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学位論文全文に代わる要約
Extended Summary in Lieu of Dissertation

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学位論文題目：
Title of Dissertation

Effects of Anti-insulin Resistance Food Materials and Endurance Exercise on Skeletal Muscular Fat Metabolism in Rats
（インスリン抵抗性改善剤および持久性運動が骨格筋脂質代謝に及ぼす影響）

学位論文要約：
Dissertation Summary

Diabetes is a chronic disease that occurs either when insulin is not enough produced by the pancreas or when insulin cannot be effectively utilized by the body. According to the report (Danaei et al., 2011), 347 million people have type 1 or type 2 diabetes in the world. Type 2 diabetes (T2DB) comprises 90% of people with diabetes around the world (WHO, 1999), and is largely the result of excess body weight, obesity, insulin resistance (IR) and physical inactivity. While the majority of patients with T2DB in Western countries are accompanied by overweight and obese, in Asia countries including Japan, 60% of patients with T2DB are characterized as non-obese and lean (Brunetti et al. 2007). Therefore, excess fat accumulation not only in the adipose tissues but also in the ectopic tissues such as skeletal muscles and liver is reviewed to be connected with IR and T2DB in humans and animals (Bosma et al. 2012).

Excess fat accumulation in skeletal muscles has been known to contribute to the development of IR and T2DB, but the regulatory mechanisms has not perfectly clarified and controversial topics are left to be unsolved. Uptake and accumulation from the endogenous and exogenous fat in skeletal muscles are regulated by some enzymes, such as lipoprotein lipase (LPL), stearoyl-CoA desaturase (SCD), and diacylglycerol transferase (DGAT). In particular, regulation of the SCD activity and triacylglycerol (TAG) accumulation in skeletal muscles by dietary nutrients has been focused.

SCD is a rate-limiting enzyme for TAG synthesis and responsible for the conversion of saturated fatty acid to their respective unsaturated fatty acids. Decrease in the SCD activity is reported to inhibit TAG synthesis and fat accumulation in the liver, skeletal muscles, and total body, which can lead to improvement of IR and T2DB. A lot of food materials have been reported to suppress the SCD activity and TAG accumulation in the liver and adipose tissues, but these effects have not been well-clarified in skeletal muscles.

DGAT also have been known to play an important role in TAG storage in adipose tissues, liver, and skeletal muscles, although the regulatory mechanisms in skeletal muscles have not been clarified (Shi and Cheng 2009). DGAT catalyzes the final step in TAG synthesis by using diacylglycerol (DAG) and FA-CoAs as substrates. DGAT1 deficient mice show a beneficial metabolic phenotype, most notably a reduction in the postprandial rise of blood TAG level, resistance to

diet-induced obesity, increased insulin sensitivity. Therefore, modulating the TAG synthesis pathways via DGAT1 inhibition as well as SCD inhibition can have tremendous therapeutic potential in the treatment of dyslipidemia, obesity, IR, and T2DB.

Recently, numerous food ingredients in order to protect from obesity, T2DB, and IR and to suppress enzymatic activities of SCD1 and DGAT1 have been focused. Dietary proteins are also not only body protein sources but also functional food factors to have an anti-IR and T2DB effects. Dietary proteins can affect IR and T2DB differently via various mechanisms, but to the present, anti-IR and T2DB effects of egg white (EW) or egg white hydrolysate (EWH) have not yet evaluated from the aspect of the skeletal muscular fat metabolism.

In the Experiment 1, I evaluated the suppressive effects of EWH on TAG accumulation and SCD index of skeletal muscles in rats with T2DB. Twelve male Wistar rats or 12 T2DB Goto-Kakizaki (GK) rats aged 5-weeks-old were divided into two dietary groups fed a casein diet or EWH diet, respectively, and the rats were fed with their respective diet for 6 weeks. At the end of the test period, the serum and several tissues were collected after a 12 h-fasting for the biochemical analyses of glucose and fat metabolism. As the results, I revealed that the higher TAG accumulation and SCD indices in the skeletal muscles of GK rats were decreased by dietary EWH in parallel with improving insulin sensitivity. On the other hand, DAG contents in the skeletal muscle were not exactly detected by the thin-layer chromatography (TLC) method because of lower contents of DAG, which was difficult to determine the DGAT activity in skeletal muscles.

