# 論 文 の 内 容 の 要 旨

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論文題目　Studies on serum L-Carnitine level and its regulatory factors in the cat
(猫の血中 L-カルニチンとその制御因子に関する研究)

**Background and Aim**

L-carnitine is an essential nutrient needed in fatty acid metabolism, that enables the translocation of long-chain fatty acids into the mitochondria. L-carnitine is actively transported *via* Organic Cation/Carnitine Transporter (OCTN)-2 into cells. In mammals, L-carnitine is usually obtained from dietary sources and biosynthesis in the body, and the skeletal muscle in the major tissue reservoir of L-carnitine. L-carnitine has been extensively used as a nutritional supplement in humans for losing body fat and/or avoiding muscle wasting. As well as in humans, metabolic problems in cats including obesity and wasting have been considered to be important health issues during recent decades. L-carnitine supplementation to obese cats has been frequently tried and was proved to accelerate body fat loss, however, there is still limited information about L-carnitine metabolism in cats. Recently, researchers have been focusing on the blood levels of L-carnitine to assess the relationship between L-carnitine metabolism and certain pathophysiological conditions. Serum L-carnitine

concentrations elevate in human patients with hepatic cirrhosis, malignancy and chronic heart failure, and also in dogs with hepatic disorders. On the other hand, the significance of serum L-carnitine concentrations in cats is entirely unknown. The present study was designed to obtain basal information about L-carnitine levels in the feline serum, and to elucidate the underlying mechanisms those change the L-carnitine levels. Chapter I describes serum concentrations of total, free and acylated L-carnitine in the sera of clinically healthy cats and diseases cats. Chapter II deals with *in vitro* models of the skeletal muscle to simulate the release of L-carnitine from skeletal muscle cells under hypoxic or oxidative conditions. In Chapter III, a microarray technique was employed to analyze the gene expressions of murine skeletal muscle cells under hypoxic or oxidative stress *in vitro* to reveal the mechanism of L-carnitine release from cells.

**Chapter I: Serum L-carnitine levels in healthy and diseased cats**

Firstly, serum total, free and acylated L-carnitine levels were measured in 41 clinically healthy cats at various ages by using commercial kits on an automatic biochemical analyzer. A positive correlation was found between the cats' ages and the serum acylcarnitine levels, whereas serum total or free L-carnitine concentrations showed little age-related changes. Secondly, serum total, free and acylated L-carnitine levels were measured in 139 randomly selected feline patients with various diseases presented at the University of Tokyo Veterinary Medical Center between 2006 and 2012. Total and acylated L-carnitine concentrations were significantly higher in cats with diabetes mellitus, neoplastic disorders and cardiac disorders than those in healthy cats. Thirdly, relationships between serum L-carnitine levels and disease markers (serum amyloid A and haptoglobin as acute phase proteins; leptin as a marker of cachexia) were analyzed in the diseased cats. A weak but significant correlation between serum total L-carnitine and haptoglobin was confirmed. Thus, the increased serum L-carnitine, which might come from the skeletal muscle, was considered to reflect insulin resistance,

inflammatory events and/or oxidative stress in cats with diabetes mellitus, malignancy and heart failure, respectively. Also, the data suggest the possibility of serum L-carnitine levels as a positive disease biomarker in cats.

**Chapter II: *In vitro* genetic analysis of L-carnitine release mechanism from murine skeletal myotubes under oxidative or hypoxic stress**

To simulate the L-carnitine release from the skeletal muscle under oxidative and/or hypoxic conditions, *in vitro* models were employed. Mouse $C_2C_{12}$ myoblasts and commercially available primary cultures of feline skeletal myocytes were used for this aim. These cells were differentiated into myotubes and pre-cultured in culture media supplemented with 250 mM L-carnitine to construct the intracellular L-carnitine pools. The L-carnitine uptake was blocked by OCTN2 inhibitors (amiodarone, carvedilol, propantheline and verapamil), indicating the myotubes well presented OCTN2. After that, mouse and feline myotubes were cultured under an oxidative model (cells cultured in the presence of 0.5 mM $H_2O_2$ for 12 hours) or a hypoxic model (cells cultured under 100% nitrogen atmosphere for 12 hours), and the intra- and extra-cellular L-carnitine amount was measured. Both in the oxidative and hypoxic models, release of L-carnitine into the extracellular space was accelerated. Additionally the OCTN2 inhibitors had no inhibitory effect on the L-carnitine release from the myotubes. In this Chapter, the disease-related release of L-carnitine from the skeletal muscle was reproduced at least in part *in vitro*, however, the efflux pathway of L-carnitine could not be identified. Intramyocellular L-carnitine might leak *via* passive permeability and/or unknown route.

**Chapter III: *In vitro* genetic analysis of L-carnitine release mechanism from murine skeletal myotubes under oxidative or hypoxic stress**

To reveal the mechanism of L-carnitine release from murine $C_2C_{12}$ myotubes, a microarray technique was employed to analyze the gene expressions under the same oxidative

or hypoxic conditions as in Chapter 2. Significant up-regulation of *Mrpl52* and down-regulation of *RNF220* were the major changes, indicating strong expression of mitochondrial transcript and suppression of the ubiquitin proteasome system, respectively. On the other hand, no significant changes in gene expression related to L-carnitine metabolism was observed. Despite the definite mechanism of intramyocellular L-carnitine release remained unclear, certain changes in mitochondrial function during energy loss might affect the maintenance of the L-carnitine pools.

**Conclusion**

This study revealed the increase in feline serum L-carnitine level in diseased cats. The increased level was considered to reflect the L-carnitine release from the skeletal muscle accompanied by insulin resistance, inflammatory events and/or oxidative stress. The release of intramuscular L-carnitine under oxidative and/or hypoxic conditions may be *via* unknown pathways including passive permeability, and may be caused by muscular energy loss.

To date, L-carnitine has been supplemented to healthy and diseased cats with little evidence and little consideration. From the standpoint of this study, in diseased cats especially with diabetes mellitus, malignancy and heart failure, the serum L-carnitine level seems to be saturated by the release of L-carnitine from the skeletal muscle. To utilize L-carnitine for diseased cats, appropriate consideration about the L-carnitine metabolism would be needed.