Intracellular lipid accumulation and insulin sensitivity in muscle and liver: Fighting against “intracellular obesity”

Yoshifumi Tamura^{1,2*}, Saori Kakehi^{1,2} and Kageumi Takeno¹

¹ *Department of Metabolism & Endocrinology, Juntendo University Graduate School of Medicine, 2-1-1 Hongo, Bunkyo-ku, Tokyo 113-8421, Japan*

² *Sportology Center, Juntendo University Graduate School of Medicine, 2-1-1 Hongo, Bunkyo-ku, Tokyo 113-8421, Japan*

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Abstract Insulin resistance induced by obesity is important in the pathogenesis of metabolic syndrome and type 2 diabetes. On the other hand, recent data suggest that ectopic fat accumulation in muscle and liver induces insulin resistance in these organs, independent of obesity. For example, short-term calorie restriction greatly decreased intrahepatic lipid levels and improved hepatic glucose metabolism in patients with metabolic disease. In addition, short-term aerobic exercise decreased intramyocellular lipid accumulation and improved insulin resistance in skeletal muscle. Similarly, physical inactivity was identified as a risk factor of intramyocellular lipid accumulation during a 3-day high fat diet. Considering the fact that metabolic diseases are frequently observed in lean subjects in East Asian countries, ectopic fat accumulation in insulin target organs may be an important pathogenesis of, as well as a therapeutic target of, metabolic diseases in non-obese subjects. Thus, it may be more appropriate to call fat accumulation in insulin target organs as “intracellular obesity” rather than ectopic fat. Furthermore, it is important to perform additional research to clarify the pathogenesis of metabolic diseases in non-obese subjects.

Keywords : ectopic fat, insulin resistance, diabetes, metabolic syndrome, physical activity

Introduction

Insulin resistance induced by obesity has been hypothesized as an important factor in the pathogenesis of metabolic syndrome and type 2 diabetes. Thus, most previous studies investigated the mechanisms underlying obesity-induced insulin resistance. Accordingly, it has been hypothesized that free fatty acid (FFA) is an important substrate linking obesity and insulin resistance. For example, serum FFA levels are higher in obese insulin-resistant subjects than healthy subjects and several hours of FFA infusion in healthy subjects induced insulin resistance in both muscle¹⁻³⁾ and liver⁴⁾.

On the other hand, fat is an important substrate for producing energy, particularly in the fasting state, and most fat is stored in adipose tissue. Adipose tissue mainly exists as subcutaneous and visceral fat; however, adipose tissue also exists around and in tissues such as heart, pancreas, and skeletal muscle. In addition, a small amount of fat is also stored in all cells. In particular, intracellular lipid accumulation in insulin-sensitive tissues such as muscle and liver is recognized as an important factor in the pathogenesis of insulin resistance. In this review, we

define the intracellular fat in muscle and liver as ectopic fat and discuss the role of ectopic fat in insulin resistance and metabolic diseases.

Intracellular lipid accumulation and insulin resistance

In obese subjects, fat exists as adipose tissue in skeletal muscle. Adipose tissue in fatty muscle is also observed, for example, in Kobe beef, which is Japanese fatty beef. On the other hand, fat also exists in the cells of skeletal muscle. Thus, to precisely measure the fat content in muscle, it is necessary to distinguish between intramyocellular lipid (IMCL) and adipose tissue in the muscle, which is defined as extramyocellular lipid (EMCL). Thus, most previous studies that performed muscle biopsies quantified the amount of IMCL by Oil Red O (ORO) staining. In 1999, Szczepaniak et al. established a non-invasive method to measure IMCL and EMCL separately using proton magnetic resonance spectroscopy (¹H-MRS)⁵⁾. In addition, they also succeeded in measuring intrahepatic lipid (IHL) by ¹H-MRS. Because ¹H-MRS is a non-invasive and valid method based on physical science, a lot of research has been performed using ¹H-MRS to examine the role of intracellular lipid on metabolism in muscle and liver.

