for CSF collection.

2. Restricted feeding (given 9:00 AM – 11:00 AM) caused postprandial changes in several parameters in plasma and CSF, indicating that energy source production via metabolism with the aid of microorganisms in the rumen seemed to be faster than expected in ruminants. It was confirmed that plasma and CSF glucose levels were markedly low in sheep and goats when compared with non-ruminants and the CSF/plasma ratio of glucose was maintained at a constant value (0.74 ± 0.01).

3. Lactic acid concentration in CSF of sheep and goats was about 5 folds of that in plasma. In non-ruminants, it has been reported that the concentration in plasma and CSF was nearly equal. This fact implies that lactic acid may play some role in energy metabolism in CNS of ruminants. No considerable changes were observed in electrolytes concentrations in plasma and CSF.

4. By fasting, diurnal alterations of glucose and lactic acid observed in animals with restricted feeding were disappeared but plasma FFA concentration was increased and maintained at an elevated level throughout the fasting period. These results suggest that metabolic system has been changed by fasting and that digestion via fermentation can be terminated relatively quickly. Mobilization of FFA did not seem to be involved in brain metabolism since the FFA concentration decreased in CSF by fasting and its actual concentration in CSF is as low as 1/20 – 1/400 of that in plasma.

5. In ruminants, a possibility was suggested that, other than glucose, alternative energy sources such as lactic acid may be ordinarily utilized especially in CNS in order to compensate for the shortage of glucose supply.

Thermogenic uncoupling proteins: tissue distribution and mRNA expression by treatment with β3-adrenergic agonist in obese mice.

Yoshiko NAKAMURA

Department of Biomedical Sciences
Laboratory of Biochemistry
Graduate School of Veterinary Medicine
Hokkaidoo University, Sapporo 060–0818 Japan

Obesity is a serious problem in small animal practice. Current treatment of obesity relies on reducing caloric intake, or increasing energy consumption by exercise. β3-Adrenergic agonist has been expected to become a new anti-obesity agent.

β3-Adrenergic receptor (AR) is localized specifically in the white and brown adipose tissue (WAT and BAT, respectively). Thus, administration of selective agonist for β3-AR is expected to induce lipolysis in WAT and thermogenesis in BAT, without side effects. Chronic treatment of genetically obese yellow KK mice with a selective β3-AR agonist, CL316,243, caused a significant reduction of body weight, associated with a marked decrease of white fat pad weight.

The mitochondrial uncoupling protein 1 (UCP1) is usually expressed only in BAT and acts as a key molecule for metabolic thermogenesis under the control of sympathetic nerve system. Recently, two UCP homologues have been cloned and called UCP2 and UCP3. Both proteins showed ability as uncoupler in yeast. In

this study, the effect of chronic treatment with CL316,243 on mRNA expression of UCP1, UCP2 and UCP3 was examined.

1) The single injection of CL316,243 (0.1 mg/kg) to mice caused an acceleration of lipolysis. Same result was obtained even after daily injection of CL316,243 (0.1 mg/kg) for 9 days.

2) Compared to UCP1 mRNA, UCP2 mRNA was expressed in all tissues examined, and its expression in the WAT, BAT and skeletal muscle of obese yellow KK mice was higher than that of C57BL control mice. UCP3 mRNA expressed abundantly in skeletal muscle and BAT, and there was no difference in its expression between yellow KK and C57BL mice.

3) Chronic treatment with CL316,243 resulted in an increased UCP1 expression in WAT and even in skeletal muscle. Moreover, CL316,243 increased both UCP2 and UCP3 mRNA expression in skeletal muscle, but not in WAT or BAT.

These results suggest that anti-obesity effect of β3-AR agonist might be attributable to increased energy expenditure not only by UCP1 but also by UCP2 and UCP3.

Canine leptin: molecular cloning, characterization and development of enzyme-linked immunosorbent assay

Miho IWASE

Laboratory of Biochemistry
Department of Biomedical Sciences
Graduate School of Veterinary Medicine
Hokkaido University, Sapporo 060–0818, Japan

Obesity is defined as a state of excessive body fat accumulation, and is due to an unbalance between energy intake and energy expenditure. Since obesity is also known as a major risk factor of a lot of number of diseases such as non-insulin dependent diabetes, atherosclerosis and hypertension, control of obesity is needed as well in small animal practices as in humans. For this purpose, it is necessary to evaluate the extent of obesity objective and quantitatively. However, no reliable method for assesment of obesity in small animals has not yet been established.

Leptin is the product of the gene responsible for obesity in the *ob/ob* mice strain. Leptin is synthesized and secreted by adipose tissues, and has been shown to regulate food intake and energy expenditure. Interestingly, the serum leptin level positively correlates with body fat content and body mass index, and is higher in obese human and rodents. Therefore, it is expected that serum leptin level in dogs could be also proportional to body fat content, and this trend may enable to assess obesity and adiposity in dogs. In the present study, in order to clarify the properties of canine leptin, cDNA molecular cloning, recombinant protein expression in *E. coli*, and its functional analysis were performed. Moreover, a new method to measure dog serum leptin levels was established.

1) Canine leptin cDNA was cloned by RACE method using mRNA from omental adipose tissue as a template ; nucleotide sequence in the coding region and the deduced amino acid sequence were 80–90% homologous to other species.

2) Northern blot analysis revealed abundant leptin mRNA expression in adipose tissues, but not in other tissues.