Metabolome study on canine insulin resistance and diabetes onset

（犬におけるインスリン抵抗性と糖尿病発症に関するメタボローム研究）

Summary

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Canine diabetes is classified as type 1 diabetes in human needing the insulin dosage of the life for treatment. Type 2 diabetes by the insulin resistance caused by the obesity is common in human, and it is thought that diabetes onset mechanism is greatly different from a dog in human. The aims of this study are to analyse a characteristic of the diabetes onset in the dog, and discover the differences between the hyperadrenocorticism (HAC) dog and the obesity dog. In this study, the metabolome analysis mainly performed on the above characterisation.

Chapter 1 Analysis of the insulin signaling gene expression of in peripheral blood neutrophil from hyperadrenocorticism dog.

Insulin signaling gene (IRS-1, IRS-2, PI3-K, Akt2, PKC-λ) of the peripheral blood leukocyte of the HAC dog were analysed as preliminary investigation to consider whether the impact statement of glucocorticoid can use a peripheral blood leukocyte.

In HAC dog, the gene expression of IRS-1 slightly decreased, and the gene expression of IRS-2, PI3-K and Akt2 decreased to half of the Control group. From these results, it was clear that the insulin signaling gene expression of peripheral blood neutrophils of the HAC group changed, and it was thought that it was proper to use a peripheral blood leukocyte to evaluate influence of the glucocorticoid. In chapter 2, the metabolites in the cells treated *ex vivo* with glucocorticoid was analysed using an isolated peripheral blood leukocyte.

Chapter 2 Metabolites analysis of canine peripheral blood mononuclear cells with addition of dexamethasone.

Using the established peripheral blood mononuclear cells of the culture method, the influence of glucocorticoid to metabolites of *in vitro* cultured cells was examined. Addition of 0 (control), 1μmol/L dexamethasone in canine peripheral blood mononuclear cells were cultured and extracted metabolites from cells. For the analysis of the metabolites capillary electrophoresis time-of-flight mass spectrometer was used.

As a result of analysis, 96 metabolites were identified, and a change was accepted as a result of pathway analysis mainly on TCA cycle and glycolysis/gluconeogenesis in the group of treated dexamethasone. In addition, it was suggested by tendency to increase in metabolites of the gluconeogenesis pathway upper reaches and tendency to decrease in intermediate of TCA cycle and pyruvic acid that the addition of the dexamethasone decreased a catabolic reaction of glucose in the culture canine peripheral blood mononuclear cells. Because the non-change of the glucose uptake ability and the

decreasing glucose catabolic reaction in the culture canine peripheral blood mononuclear cells by dexamethasone, an intracellular glucose concentration is maintained, and the glucose uptake to a cell is unnecessary, and it is thought that it leads to hyperglycosemia.

Chapter 3 The influence of dexamethasone and TNF-α to cultured canine skeletal muscle cells.

The glucocorticoid increasing in blood of HAC and the TNF-α increasing in blood of obesity brings about insulin resistance. Therefore dexamethasone and TNF-α were added in the culture medium of the normal skeletal muscle cell for the purpose of examining the influence that HAC and obesity gave to a skeletal muscle. Using a myotube-like cells provided by the differentiation instruction of the normal canine skeletal muscle cells, metabolites were measured gas chromatograph mass spectrometer (GC-MS) and liquid chromatograph tandem mass spectrometer (LC-MS/MS). The glucose uptake ability was evaluated by measuring quantity of intracellular 2-deoxyglucose-6-phosphate by LC-MS/MS and IRS-1, PI3-K and Akt2 gene expression were measured by quantitative PCR method.

The addition of the dexamethasone showed the decrease in much metabolites and a tendency to decrease of the glucose uptake ability, and it was suggested that a catabolic reaction of glucose in the cell decreased like the experiment using the culture canine peripheral blood mononuclear cells which I described in chapter 2. In addition, the decrease in branched-chain amino acid (BCAA) in particular was remarkable, and, as for this, it was thought with a thing by two action with glucocorticoid; 1) resolution promotion of BCAA in the cell, 2) BCAA transportation decrease in the cell. Because the decrease of the quantity of BCAA in the cell inhibits protein translation system, the atrophy of the skeletal muscle is known to happen. Because the skeletal muscle is a maximum glucose uptake organ in the living body, the atrophy of the skeletal muscle to occur because of dexamethasone is connected for decrease of the glucose uptake quantity, and it is thought that it is with one of the factors to cause hyperglycosemia in HAC.

