Etiology of Insulin Resistance in Asian Non-Obese Subjects
–Juntendo Sportology Center Core Study–

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Obesity and its associated metabolic disorders are rapidly disseminating all over the world. While they became serious health problem, such diseases in Asians shows different features from those in other races such as Caucasian and African. One of the important features is a susceptibility to develop to type 2 diabetes even without excess body mass index. To search for the mechanism underlying this feature is important to find the suitable therapeutic strategy for Asian obesity. Thus, as a core study of the Sportology Center of Juntendo University Graduate School of Medicine, we assessed tissue-specific insulin resistance in Japanese non-obese subjects. In this study, we recruited non-obese and non-diabetic Japanese subjects and measured insulin sensitivity in muscle and liver by a 2-step hyperinsulinemic-euglycemic clamp with glucose tracer and ectopic fat content in muscle and liver by proton magnetic resonance spectroscopy. So far, our data suggests that muscle insulin resistance may play a central role in future onset of the diseases that could make the healthy people to disable people.

**Key words:** insulin sensitivity, euglycemic–hyperinsulinemic clamp, ectopic fat, MRS

**Abbreviations**

BMI; body mass index, FABPpm; plasma membrane-associated fatty acid-binding protein, IMCL; intramyocellular lipid content

**Pathophysiology of Asian type 2 diabetes mellitus**

Two main defects observed in type 2 diabetes mellitus are insulin resistance and beta cell dysfunction. The combination of these two defects causes decreased effect of insulin that is a sole hormone to reduce blood glucose level, thus, results in hyperglycemia. In each patient with type 2 diabetes mellitus, the different ratio of these two defects seems to present. Accordingly, type 2 diabetes mellitus is considered to be a heterogeneous disease. Given that the onset of most diseases is affected by genetic factors and environmental factors, environmental factors are believed to strongly affect the onset of insulin resistance. The key environmental factor is westernized life style such as high fat intake and less exercise. The exposure of westernized life style causes overnutrition that changes the systemic adiposity. The abnormal adiposity such as enhanced visceral fat accumulation is regarded as the main cause of insulin resistance. Abnormal adiposity with signs of insulin resistance is defined as metabolic syndrome and the metabolic syndrome is regarded as a risk factor for cardiovascular diseases and also for type 2 diabetes. However, the presence of type 2 diabetes mellitus is a stronger risk factor for cardiovascular diseases and also causes a characteristic complication of diabetes such as retinopathy, nephropathy, and neuropathy (Figure-1).

According to the latest report from International Diabetes Federation, the number of the patients with diabetes is increasing. In 2013, there are 382 million people with diabetes mellitus in the world. In addition, whereas mean body mass index (BMI) are variable among the countries, the prevalence rate of

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diabetes is similar among the countries\textsuperscript{1).} Given that Asian patients with diabetes are relatively lean, Asian is susceptible to develop to type 2 diabetes even with normal body mass index (BMI) (<25 kg/m\textsuperscript{2})\textsuperscript{1-3}. Thus, a recent position statement from American Diabetic Association indicated that testing for diabetes should be considered for all Asian American adults who present with a BMI of $\geq 23$ kg/m\textsuperscript{2}\textsuperscript{3}. In addition, Asian also easily developed to metabolic syndrome compared with BMI matched non-hispanic Whites and African Americans\textsuperscript{4}. One possible explanation of this feature is that beta cell function in Asian is weaker than that in other races. However, a recent study comparing the beta cell function between Japanese and Caucasian could not find the distinct ethnic difference of beta cell function considering the state of insulin resistance\textsuperscript{5).}

**Insulin resistance and ectopic fat accumulation**

Another possibility is that susceptibility of insulin resistance by overnutrition could be different between Caucasian and Japanese. Overnutrition results in accumulation of excess energy storage in whole body. Adipose tissue, especially subcutaneous adipose tissue is regarded as a suitable place to store the excess energy as a form of triglyceride. However, the accumulation of triglyceride in different places causes different systemic metabolic state. Indeed, increased size of visceral, not subcutaneous adipose tissues is known to cause systemic insulin resistance. In addition, ectopic fat accumulations in muscle and liver are observed in such cases and are considered as a cause of insulin resistance in each organ\textsuperscript{6}. These facts suggest that the feature of adiposity may play a key role on the susceptibility for type 2 diabetes in Asian.

