

## REVIEW

# Saturated fatty acids and insulin resistance

Makoto Funaki

*Clinical Research Center for Diabetes, Tokushima University Hospital, Tokushima, Japan ; and Department of Physiology, Institute for Medicine and Engineering, University of Pennsylvania, Philadelphia, PA, USA*

**Abstract :** Insulin resistance is one of the pathophysiological features of obesity and type 2 diabetes. Recent findings have linked insulin resistance to chronic low-grade inflammation in white adipose tissue. Excess storage of saturated fat in white adipose tissue due to a modern life style causes hypertrophy and hyperplasia of adipocytes, which exhibit attenuated insulin signaling due to their production and release of saturated fatty acids. These adipocytes recruit macrophages to white adipose tissue and, together with them, initiate a proinflammatory response. Proinflammatory factors and saturated fatty acids secreted into the bloodstream from white adipose tissue impair insulin signaling in non-adipose tissues, which causes whole-body insulin resistance. *J. Med. Invest.* 56 : 88-92, August, 2009

**Keywords :** saturated fatty acid, insulin resistance, inflammation, white adipose tissue, adipocyte

## PHYSIOLOGICAL ROLE OF ADIPOSE TISSUE IN ENERGY BALANCE

Evolutionally, humans have developed the ability to store excess calorie intake in adipose tissue as fat, which can be used in times of famine (1). Dietary fat absorbed into epithelial cells of the intestines is assembled into one type of lipoprotein, chylomicrons, enters the bloodstream, and is transported to peripheral tissues. Lipoprotein lipase (LPL) in adipocytes, one of the main destinations of chylomicrons, hydrolyses triglyceride (TG) in chylomicrons into free fatty acids (FFAs) and glycerol. Adipocyte LPL also hydrolyses TG in VLDL (very low density lipoprotein), supplied from the liver and in remnants of both chylomicrons and VLDL, into FFAs and glycerol. Adipocytes take up FFAs and glycerol and reassemble them into TG and store it in lipid

droplets (2). When necessary, stored TG in adipocytes can be hydrolysed by their adipose triglyceride lipase (ATGL) and hormone-sensitive lipase (HSL) (3), and used as energy, which may help individuals in situations where the caloric intake falls short of its demand. Due to a robust food supply and sedentary life style in industrialized nations, the amount of caloric intake exceeds that of calorie demand in many individuals. Thus, white adipose tissue (WAT) in these individuals keep accumulating TG and growing both in size and in number, resulting in obesity and even type 2 diabetes through the development of insulin resistance (4).

## PHYSIOLOGICAL ROLE OF ADIPOSE TISSUE IN ENDOCRINE SYSTEM

Recent studies have revealed that adipose tissue not only serves as a storage site for fat but also functions as an endocrine organ by secreting a wide range of hormones and cytokines (2, 5). For instance, leptin production by adipocytes, which is upregulated in large adipocytes (6), regulates food

Received for publication July 6, 2009 ; accepted July 21, 2009.

Address correspondence and reprint requests to Makoto Funaki, Clinical Research Center for Diabetes, Tokushima University Hospital, Tokushima, Japan and Fax : +81-88-633-9679.

intake and fat mass through its action in the hypothalamus, resulting in decreased hunger and stimulated energy expenditure (7, 8). Leptin also increases lipid oxidation in the liver and lipolysis in skeletal muscle and adipocytes to control whole-body energy balance (9, 10). Adiponectin is exclusively produced by adipocytes and secreted into the bloodstream, where it self assembles into larger structures (trimer, hexamer and high-molecular weight forms). A proteolytic cleavage product of adiponectin, known as globular adiponectin, also circulates in human plasma (11). Although the biological activities of these isoforms are controversial, it appears that high-molecular weight adiponectin has a mainly beneficial role in humans and rodents (12, 13). In these studies, high-molecular weight adiponectin improved insulin sensitivity by inhibiting hepatic glucose production and enhancing glucose uptake into skeletal muscle. It also increased lipid oxidation in both the liver and skeletal muscle (14).