Next, in the Experiment 2, I evaluated the suppressive effects of dietary EW and EWH on fat accumulation in total body, liver, and skeletal muscles of rats *ad libitum* fed with a high-fat and high-sucrose diet (HFSD). Twenty male Wistar rats aged 4-weeks-old were divided into three dietary groups fed a casein diet (C), EW diet (E), and EWH diet (EH). The rats were *ad libitum*-fed with their respective HFSD for 8 weeks. During the final 3 days of the test period, the all excreted feces were collected, dried, weighed, and then powdered for fecal fat analyses. At the end of the test period, all the rats were euthanized after a 12 h-fasting to collect the serum and several tissues for the analyses of glucose and fat metabolism. As the results, dietary EW and EWH significantly or slightly suppressed TAG accumulation in skeletal muscles, liver, and total body by not only inhibiting the food intake and fat absorption but also inhibiting the SCD indices in the skeletal muscles and liver. The clear mechanism for lower food intake in the rats fed with EW has not been clarified, but the marked lower level of serum leptin can be related as a possible mechanism.

Next to the Experiment 2, I evaluated the suppressive effects of dietary EW and EWH on fat accumulation in total body, liver, and skeletal muscles of rats pair-fed with the HFSD (Experiment 3). Eighteen male Wistar rats aged 4-weeks-old were placed into three dietary groups fed with the casein diet (C), EW diet (E), and EWH diet (EH). The rats of the C and E groups were pair-fed with their respective HFSD for 8 weeks. The experimental protocol was the same as the Experiment 2. As the results, the suppressive effects of EW and EWH on fat absorption and TAG accumulation were also observed in the liver and skeletal muscles, but not adipose tissues, carcass, and total body. As well as the lower leptin level of rats fed the EW diet, the serum leptin level was lower in the E

group than the C group.

These Experiments1~3 concluded that dietary EW and EWH can be effective protein materials to protect from ectopic fat accumulation in the skeletal muscles and liver, IR, and T2DB in spontaneous T2DB or HFSD-fed rats.

On the other hand, thiazolidinediones (TZDs) is well-known as a kind of medications used in the treatment of T2DB and IR. TZDs are known to stimulate peroxisome proliferator receptor gamma (PPAR-γ) and facilitate the differentiation of adipocytes and generate small adipocytes, resulting in the improvement of adiponectin secretion and insulin sensitivity. Unlike anti-obesity materials such as EW and EWH described above, TZDs are well-known to facilitate TAG accumulation in adipose tissues and induce obesity in parallel with improving adiponectin secretion and insulin sensitivity. As excess body fat accumulation had closely associated to the development of IR and T2DB, this has been controversial and the regulatory mechanisms in skeletal muscles need to be clarified.

Therefore, in the Experiments 4 and 5, I evaluated the effects of repeated administration of pioglitazone (PIO), a kind of TZDs, on IR and TAG accumulation in skeletal muscles in rats fed with the HFSD. Thirteen or seventeen male Wistar rats aged 4-weeks-old in the Experiment 4 and 5, respectively were divided into the control (C) or PIO (P) group. All the rats were fed with a HFSD. The rats in the P group were orally administrated with PIO (1 mg/kg, Experiment 4; 3 mg/kg, Experiment 5) once a day for 8 weeks. At 8th week of the test period, oral glucose tolerance test (OGTT) was performed. At the end of the test period, the serum and various tissues were rapidly removed after 12 h-fasting, weighed, and stored until biochemical analyses. As the results, higher administration of PIO (3 mg/kg) improved glucose tolerance and increased serum adiponectin level, TAG accumulation and SCD indices in skeletal muscles. I also partially revealed their regulatory mechanism that the increase in TAG accumulation induced by PIO was closely correlated with LPL activity in adipose tissues, but not correlated with LPL activity in the skeletal muscles. On the other hand, DAG contents in the skeletal muscle were not exactly detected by the TLC method because of lower contents of DAG, which was difficult to determine the DGAT activity in skeletal muscles. No significant differences between the C and P groups were observed in the case of lower administration of PIO (1 mg/kg) in the Experiment 4.