Regarding this possibility, Azuma \textit{et al}, compared amount of visceral and liver fat accumulation considering BMI level between Japanese and Caucasian. Intriguingly, while visceral fat accumulation of Japanese is similar to that of Caucasian after
adjustment of BMI level, Japanese easily develop to fatty liver compared with Caucasian. These data suggest that susceptibility for ectopic fat accumulation may play important role in the progression of insulin resistance in Asian with normal BMI level.

Therefore, we have investigated the etiology of ectopic fat accumulation and its relation to insulin resistance in Japanese. The study of high fat diet intervention for normal subjects revealed that high molecular weight adiponectin and daily physical activity are determinants of intramyocellular lipid content (IMCL) accumulation by a high-fat diet. Similar intervention with the analysis of mRNA expression in muscle sample revealed that IMCL accumulation and impaired insulin sensitivity after high fat diet are closely associated with changes in the expression of genes related to lipid metabolism in muscle. In addition, moderate exercise intervention study in the patients with type 2 diabetes mellitus revealed that exercise decrease IMCL and improve insulin resistance. Furthermore, diet restriction in subjects with impaired glucose tolerance showed that diet therapy decrease intrahepatic accumulation of triglyceride and enhance insulin sensitivity. These data suggest that in Japanese subjects, ectopic fat accumulation seems to be tightly associated with insulin resistance (Figure-2).

Sportology Center Core study

To investigate the etiology and the role of tissue specific insulin resistance in Japanese non-obese men especially further focusing on ectopic fat accumulation, we planned Sportology Center Core study supported by High Technology Research Center Grant from MEXT. In this study, we recruited subjects who were non-diabetic and aged between 30 and 50 years men and obese men with metabolic syndrome as positive control of metabolic disorders with obesity. In this study, we assessed dietary composition and alcohol intake by questionnaire, physical activity by accelerometer, IMCL, and intrahepatic lipid by proton magnetic resonance spectroscopy, maximum oxygen uptake by ergometer, visceral fat area by computer tomography and high molecular weight adiponectin, free fatty acids and other biochemical tests by standard measurement. In addition, we also assessed 2-step hyperinsulinemic euglycemic clamp with glucose tracer. We used the endogenous glucose production suppression at 1st step as an index of hepatic insulin sensitivity and rate of disappearance at 2nd step as an index of muscle insulin sensitivity (Figure-3).

So far, we found the subjects showing various pattern of insulin resistance, even in non-obese, non-diabetic subjects; subjects with neither, or either or both of muscle and liver insulin resistance.
We found that alcohol intake is one of the major determinants of liver insulin resistance. On the other hand, as the determinants of muscle insulin resistance, not only the factors associated visceral fat accumulation but also, factors related to quality of the muscle, factors associated with systemic microinflammation, and factors associated with daily diet and exercise were identified. These results clearly suggest the different etiology of muscle and liver insulin resistance in Japanese non-obese, non-diabetic subjects.

Regarding the relation between ectopic fat accumulation and tissue specific insulin resistance, liver fat accumulation is associated well with muscle insulin resistance. On the other hand, muscle fat accumulation did not show significant association with muscle insulin resistance. Intriguingly, we found modest association between muscle insulin resistance and muscle fat accumulation by excluding the subjects whose VO$_2$ max is high. Also, we found that some subjects with high muscle fat accumulation show high insulin sensitivity. Thus, we focused the subjects with high fat accumulation in muscle, we compared mRNA expression in muscle between the subjects with insulin resistance and those without insulin resistance. As a result, several lipid oxidation genes in muscle were up-regulated in the subjects without insulin resistance, and this was associated with increased expression of higher plasma membrane–associated fatty acid–binding protein (FABPpm) and decreased expression of fatty acid transport protein–1. Over-expression of FABPpm in culture myocytes increased fatty acid oxidation coupled with the elevated expression of genes related to fatty acid oxidation. These results suggest that the level of FABPpm expression may play an important role in insulin sensitivity in the fat accumulated muscle$^{13}$ (Figure–4).

Using the data of Sportology Center Core study, we tried to estimate the outcome of insulin resistance. The presence of insulin resistance in muscle highly associated with cardiovascular risk factors. In addition, it also highly associated with fatty liver that is the well–known basis of liver cancer. Furthermore, our study revealed that insulin resistance is also associated with white matter alteration that may be linked with future onset of dementia$^{14}$. Taken together, muscle insulin resistance may play a central role for future onset of the diseases that could make the healthy people to disable people (Figure–5). Thus, to find the factors that cause insulin resistance and to prevent the onset of insulin resistance seems to be efficient method to prevent the future onset of the disease that makes healthy people disabled.

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References

8) Sakurai Y, Tamura Y, Takeno K, et al: Determinants of