The addition of the TNF-α showed the tendency to decrease of IRS-1 gene expression and the non-change of the quantity of sugar uptake. Glucose uptake is inhibited in rodent myotube cells with TNF-α, but even if TNF-α was added, as for the canine myotube-like cells which I used in this study, the glucose uptake ability was not restrained. Furthermore, β-amino-isobutyric acid markedly increased in canine myotube-like cells with addition of TNF-α. Glucose metabolism abnormality is known to be improved when β-amino-isobutyric acid was added in the culture medium of rodent myotube cells. Therefore, it is thought that the intracellular β-amino-isobutyric acid increase in this experiment by the

TNF-α addition is one of the factors that is hard to cause glucose metabolism abnormality in the obese dogs.

Chapter 4 The influence on serum metabolite by serum insulin and glucose levels in dogs.

Serum amino acid were analysed by GC-MS while an insulin secretion promoted by intravenous glucose tolerance test to healthy dogs

Leucine, isoleucine and valine (three amino acid is BCAA) and phenylalanine were significantly decreased in 0-60 minutes that insulin concentration showed a peak, and it was thought that it was the amino acid which reflected an insulin change precisely. These amino acids are useful for the risk evaluation of diabetes in human, in addition BCAA is taken in depending on insulin to skeletal muscle. Thus, possibility to become a useful marker to evaluate the decrease of the insulin action in the dog was shown.

Chapter 5 Comparison of serum metabolites between hyperadrenocorticism and obese affected dogs.

Serum metabolites of the HAC of the dog (HAC group) which reported in that diabetes develops following insulin resistance and the obesity of the dog (Obesity group) which the onset of diabetes of the thing which insulin resistance set up is not reported in were analysed. In this way, the difference of the metabolites in the insulin-resistant from the both obesity and HAC were analysed.

In the HAC group ALP and ALT significantly increased in comparison with the Obesity group, and these changes are in agreement with published data. It is reported that a strong positive correlation between blood cystine concentration and body-mass index in humans. In this study, cystine significantly increased in the HAC group in comparison with Control group and, on the other hand, was a tendency to decrease in the Obesity group. Thus, it was shown that there was the change that was different from human. Besides, it is reported that excessive cystine leads to insulin secretion inhibition and insulin signaling down-regulation in human. Additionally, In HAC group, serum glutamine significantly decreased comparison with Obesity group, and it is thought that the decrease of glutamine is connected for the decrease of the insulin sensitivity in human and rodent. Furthermore, in HAC group, stearoyl-Coenzyme A desaturase 1 (SCD-1) activity significantly increased comparison with Obesity group. SCD-1 activity was an index indicating what the gluconeogenesis in the liver increased in human, and gluconeogenesis sthenia was suggested in HAC group. Valine and isoleucine which are BCAA were significantly increased in both HAC group and Obesity group in comparison with the Control group.

Blood BCAA increases in a type 2 diabetes patient of human. It is explained that this is because the BCAA uptake in the cell is inhibited when insulin resistance generated. Therefore, it is thought that insulin resistance generated in both HAC group and the Obesity group together in this study.

From these, insulin resistance generated in comparison with Control group in both groups of HAC group and Obesity group, but in the HAC group, the decreased of the insulin sensitivity and gluconeogenesis sthenia were suggested in comparison with Obesity group, and it is thought that hyperadrenocorticism is in condition that diabetes is easy to on set.

In conclusion, serum BCAA decreased with serum insulin increase in the normal dogs, and the decrease of BCAA showed what was taken in a cell depending on insulin. In canine myotube-like cells which added dexamethasone, because intracellular BCAA in comparison with control group significantly decreased, it is suggested that insulin resistance increased. Furthermore, in canine myotube-like cell which added dexamethasone, glucose uptake ability was inhibited, and a catabolic reaction of the glucose tended to decrease. Also the decrease of glucose catabolic reaction was shown by the analysis result of the metabolites of canine peripheral blood mononuclear cells which added dexamethasone. On the other hand, with canine myotube-like cells which added TNF-α, β-amino-isobutyric acid reinforcing insulin sensitivity significantly increase, and it is thought that it is action to compensate insulin resistance. In addition, serum metabolites of HAC group and the Obesity group compared it with the Control group, and serum BCAA showed significantly high value. This suggests that insulin resistance generated in comparison with Control group in both groups of HAC group and Obesity group, but in the HAC group, the decreased of serum glutamine which is an index of the insulin hyposensitivity and gluconeogenesis sthenia were showed in comparison with Obesity group. Difference of these metabolites which it is recognized for HAC, but are not recognized for the obesity shows the cause that the HAC of the dogs leads to the diabetes onset, and the cause that the obesity of the dogs does not lead to diabetes. Thus, these results may be useful for further study of the diabetes onset mechanism in dogs. In addition, it is thought that the metabolome study using the cultured canine skeletal muscle cells is useful means to parse diabetes onset mechanism peculiar to the dogs.