## ADIPOSE TISSUE DYSFUNCTION IN OBESITY

A negative impact of excess plasma free fatty acids or triglycerides, including cellular dysfunction and programmed cell death, has been reported in a number of non-adipose tissues (15, 16). Thus, hypertrophy (increased cell size) and hyperplasia (increased cell number) of WAT to store more TG in their intracellular lipid droplets is a protective mechanism against calorie overload. However, the capacity of WAT to synthesize TG from FFAs is not infinite. Challenging adipocytes with an excess amount of FFAs saturates the biosynthetic pathway of TG in adipocytes, and FFAs start to accumulate in adipocytes. FFAs accumulated in adipocytes pose endoplasmic reticulum stress (ER stress) and oxidative stress at the level of the mitochondrion, both of which cause dysfunction in adipocytes (17). Affected adipocytes have decreased TG synthesis and increased lipolysis, which results in the systemic release of FFAs. In addition, hormones and cytokines produced and released by these adipocytes are different from those by healthy adipocytes. Secretion of adiponectin is attenuated, whereas that of proinflammatory cytokines such as interleukin-6 (IL-6), tumor necrosis factor  $\alpha$  (TNF $\alpha$ ) and monocyte chemoattractant protein-1 (MCP-1) is elevated, which recruits macrophages into WAT, causing chronic low-grade inflammatory responses (18). Dysfunctional

adipocytes due to overloading of FFAs die and release their contents, which recruits neutrophils and macrophages into WAT and promotes a chronic low-grade inflammatory response in WAT (19, 20). As a result, the plasma level of inflammatory cytokines goes up and affects other tissues as well, such as liver, skeletal muscle, cardiac muscle and blood vessels, which eventually leads to insulin resistance and atherosclerosis (17).

## SATURATED FATTY ACID CONSUMPTION

Among dietary fat constituents, saturated fat, which contains saturated fatty acids (SFA), is drawing particular attention these days, since a strong correlation between SFA intake and the metabolic syndrome, which exhibits insulin resistance, has been reported (21-23). Foods that contain a high level of SFA are dairy products, fatty meats, palm oil, coconut oil and some processed foods (24). Although overconsumption of FFAs generally leads to chronic low-grade inflammation in WAT, as described above, SFAs exhibit a particularly strong effect to induce WAT inflammation (25).

## SFA-INDUCED INSULIN RESISTANCE AND INFLAMMATORY RESPONSE IN WAT

Overloading of adipocytes with SFAs, transported from the bloodstream presumably by CD36/FAT, results in accumulation of diacylglycerol (DAG), which in turn activates protein kinase C (PKC)  $\theta$  and desensitizes adipocytes to insulin stimulation (26, 27). PKC $\theta$  activates the I-kappa-B kinase (IKK) and c-Jun N-terminal kinase (JNK) pathways, which induces serine phosphorylation and degradation of IRS-1 and stimulates production and secretion of proinflammatory cytokines (28). Overloading SFAs also results in accumulation of ceramide in adipocytes, which is reported to activate IKK and JNK, as well (29, 30). Accelerated  $\beta$ -oxidation of SFAs causes excess electron flux in the mitochondrial respiratory chain, resulting in increased production of reactive oxygen species (ROS), which was reported to cause insulin resistance and an inflammatory response in adipocytes (31). Furthermore, SFAs activate Toll-like receptor 4 (TLR4) on the surface of adipocytes, which activates the NF $\kappa$ B pathway and JNK pathway (32, 33). Thus, SFAs both transported into adipocytes and bound to TLR4 impair insulin

signaling and stimulate secretion of proinflammatory factors from adipocytes.