It has also been reported that endurance athletes exhibit excess TAG accumulation in skeletal muscles despite preserved high insulin sensitivity in the same case as the PIO administration as described in the Experiment 5. The regulatory mechanisms about excess TAG accumulation and impaired insulin sensitivity have not yet fully clarified, but swimming-endurance exercise (Ex) in combination with dietary PPAR-γ agonists can much more improve IR and T2DB.

In the following experiments, I focused on *Kaempferia parviflora* (black ginger, BG) and resveratrol (RES) as PPAR-γ agonists, and evaluated the effects of BG and RES on TAG accumulation in skeletal muscles, IR, and T2DB in rats.

In the Experiment 7, I evaluated the short-term dietary effects of BG and RES on serum biochemical components in rats previously fed with the HFSD. Twenty male Wistar rats previously fed with the HFSD for 9-10 weeks were divided into the control (CON), PIO, BG, and RES. The all

rats were fed with their respective HFSD for 8 days. The rats of the PIO group were orally administrated with the PIO (3 mg/kg) at once a day for 8 days. The rats of the BG and RES groups were fed with the HFSD containing 1.0% BG and 0.5% RES, respectively for 8 days. The non-fasting plasma was taken at day of 0 (before this experiment), 2, 3, 4, 5, 6, 7, and 8 for biochemical analyses. As the results, the short-term dietary intake of BG and RES improved the glucose metabolism in rats adiponectin-dependently or adiponectin-independently.

Next to the Experiment 7, I evaluated the long-term dietary effects of BG and RES on glucose tolerance, serum components, and TAG accumulation and SCD indices in skeletal muscles in HFSD-fed rats in combination with the swimming endurance training. Forty one male Wistar rats aged 4-weeks-old were divided into sedentary groups or Ex groups. The rats in the S groups were furthermore divided into control (C), PIO (P), BG (B), or RES (R) group. The rats in the Ex groups were also furthermore divided into control (Ex), BG (Ex + BG), or RES (Ex + RES) group. The rats of the PIO group were orally administrated with the PIO (3 mg/kg) at once a day for 8 weeks. The rats of the C, P, and Ex groups were fed with the HFSD for 8 weeks. The rats of the BG and RES groups were fed with the HFSD containing 1.0% BG and 0.5% RES, respectively for 8 weeks. The rats in the Ex groups were subjected to the 1-h Ex every second day. At 8th week of the test period, OGTT was performed. At the end of the test period, serum and various tissues were rapidly removed after 12 h-fasting, weighed, and stored until biochemical analyses. As the results, the long-term dietary intake of BG improved the serum glucose metabolic parameters, but did not remarkably increase the SCD indices and TAG accumulation in skeletal muscles, which did not mean that dietary BG improved IR by regulating skeletal muscular fat metabolism. On the other hand, Ex with or without BG or RES dramatically improved IR, but decreased TAG accumulation in skeletal muscles contrary to my expectations.

In conclusion, dietary EW and EWH improved IR and T2DB by increasing fat excretion and suppressing food intake, fat accumulation, SCD activity indices and TAG accumulation in skeletal muscles. On the other hand, PIO improved IR by increasing SCD index and TAG accumulation in skeletal muscles. Ex also improved IR, but the mechanism was by suppressing fat accumulation in the total body and skeletal muscles and SCD indices in the skeletal muscles contrary to my expectations. Dietary BG, a PPAR-γ agonist, improved IR, but their regulatory mechanisms were not clarified in my study.

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