.06 ± 1.15	5.06 ± 0.73
13%	118.86 ± 6.90[a]	285.06 ± 19.08	5.92 ± 0.39	20.07 ± 1.16	11.99 ± 1.13[b]	6.33 ± 0.77[b]
18%	128.58 ± 11.64[a]	285.06 ± 19.08	8.21 ± 0.54	15.66 ± 1.41	12.41 ± 0.70[a]	5.87 ± 0.74[c]

1. Values are means ± SD; n = 11.
a. Significantly different from 8% group, $p < 0.001$.
b. Significantly different from 8% group, $p < 0.005$.
c. Significantly different from 8% group, $p < 0.025$.

nificantly lowered in 8% protein diet group. Rats given 8% protein diet *ad libitum* consumed 63.44 *g/kg* body weight/day, and intake of 52.51 *g/kg* body weight/day and that of 49.62 *g/kg* body weight/day were observed for 13% and 18% groups respectively. The weight gains per gram N consumed were 23.50 *g* for 8% protein diet group, 20.07 *g* for 13% and 15.66 *g* for 18%.

Even though there were statistically different values of body and tail length increment in 13% and 18% groups, body length decrease was only 10% less than 13% or 18%. There were 31.95% decrease in weight changes.

Carcass analysis showed that total body fats were 17.48 *g*, 19.85 *g* and 18.99 *g* per carcass for 8%, 13% and 18% casein groups (Table 3). When the fat content was expressed as a percentage of body weight, a value of 14.16% was obtained for 8% group, and those of 13.07% and 11.89% for 13% and 18% diet groups. There was no significant effect of dietary protein level on the carcass fat content at the same level of energy intake. A slight difference was noted in carcass fat percentage between 8% and 18% groups. Fat free body mass was calculated to be 110.90 ± 7.41 *g*, 131.58 ± 5.82 *g* and 140.75 ± 12.30 *g* for each of 8, 13 and 18% protein groups. A significant difference was found in N deposition of 18% protein diet group compared to that of 8% group. The carcass N percentages of higher protein groups were not different from that of 8% group.

The results of energy balance study of the animals are presented in Table 4. All the animals consumed on average 5,699.73 kJ of energy during the experimental period. The estimated energy expenditure for maintanance and tissue deposition of rats fed 8% protein was 4,576.61kJ. As the protein contents of the diet were increased to 13% and 18%, energy expenditure was also increased to 5,440.80 kJ for 13% group and 5,607.67 kJ for 18% group. The eight percent protein group showed the highest energy dissipation. The diffe

Table 3. Body composition of rats[1)]

Group	Total lipid (*g*)	% Fat	Total N (*g*)	% N	FFBM (*g*)
8%	17.48 ± 2.92	14.16 ± 2.10	2.86 ± 0.24	2.24 ± 0.15	110.90 ± 7.41
13%	19.85 ± 3.41[d]	13.07 ± 1.91[d]	3.52 ± 0.25[a]	2.32 ± 0.13[d]	131.58 ± 5.82[a]
18%	18.97 ± 2.86[d]	11.89 ± 1.61[c]	3.43 ± 0.36[b]	2.15 ± 0.16[d]	140.75 ± 12.30[a]

1. Values are means ± SD; n = 11.
a. Significantly different from 8% group, $p < 0.001$.
b. Significantly different from 8% group, $p < 0.005$.
c. Significantly different from 8% group, $p < 0.02$.
d. Not significantly different from 8% group.

Table 4. Energy balance[1)]

Group	Energy intake	Energy expenditure[a]	Maintenance energy requirement	Energy used for tissue deposition[b]	
				Fat	Protein
	kJ	kJ	kJ	kJ	kJ
8%	5699.73	4576.61	3892.21	487.79	196.61
13%	5699.73	5440.80	4613.00	580.86	247.02
18%	5699.73	5607.67	4797.32	546.94	263.43

1. Expressed kJ/experimental period.
Data were obtained by the calculation utilizing the maintenance energy requirement per unit weight and energy consumed per unit tissue deposition (for the details, see materials and methods), and body composition analysis.
a. Energy expenditure = maintenance energy requirement + energy used for tissue deposition.
b. Final energy content − initial energy content.

