FSP27 is a potent player regulating lipid storage in liver as well as adipose tissue

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Obesity has increased dramatically worldwide and leads to numerous disorders including cardiovascular diseases, type 2 diabetes, dyslipidemia and cancers. These pathological states are closely associated with hyperinsulinemia or insulin resistance. Obesity itself is characterized by excessive triacylglycerol (TAG) storage as lipid droplets (LDs) in adipose tissue due to an increase in nutrient intake and insufficient energy expenditure. Excessive TAG storage in adipocytes causes adipose tissue inflammation and deregulation of adipokines, both of which induce systemic insulin resistance. At the same time, a hallmark of obesity is TAG accumulation in the liver and skeletal muscle, which is also important in inducing insulin resistance in these tissues. These findings underline the importance of lipid accumulation in insulin-sensitive organs in the development of insulin resistance and type 2 diabetes.

Lipid droplets are comprised of a central core of neutral lipids containing TAG and cholesterol ester and surrounded by a phospholipid monolayer. This monolayer of LDs is decorated with a variety of proteins, which contribute to the formation of the droplets, the synthesis and hydrolysis

of lipids, and the movement of these lipids to specific intracellular and secretory pathways. The ability to store TAG in LDs is evolutionarily conserved in yeast, plants, invertebrates and vertebrates. In mammals, excess energy is primarily stored as TAG in LDs in adipose tissue. Especially white adipocytes are specifically differentiated cells for storing TAG as the large unilocular LDs that occupy most of the cell volume. The LD size can be in the 100 µm range. Such TAG storage in LDs serves a vital role as the body's major stored energy supply. In the case of energy demand, such as starvation and exercise, TAG reserves are hydrolyzed by lipolysis to supply free fatty acid (FFA) to a variety of tissues. LDs are also recognized in non-adipose cells; however, non-adipocyte LDs are usually much smaller than those of adipocytes. Such TAG storage in liver, skeletal muscle and heart is thought to serve as a local energy supply. Recently, a fat-specific protein of 27 kDa (FSP27) was found to be the determining factor for LD size and critical for large unilocular LD formation in white adipocytes [1–3].

FSP27 was first identified in 1992 as a fat-specific protein whose expression was regulated by C/EBP, although its function in adipocytes remained elusive then [4]. Subsequently, FSP27 was reported to be a member of the CIDE family of proteins, comprised of CIDE-A, CIDE-B and FSP27, which had homology with the amino-terminal domain of DFF-45 (45-kDa subunit of the DNA fragmentation factor) [5]. Later, CIDE-A was shown to be expressed in brown adipose tissue in mice and to inhibit energy expenditure [6]. CIDE-B is expressed mainly in the liver and kidney and is important for the maturation of VLDL as apolipoprotein B binding protein in the liver [7]. As for FSP27, functional analysis of FSP27 has robustly progressed since knockout (KO) mice were generated in 2008 [1, 3], although FSP27 was already found to be a LD

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protein in 2004 [8]. FSP27 was abundantly expressed in adipose tissues. The amount of white adipose tissue in FSP27 KO mice was much smaller than that in wild-type mice, and white adipocytes of FSP27 KO mice showed multilocular LD formation along with increased mitochondrial biogenesis such as brown adipocytes [1]. FSP27 KO mice displayed an increased metabolic rate and were protected from diet-induced obesity. Thus, FSP27 was assumed to render energy metabolism to the energy-storing state by efficiently accumulating TAG as unilocular LD in adipocytes. Because unilocular LD is the form with the minimum surface area, lipolysis from the LD surface by lipase accession can be repressed to a maximum degree. The detailed mechanism by which FSP27 promotes unilocular large LD growth still remains unclear. However, it has been revealed that FSP27 is highly enriched at LD-LD contact sites and mediates lipid transfer between these LDs, suggesting the promotion of each LD fusion [9]. Recently, it was also reported that FSP27 promoted the enlargement of LD in concert with perilipin 1, known as another major LD protein expressed abundantly in adipocytes [10, 11].

In human studies, adipose FSP27 expression has also been shown to be involved in insulin resistance and type 2 diabetes. FSP27 expression in subcutaneous adipose tissue was negatively correlated with body mass index (BMI) and fat mass, but not with insulin sensitivity [12]. However, in obese patients, FSP27 expression in white adipose tissue was correlated with insulin sensitivity [13]. Given that FSP27 expression was decreased by proinflammatory cytokine $\text{TNF}\alpha$ [1], which was induced in adipose tissue in obesity, the decrease in adipose FSP27 expression in obesity may be a result of obesity, but not a cause of it. In 2009, the homozygous premature truncation mutation of the CIDE-C gene, an ortholog of FSP27 in humans, was found to cause partial lipodystrophy, white adipocytes with multiloculated LDs and insulin-resistant diabetes [14]. This mutation failed to increase the LD size in the cultured cells. However, the metabolic phenotype of CIDE-C deficiency in humans was different from that in FSP27 KO mice, which were protected against diet-induced obesity and insulin resistance. Thus, the physiological role of FSP27 in glucose and energy metabolism may be different between mouse and human, although the effect on LD morphology is the same.