## CROSSTALK BETWEEN ADIPOCYTES AND MACROPHAGES IN SFA-INDUCED INSULIN RESISTANCE

In obesity, hypertrophied adipocytes in WAT secrete proinflammatory cytokines (called adipokines, as they are produced in and released from adipocytes), such as MCP-1, which recruit macrophages into WAT. SFAs released from hypertrophied adipocytes bind to TLR4 on the surface of macrophages, and together with adipokines, activate macrophages. As a result, active macrophages in WAT stimulate the NF $\kappa$ B pathway to produce and secrete cytokines that impair the insulin signaling and further potentiate inflammation in adipocytes (33). In fact, TLR4 knockout mice failed to develop high fat diet-induced insulin resistance, due to a lack of NF $\kappa$ B activation, both in adipocytes and macrophages (34). Thus, infiltration of macrophages into WAT and crosstalk between adipocytes and macrophages through their SFA-TLR4-NF $\kappa$ B pathways are crucial for the development of SFA-induced chronic low-grade inflammation and insulin resistance in WAT.

## SFA-INDUCED CHRONIC LOW-GRADE INFLAMMATION IN WAT CAUSES INSULIN RESISTANCE IN NON-ADIPOSE TISSUES

SFA-induced chronic low-grade inflammation and insulin resistance in WAT affects other tissues and impairs their sensitivity to insulin, as well. SFA overflow from WAT is taken up by myotubes and hepatocytes, and accumulates in them. Similar to adipocytes, SFAs in myotubes activate signal transduction pathways through IKK and JNK, which causes serine phosphorylation and degradation of IRS-1 and desensitizes myotubes to insulin stimulation (35-38). NF $\kappa$ B activation by binding of SFA to TLR4 is also reported in myotubes (39). Since skeletal muscle accounts for the majority of insulin-stimulated whole body glucose uptake, impaired insulin signaling in skeletal muscle causes marked insulin resistance (40).

SFA-induced serine phosphorylation and degradation of IRS-1 are also observed in hepatocytes, which lead to less insulin-regulated inhibition of

hepatic glucose production and increased fasting glycemia (41). SFA is also reported to induce apoptosis of hepatocytes through the JNK pathway (42).

## CONCLUSION

Cellular dysfunction of adipocytes in WAT due to overloading of SFAs into them, designated 'lipotoxicity', is a chronic low-grade inflammation of WAT. It not only impairs insulin signaling in WAT, but also affects insulin signaling in remote tissues, resulting in whole body insulin resistance. Lipotoxicity by SFAs is one of the underlying pathophysiological mechanisms of obesity and type 2 diabetes.

## REFERENCES

1. Neel JV, Weder AB, Julius S : Type II diabetes, essential hypertension, and obesity as "syndromes of impaired genetic homeostasis" : the "thrifty genotype" hypothesis enters the 21st century. *Perspect Biol Med* 42 : 44-74, 1998
2. Hajer GR, van Haeften TW, Visseren FL : Adipose tissue dysfunction in obesity, diabetes, and vascular diseases. *Eur Heart J* 29 : 2959-2971, 2008
3. Miyoshi H, Perfield JW 2nd, Obin MS, Greenberg AS : Adipose triglyceride lipase regulates basal lipolysis and lipid droplet size in adipocytes. *J Cell Biochem* 105 : 1430-1436, 2008
4. Kiess W, Petzold S, Topfer M, Garten A, Bluher S, Kapellen T, Korner A, Kratzsch J : Adipocytes and adipose tissue. *Best Pract Res Clin Endocrinol Metab* 22 : 135-153, 2008
5. Lago F, Dieguez C, Gomez-Reino J, Gualillo O : Adipokines as emerging mediators of immune response and inflammation. *Nat Clin Pract Rheumatol* 3 : 716-724, 2007
6. Considine RV, Sinha MK, Heiman ML, Kriauciunas A, Stephens TW, Nyce MR, Ohannesian JP, Marco CC, McKee LJ, Bauer TL, et al. : Serum immunoreactive-leptin concentrations in normal-weight and obese humans. *N Engl J Med* 334 : 292-295, 1996
7. Yamauchi T, Kamon J, Waki H, Terauchi Y, Kubota N, Hara K, Mori Y, Ide T, Murakami K, Tsuboyama-Kasaoka N, Ezaki O, Akanuma Y, Gavrilova O, Vinson C, Reitman ML, Kagechika H, Shudo K, Yoda M, Nakano Y, Tobe K, Nagai R, Kimura S, Tomita M, Froguel P, Kadowaki