Table 5. Hepatic malic enzyme activity[1)]

Group	Malic enzyme activity	Liver weight
	U per *mg* protein	*g*
Initial	662.92 ± 50.3^{a} (3)	-
8%	281.72 ± 60.34 (6)	6.59 ± 1.09
13%	259.42 ± 64.83^{b} (6)	5.51 ± 0.46^{b}
18%	240.31 ± 83.37^{b} (6)	6.05 ± 0.70^{b}

1. Values are means $\pm$ SD ; parenthesis indicates number of rats
a. Significantly different from 8% group, $p < 0.01$.
b. Not significantly different from 8% group.

rences in energy dissipation between 8% and 18 % groups appeared to be 1,031 kJ.

Regardless of dietary treatment, levels of malic enzyme activity were not significantly different among three dietary groups (Table 5). However, the levels of enzymatic activity were greatly lowered from that of initial weaned rats.

DISCUSSION

The present study demonstrates that diet in low protein content can cause growth restriction not because of energy insufficiency, but due to the suboptimal intake of protein, since the energy balance study shows that at least 1,031 kJ of energy appeared to be lost in rats received lower protein diet. Differences in energy utilization may be attributed to differences in physical activities.

Although there were significant reductions in body weight gain, and body and tail lengths increment in response to the intake of lower protein diet, isocaloric intake of different protein level did not seem to cause significant differences in carcass fat content. This is contrast to adult rats, since Donald et al.[13)] have demonstrated that rats fed isocaloric diets containing 15 and 25 percentage of protein for the period of 9.5 weeks gained 100 *g* excess body weight and this increase was resulted from body fat deposition. Food intakes were not different among low and high protein groups. The fact that any noticeable increase in body lipid deposition was not found in high protein groups in the present experiment may indicate that rats with high protein diets were under the slight restriction of energy required for the further fat accumulation. With *ad libitum* feeding, there were significantly higher fat content in rats fed high protein diet[14)]. Morgan and Naismith[15)] had shown that with the marginally reduced intake of energy, high protein diet cause hyperplastic growth, while there were small increases in body fat. Feeding the low protein diet or underfeeding in early life affects the rate of cell differentiation, especially fat cell differentiation[16)]. If, in the present study, fat cell differentiation had been affected in low protein group, it is possible that rats fed low protein diet may have a fewer fat cells with larger size. It has been demonstrated in pigs that the administration of 6% protein diet beginning at 1.8*kg* weight resulted in increased fat cell size and a lower number of fat cells in adipose tissue compared to 15% protein diet[17)]. The possibility that high protein diet at the restricted energy intake may cause the greater number of fat cells in small size remains to be elucidated. Also it would be interesting to find out that, between fat cell differentiation and muscle cell differentiation, which is influenced in a geater degree by energy restricted high protein diet. The increased fat cell differentiation followed by feeding high protein diet with high energy may result in obesity in adult. Once adipose cells are differentiated into a great deal of numbers, there seems to be an alternation in energy and fat metabolism. Obese animals were found to be more efficient in energy retention[18)] and obese rats preferentially convert available metabolites into body lipid[19)20)21)], even at the expense of lean body mass[22)23)]. Relationship between protein levels, and energy expenditure and altered fat deposition in accelerated fat cell differentiation requires further studies.

The mean value of malic enzyme activity appears to be slightly increased in 8% group, but there were no significant differences among three

groups. This may indicate that the similar rate of lipogenesis is occuring among three protein groups, which may account for the similarity of total body fat content. The reason why there are elevated response of malic enzyme activity in initial group can be explained by age differences, since the activity of malic enzyme is progressively decreased as rats get older[24], and this is related to the decrease in the m-RNA coding for this enzyme[25].

Carcass analysis showed that low protein group synthesized markedly less body tissue than high protein group. This means high protein utilized the gseater portion of protein or energy for tissue deposition. Therefore, low protein fed rats must have had a higher energy loss. There were many attempts to explain the origin of excess energy loss in rats fed low protein diets. There were slight variation in the heat production of rats with differences in dietary protein concentrations[26]. Tulp et al.[27] and Rothwell et al.[28] have shown that the increased rate of diet-induced thermogenesis in brown adipose tissue of weanling rats fed 8 percent protein compared to 22 percent protein diet.

Although the low protein diet may not achieve full growth potential, considering the fact that the dietary factors responsible for the acceleration of growth, especially acceleration of fat cell differentiation, may be involved in the failure to attain the full longevity[29], one should not overlook its long term effect.

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