*Correspondence: ys-tamur@juntendo.ac.jp

IMCL and insulin resistance

It has been shown that intracellular fat accumulation induces insulin resistance in muscle and liver *in vitro* and in a rodent model. However, the role of intracellular fat accumulation in insulin resistance in humans has not been clarified, because tissue biopsy is generally required to test the hypothesis. Accordingly, a cross-sectional study using ^1H -MRS revealed a significant negative correlation between IMCL level and insulin sensitivity in skeletal muscle of 14 non-obese subjects with a family history of diabetes⁶. In addition, elevated serum FFA levels in humans, which was induced by infusing a fat emulsion with heparin, increased IMCL and decreased insulin sensitivity in muscle. This study also found a negative correlation between IMCL and insulin sensitivity in muscle during FFA infusion². These data suggest that in obese subjects, FFA released from enlarged adipocytes flows into muscle tissue and accumulates as IMCL, thus inducing insulin resistance.

IMCL exists mostly as triacylglycerol (TAG), which may not impair insulin sensitivity in muscle. An important substrate of lipids that is considered to link IMCL accumulation with insulin resistance is diacylglycerol (DAG). Intramyocellular DAG concentration generally increases in parallel with the amount of intramyocellular TAG. DAG is known to activate protein kinase C (PKC), and activated PKC phosphorylates the serine residues of insulin receptor substrate (IRS)-1, which subsequently impairs insulin signal transduction^{3,7}. In fact, FFA infusion in humans promoted DAG accumulation and PKC activation in skeletal muscle³.

Taken together, in obese subjects, FFA is released from enlarged adipocytes, and then flows into muscle tissue and accumulates in myocytes. DAG accumulation in myocytes activates PKC and impairs insulin signal transduction. This cascade may be at least partly involved in the mechanisms of obesity-induced insulin resistance in skeletal muscle.

Effects of calorie restriction and exercise on ectopic fat content and insulin resistance in muscle and liver

As previously described, circulating FFA may be an important source of IMCL accumulation. However, IMCL levels can change without alterations in circulating FFA levels. We previously examined the effects of calorie restriction and exercise on ectopic fat content of muscle and liver in type 2 diabetic patients⁸. We recruited 14 type 2 diabetic patients and divided them into two groups: a 2-week calorie restriction group and an exercise therapy group (mainly brisk walking in addition to the same calorie restriction protocol). Before and after treatment, we measured IMCL and IHL by ^1H -MRS, and glucose uptake in muscle and liver using the hyperinsulinemic-euglycemic clamp. After the intervention, body weight was

slightly but significantly reduced (by 2%) in both groups, whereas serum FFA levels at the fasting state were unchanged. The IHL levels were significantly decreased in both groups by ~30%, whereas hepatic glucose uptake was increased. On the other hand, exercise therapy with calorie restriction decreased the IMCL level by 19% and increased peripheral insulin sensitivity by 57%, whereas these changes were not observed in the calorie restriction-only group. We also observed that changes in physical activity level were negatively correlated with changes in IMCL level.

We also analyzed 13 non-diabetic obese subjects on a calorie restriction program⁹. After a 3-month intervention, we observed a 6.6% body weight reduction as well as improved parameters of metabolic syndrome, such as dyslipidemia and blood pressure. Glucose tolerance evaluated by the oral glucose tolerance test was also improved, with patients exhibiting lower insulin levels, suggesting improved insulin resistance. However, the IMCL levels and peripheral insulin sensitivity levels were not significantly changed. On the other hand, the IHL level was decreased by ~40% and hepatic glucose uptake was increased by 140%. In this study, we also did not observe any reduction in fasting FFA level after the body weight reduction.

From these results, it is suggested that a short period of calorie restriction greatly decreases IHL and improves hepatic glucose metabolism during moderate weight loss. On the other hand, exercise therapy may decrease IMCL and improve insulin resistance in skeletal muscle. Interestingly, these changes occurred without any changes in circulating FFA levels. Thus, it is thought that short term dietary and exercise intervention in metabolic diseases may directly decrease ectopic fat and improve metabolic states in liver and muscle, respectively, independent of circulating FFA levels.

