

COMBINATION EFFECT OF PROTEIN NUTRITION AND TREAD-MILL EXERCISE ON THE CONCENTRATION OF SERUM IGF-1 AND TRANSFERRIN IN GROWING MALE RATS

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Backgrounds and Objectives

Meat is an excellent proteinaceous food rich in well-balanced amino acids and vitamin B group in spite of the lower content of vitamin C and carbohydrate. However, since red meat usually contains large amounts of cholesterol and fat rich in saturated fatty acids, impression of red meat imagined by consumers, especially of beef, is inferior than white meat (chicken and fish meat), judging from the standpoint of human health. Recently, we have shown that accumulation of fat in rat body fed with a diet containing beef tallow as a fat source is less than that of rats fed with a diet containing vegetable oils. Based on this result and also on a general idea that saturated fatty acids are more resistant against oxidation than polyunsaturated fatty acids, we have suggested that beef tallow is less hazardous to health than vegetable oils (Tajima et al., 1995; Kawahara et al., 1997). On the other hand, there have been almost no animal studies investigating nutritional value of beef protein on the growth of animals, so far. As a matter of fact, nutritional studies of proteins have been made usually by using casein as a protein source. Therefore, real nutritional and physiological contributions of beef proteins to the growth and health of animals and human beings have not been elucidated so far.

It is well known that the growth of muscle, i.e., acceleration of muscle protein synthesis and inhibition of protein degradations, is regulated by the following three factors: (1) sufficient circulatory supply of amino acids to muscle cells, (2) frequent contraction of muscle, and (3) adequate concentration of serum insulin (Fulks et al., 1975). It has been shown the stretch overloading of chicken muscle results in an increase in protein content of the muscle (Carson et al., 1995). IGF-1 is another growth factor which stimulates the uptake of glucose and amino acids into muscle cells and increase the synthesis of protein and nucleic acids (Turkalji et al., 1992; Monier et al., 1981; Turkalji et al., 1992). It has also been demonstrated that IGF-1 is a strong inhibitor of the degradation of muscle proteins both in vivo and in vitro (Ballard et al., 1981; Turikalji et al., 1992). The concentration of serum IGF-1 is also dependent on nutrition, especially on protein nutrition (Takahashi et al., 1990).

The objectives of the present study were to investigate combination effect of beef protein nutrition and a voluntary exercise on the growth of male rats and on the concentration of serum insulin-like growth factor-1 (IGF-1) of growing male rats in order to know whether a voluntary exercise is able to contribute to the growth of rats or not, when rats were fed with two different protein diets, beef protein and soybean protein.

Materials and Methods

Animals and diets

Twenty four of Brown-Norway (BN) rats (male, 5 weeks old) were purchased from a commercial animal breeder (Seac Ltd., Fukuoka, Japan). After the rats were raised with a commercial diet (Charles River CRF-1) for 1 week in Kyushu University Biotron in order to accustom to the conditions of 20 °C in temperature, 60 % in humidity and 12 hr in lightening, 24 rats were weighed and then divided into 4 groups as follows by adjusting total weight of each group of rats to almost the same weight as possible as we could: (a) 6 rats fed with a diet containing 20% (wt/wt) beef powder as a protein source with 30 min/day voluntary exercise {Each rat was put on a treadmill (Kori Seiki Co. Ltd, Tokyo) and the rat was subjected to run freely on the treadmill for 30 min. During the running rotated counts of the treadmill was checked every two min. Also during the treadmill running, a back-ground music (waltz) was beamed from a tape recorder}, (b) 6 rats fed with the same diet as in (a) without the voluntary exercise, (c) 6 rats fed with another diet containing soybean protein powder (Fuji-Pro, Fuji Oil Ltd., Osaka, Japan) as protein source with the voluntary exercise, and (d) 6 rats fed with the diet as in (c) without the voluntary exercise. The composition of beef powder (freeze-dried) was 82.5% protein, 7.0% lipid, 5.8% ash and 2.0% water, while that of soybean protein powder was 90.0% protein, 1.0% lipid, 3.7% ash and 4.7% water. During 4 weeks' feeding, each rat in a separate stainless-steel wire cage was freely accessible to the diet (30 g/rat/day) and water. After the feeding each rat was anaesthetized with ether and was killed according to the guideline for Animal Experiment in the Faculty of Agriculture and the Graduate Course, Kyushu University and the Law [No.105] and Notification [No.6] of the

Government. Serum, hind leg muscles, and liver were collected and were stored at -80 °C until use.

Serum IGF-1 and transferrin

The concentration of serum IGF-1 was determined according to a method of Uchijima et al. (1990). The principle of this method is to get a free IGF-1 by passing the complex of IGF-1 and IGF-1-BPs (IGF-1 binding proteins) in the serum through a HPLC (Waters Protein-pak 125, 0.78 × 30 cm, MILLIPORE) gel filtration column at acidic pH (pH 2.8). The resulting free IGF-1 was assayed by a usual radio-immuno-assay technique. The concentration of serum transferrin was determined by ELISA (Hornbech, 1991).