Besides adipose tissue, FSP27 is also expressed in muscle and liver, although its expression level is much smaller compared to adipose tissue. Liver plays a very important role not only in carbohydrate metabolism, but also in lipid metabolism. Calorie overload increases the delivery of FFA to the liver, leading to hepatic steatosis, in which intrahepatic LDs are enlarged with increased expression of LD proteins. In fact, increased hepatic FSP27 expression was closely associated with steatosis of leptin-

deficient genetically obese ob/ob mice [15]. This suggests the importance of FSP27 in liver for efficient intracellular TAG storage. Indeed, FSP27 was known to induce hepatic steatosis in a $\text{PPAR}\gamma$ -dependent manner in mice [15, 16]. However, little was known about the physiological role of FSP27 in the liver. The important role of the liver is producing the glucose needed to maintain normoglycemia in fasting. During the early stage of fasting, the liver produces glucose mainly through glycogenolysis. During the later stage of fasting, the liver produces glucose by gluconeogenesis. Liver uses FFA originating from adipose tissue as a source of energy to support this latter process. Very recently, a novel concept was presented by Vilà-Brau et al. [17]: hepatic FSP27 expression could be regulated nutritionally. During the initial stages of fasting (6–15 h fasting), FSP27 was induced at the maximum level, while during the latter phase of fasting (24 h fasting), its expression decreased [17]. This early increase in FSP27 with fasting can be explained by activation of a canonical PKA-CREB-CRTC2 signaling pathway possibly induced by glucagon. On the other hand, the latter decrease of FSP27 is supposed to result from Sirt1 activation, which is thought to be a molecular switch between an early and late fasting signaling program in liver [18]. Such a change may reflect the adaptation that hepatocytes efficiently store the delivered FFA from adipose tissue as intracellular TAG for the energy source in preparation for further fasting, because liver can produce glucose by glycogenolysis in the early phase of fasting (Fig. 1). Indeed, it is well known that fasting in mice leads to TAG accumulation in liver. Thus, the increased expression of hepatic FSP27 in this period consequently works favorably for the TAG storage in hepatocytes. In addition, FSP27 depressed the mitochondrial β -oxidation activity in hepatocytes [15], which is also supposed to restrict the consumption of FFA as an energy source. Moreover, inhibition of FFA oxidation increased FSP27 expression in turn [17]. These responses suggest that the increase in FSP27 is important for transient and thrifty TAG storage in early fasting liver. However, in the longer phase of fasting in which hepatic glycogen has been decreased or depleted, the liver obtains the energy required for gluconeogenesis through the mitochondrial β oxidation of FFA, which was provided from adipose tissue by lipolysis. In that period, hepatocytes do not yet need to store FFA delivered from adipose tissue intracellularly. Thus, a decrease in FSP27 expression in this period is supposed to be advantageous for the hepatic consumption of FFA (Fig. 1). Such nutritional regulation of hepatic FSP27 expression may thus be important for effective energy metabolism in the liver. These findings may show that FSP27 plays an important role not only in efficient TAG storage as unilocular LDs in white adipocytes, but also in transient TAG storage in the initial stage of fasting

Fig. 1 Hepatic FSP27 contributes to the formation of the intracellular reservoir for TAG in the liver in preparation for long-term fasting. In the early phase of fasting, glucose output is supposed to be carried out mainly by glycogenolysis. Thus, FFA delivered from adipocyte is reesterified to TAG, which is stored efficiently in LD by FSP27. In the

late phase of fasting, glucose output is changed to gluconeogenesis. In this period, hepatic FFA is utilized in mitochondrial β -oxidation as the energy source for gluconeogenesis, which requires high energy. A decrease of hepatic FSP27 expression is favorable for converting lipid metabolism from FFA storage to FFA consumption

by forming LDs in hepatocytes. Disturbance of such physiological regulation of hepatic FSP27 may cause liver steatosis or hepatic insulin resistance. For example, it remains elusive whether hepatic FSP27 expression is associated with the development of fatty liver diseases in humans.

In summary, LDs have gained recognition as important organelles that are closely associated with cellular metabolism, although they were previously assumed to be merely passive organs for lipid storage. Furthermore, recent studies revealed the importance of small LDs in non-adipose cells as well as large LDs in adipocytes in whole-body energy metabolism. FSP27 regulates the formation of both LDs according to whole body metabolic states. Future studies will surely unveil the detailed mechanism by which FSP27-mediated LD formation in insulin-sensitive tissues controls the systemic glucose, lipid and energy metabolism.

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