Cause of IMCL accumulation: "Susceptibility to fat loading"

IMCL levels are affected by dietary composition. Several groups reported that a high-fat diet increases IMCL levels in healthy subjects^{2,10,11} as well as endurance runners^{10,12}. We recruited 7 endurance runners and 7 sprinters and investigated the effect of high-fat loading on IMCL level in the tibialis anterior muscle (TA). We found that 3 days of fat loading significantly increased IMCL levels in endurance runners, but not in sprinters¹². Bachmann et al.² also reported that a 3-day high-fat diet increased IMCL levels in the TA and decreased insulin sensitivity. They also reported a high interindividual variation in IMCL changes after fat loading. These data suggest the existence of interindividual differences in susceptibility to the accumulation of IMCL upon fat loading, and this susceptibility may modulate insulin sensitivity during high-fat diet intake.

To search for determinants of IMCL accumulation by high-fat loading, we recruited 37 non-obese men and provided a high-fat diet for 3 days¹³. We observed increases in IMCL in the TA and soleus muscle (SOL) of ~30% and ~20%, respectively, and the increase in SOL-IMCL negatively correlated with a change in muscle insulin sensitivity by the high-fat diet. These data suggest that the susceptibility to IMCL accumulation upon fat loading may influence the changes in insulin sensitivity after a high-fat diet. Next, we searched for determinants of susceptibility to IMCL accumulation after high-fat loading, and found that subjects with lower levels of high-molecular weight (HMW) adiponectin had higher susceptibility to IMCL accumulation after a 3-day high fat diet. This data is consistent with a previous report showing that adiponectin is secreted from adipocytes and increases AMP-activated protein kinase (AMPK) activity and decreases IMCL levels, resulting in improved insulin sensitivity in animal models¹⁴. We also observed that lower daily physical activity in sedentary subjects was closely associated with more IMCL accumulation after high-fat loading. This relationship is consistent with previous observations showing that physical activity rapidly decreases IMCL levels in healthy subjects^{10,15}.

Taken together, a high-fat diet, physical inactivity, and lower HMW adiponectin may coordinately increase IMCL and impair insulin sensitivity in skeletal muscle, independent of obesity. This cascade may be important in the pathophysiology of metabolic diseases in East Asian countries, because type 2 diabetes can develop relatively easily in people with a normal body mass index (<25 kg/m²) in these countries.

Athlete's paradox phenomenon

Accumulation of IMCL is also observed in endurance athletes, although their insulin sensitivity level is high¹⁶. This phenomenon is known as the athlete's paradox^{16,17}. Although the exact mechanisms of athlete's paradox is not fully understood, it has been suggested that diacylglycerol acyltransferase (DGAT)-1 may be a key molecule. DGAT catalyzes the formation of TAG from DAG. One bout of aerobic exercise was found to be sufficient to increase the expression level of DGAT1 in muscle and to prevent FFA-induced muscle insulin resistance in healthy humans¹⁸. Consistent with DGAT1 expression levels, one bout of exercise after FFA infusion was found to increase TAG levels and decrease DAG levels in skeletal muscle¹⁸. Overexpression of muscle-specific DGAT1 also exhibited similar phenotypes¹⁹. DAG is known to impair insulin signal transduction through PKC activation^{3,7}, and it has been hypothesized that DAG1 expression and decreased DAG levels play roles in the mechanisms involved in the athlete's paradox phenomenon.

It is also hypothesized that the oxidative capacity of skeletal muscle may be an important modulator of the

association between IMCL accumulation and insulin sensitivity^{16,20}. It has been reported that endurance athletes with IMCL accumulation have a higher oxidative capacity in skeletal muscle than insulin-resistant subjects with similar IMCL levels¹⁶.