SDS-PAGE

SDS-PAGE of myofibrillar and sarcoplasmic proteins prepared from hind leg muscles of each rat was performed according to the procedure of Laemmli (1970) using a gradient gel of 7.5~20% polyacrylamide with Tris-glycine buffer system. Staining of the resulting bands in the gels was made with CBB.

Statistical analysis

Statistical analysis was carried out by means of a Student's *t*-test.

Results and Discussion

Body weight of all of the rats' groups increased almost linearly throughout the course of the animal feeding experiments and increasing rate was slightly higher in the rats fed with the diet containing beef powder than in the rats fed with soybean protein powder. Especially, the difference was prominent between the exercised group {(a) and (c)}, and non-exercised group {(b) and (d)}. There was a difference in total amount of intaked feed among the groups (a)-(d). Thereby, a consistent tendency was observed in feed efficiency of the diets examined, i. e., feed efficiency was usually higher in the rats fed with beef powder {groups (a) and (b)} than in the rats fed with soybean protein powder {groups (c) and (d)}. Especially, such tendency in feed efficiency was prominent in the rat group with the voluntary exercise {group (a)}. Therefore, the difference between group (a) and groups (b) - (d) was statistically significant. Also, the difference between group (c) and groups (b) and (d) was significant. This result indicates that the combination of beef protein nutrition and exercise is effective in increasing feed efficiency.

Thereby, the combination of the two factors is effective in increasing body weight and possibly in increasing muscle weight which occupies the most largest proportion in the body. In order to investigate the difference in the proportion of proteins among hind leg muscles of the four rat groups, sarcoplasmic and myofibrillar proteins were prepared from hind legs of the rats of the four groups and were subjected to SDS-PAGE. A band in sarcoplasmic protein fraction, whose molecular weight was 17,000 Da corresponding to myoglobin was strongly detected in electrophoretograms of every rat of the group (a) where the rats were fed with beef powder and with the voluntary exercise. In addition, there were considerable differences in the intensity of some bands whose molecular weights were 30,000~35,000 in sarcoplasmic protein fraction among the rat groups with and without the voluntary exercise. In considering a fact that there are many kinds of metabolic enzymes in the range of those molecular weights, the present results suggest the importance of the combination effect of beef protein nutrition and exercise in accelerating the growth of rats. The concentration of serum transferrin of the rats of the groups (a) and (b) where the rats were fed with beef powder was slightly higher than that of the rats of the groups (c) and (d) where the rats were fed with soybean protein powder. However, the difference was not statistically significant. The concentration of serum IGF-1 of the rats of the groups (a) and (c), where the rats were subjected to the voluntary exercise, was higher than that of groups (b) and (d), where no exercise was subjected. Especially in the case of the rats of group (a), the concentration of serum IGF-1 was most significantly higher than that of the rats of the groups (b) and (d). This result is in well accordance with the results of body weight. Therefore, we tentatively concluded that the combination of beef protein nutrition and daily exercise possibly contribute to the increase in the concentration of serum IGF-1, resulting in the increase in body weight of the animals.

References

- Ballar, J. F., Nield, M. K., Francis, G. L. and Knowles, S. E., *Acta Biol. Med. Ger.*, **40** : 1293-1300 (1981).
- Fulks, R. M., Li, J. B. and Goldberg, A. L., *J. Biol. Chem.*, **250** : 290-298 (1975).
- Hornbech, P., "Current Protocols in Immunology", Vol. 1, Eds. Colligan, J. E., Kruisbeck, A. M., Margulies, D. H., Shevach, E. M. and Strober, W., Wiley & Sons, New York, 2.1.1 - 1.2.8 (1991).
- Kawahara, S., Inoue, K., Hayashi, T., Masuda, Y. and Ito, T., *Nutr. Res.*, **17** : 1013-1023 (1997).
- Laemmli, U. K., *Nature* **227** : 680-685 (1970).
- Moxley, R. T., Rner, P., Moss, S., Skottner, A., Fox, M., James, D. and Livingston, J. N., *Am. J. Physiol.*, **259** : E561-E567 (1990).
- Monier, S., LeMarchand-Bristel, Y. and Zapf, J., *J. Mol. Cell Endocrinol.*, **37** : 109-114 (1984).
- Tajima, A., Kawahara, S., Shin, K., Imaizumi, K., Nakamura, T. and Ito, T., *Nutr. Res.*, **15** : 1429-1436 (1995).
- Takahashi, S., Kajikawa, M., Umezawa, T., Takahashi, S., Kato, H., Miura, Y., Nam, T. J., Noguchi, T. and Naito, H., *Brit. J. Nutr.*, **63** : 521-534 (1990).
- Turkalj, I., Keller, U., Ninnis, R., Vosmer, S. and Stauffacher, W., *J. Clin. Endocrinol. Metab.*, **75** : 1186-1191 (1992).
- Uchijima, Y., Takenaka, A., Takahashi, S. and Noguchi, T., *Bioschi. Biotechnol. Biochem.*, **59** : 1503-1515 (1995).