It was also reported that muscle oxidative capacity is a better predictor of insulin sensitivity than IMCL accumulation²⁰. In addition, in sedentary obese subjects, 16 weeks of aerobic exercise increased IMCL and insulin sensitivity, which was concomitant with increased oxidative capacity²¹. Thus, the athlete's paradox phenomenon can occur in non-athlete subjects, and differences in oxidative capacity may be a determinant of insulin sensitivity.

Recently, we have shown the possibility that differences in the gene expression of fatty acid transporters lead to differences in insulin sensitivity and oxidative capacity in IMCL-accumulated subjects²². We recruited 36 non-obese healthy men and compared gene expression levels in the vastus lateralis muscle between IMCL-accumulated high insulin sensitivity subjects (H-GIR, n = 8) and low insulin sensitivity subjects (L-GIR, n = 9). Consistent with the athlete's paradox theory, maximum oxygen uptake was higher in the H-GIR group than in the L-GIR group. We also found that several lipid oxidation genes were up-regulated in the H-GIR group, and this was associated with higher expression levels of plasma membrane-associated fatty acid-binding protein (FABPpm) and lower expression levels of fatty acid transport protein (FATP)-1. Interestingly, a previous report demonstrated that FABPpm overexpression in skeletal muscle induced similar fatty acid transport rates, but enhanced fatty acid oxidation compared with FATP1-overexpressed muscle²³. It has also been demonstrated that endurance training increases FABPpm expression²⁴ while decreasing FATP1 expression²⁵. These data suggest that endurance exercise changes the expression levels of fatty acid transporters, thus regulating oxidative capacity in skeletal muscle. To examine the effect of fatty acid transporters, we overexpressed either the FABPpm or FATP1 gene in C2C12 myotubes, and found that overexpression of FABPpm increases fatty acid oxidation coupled with an increase in the expression of genes related to fatty acid oxidation; however, these changes were not observed in FATP1-overexpressed myotubes. Taken together, it is hypothesized that gene expression patterns of fatty acid transporters may influence oxidative capacity in skeletal muscle as well as IMCL accumulation and insulin sensitivity. Because endurance exercise increases the expression level of FABPpm in skeletal muscle²⁴, FABPpm is a candidate gene involved in the athlete's paradox phenomenon.

Conclusions

Obesity and insulin resistance have been recognized as important factors involved in the pathogenesis of metabolic diseases such as type 2 diabetes and metabolic syn-

drome. In most Asian countries, metabolic diseases develop relatively easily even in lean subjects; however, the pathogenesis of metabolic diseases in non-obese subjects has not been fully understood. Recent data demonstrated that lifestyle factors, such as diet and physical activity, can directly regulate intracellular lipid accumulation and insulin sensitivity in muscle and liver independent of body weight change. These data suggest that ectopic fat accumulation in insulin target organs may be an important therapeutic target of metabolic diseases in non-obese subjects. Thus, we should call fat accumulation in insulin target organs as “intracellular obesity” rather than ectopic fat, and perform further research towards revealing the pathogenesis of metabolic diseases in non-obese subjects.

Conflict of Interests

Yoshifumi Tamura has received lecture fees from Takeda Pharmaceutical Co., MSD, Boehringer Ingelheim, Sanofi-Aventis, Eli Lilly, Novartis Pharmaceuticals, Kissei Pharmaceutical, Ono Pharmaceutical and Astrazeneca. Yoshifumi Tamura, Saori Takehi and Kageumi Takeno have received research funds from Boehringer Ingelheim, Pfizer, Mochida Pharmaceutical Co. Sanofi, Novo Nordisk Pharma, Novartis Pharmaceuticals, Sanwakagaku Kenkyusho, Terumo Corp., Eli Lilly, Mitsubishi Tanabe Pharma, Daiichi Sankyo Inc., Takeda Pharmaceutical Co., MSD, Shionogi Pharma, Dainippon Sumitomo Pharma, Kissei Pharma, Johnson and Johnson, Bristol-Myers Squibb Company, Taisho Toyama Pharmaceutical, Ono Pharmaceutical, Kowa and Astrazeneca.

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