- T : The fat-derived hormone adiponectin reverses insulin resistance associated with both lipotrophy and obesity. *Nat Med* 7 : 941-946, 2001
8. Saladin R, De Vos P, Guerre-Millo M, Leturque A, Girard J, Staels B, Auwerx J : Transient increase in obese gene expression after food intake or insulin administration. *Nature* 377 : 527-529, 1995
  9. Cheung CC, Clifton DK, Steiner RA : Proopiomelanocortin neurons are direct targets for leptin in the hypothalamus. *Endocrinology* 138 : 4489-4492, 1997
  10. Long YC, Zierath JR : AMP-activated protein kinase signaling in metabolic regulation. *J Clin Invest* 116 : 1776-1783, 2006
  11. Fruebis J, Tsao TS, Javorschi S, Ebbets-Reed D, Erickson MR, Yen FT, Bihain BE, Lodish HF : Proteolytic cleavage product of 30-kDa adipocyte complement-related protein increases fatty acid oxidation in muscle and causes weight loss in mice. *Proc Natl Acad Sci U S A* 98 : 2005-2010, 2001
  12. Kobayashi H, Ouchi N, Kihara S, Walsh K, Kumada M, Abe Y, Funahashi T, Matsuzawa Y : Selective suppression of endothelial cell apoptosis by the high molecular weight form of adiponectin. *Circ Res* 94 : e27-31, 2004
  13. Hara K, Horikoshi M, Yamauchi T, Yago H, Miyazaki O, Ebinuma H, Imai Y, Nagai R, Kadowaki T : Measurement of the high-molecular weight form of adiponectin in plasma is useful for the prediction of insulin resistance and metabolic syndrome. *Diabetes Care* 29 : 1357-1362, 2006
  14. Kadowaki T, Yamauchi T : Adiponectin and adiponectin receptors. *Endocr Rev* 26 : 439-451, 2005
  15. Schaffer JE : Lipotoxicity : when tissues overeat. *Curr Opin Lipidol* 14 : 281-287, 2003
  16. Unger RH, Orci L : Lipoapoptosis : its mechanism and its diseases. *Biochim Biophys Acta* 1585 : 202-212, 2002
  17. de Ferranti S, Mozaffarian D : The perfect storm : obesity, adipocyte dysfunction, and metabolic consequences. *Clin Chem* 54 : 945-955, 2008
  18. Guilherme A, Virbasius JV, Puri V, Czech MP : Adipocyte dysfunctions linking obesity to insulin resistance and type 2 diabetes. *Nat Rev Mol Cell Biol* 9 : 367-377, 2008
  19. Elgazar-Carmon V, Rudich A, Hadad N, Levy R : Neutrophils transiently infiltrate intra-abdominal fat early in the course of high-fat feeding. *J Lipid Res* 49 : 1894-1903, 2008
  20. Weisberg SP, McCann D, Desai M, Rosenbaum M, Leibel RL, Ferrante AW Jr : Obesity is associated with macrophage accumulation in adipose tissue. *J Clin Invest* 112 : 1796-1808, 2003
  21. Cnop M : Fatty acids and glucolipotoxicity in the pathogenesis of Type 2 diabetes. *Biochem Soc Trans* 36 : 348-352, 2008
  22. Galgani JE, Uauy RD, Aguirre CA, Diaz EO : Effect of the dietary fat quality on insulin sensitivity. *Br J Nutr* 100 : 471-479, 2008
  23. Johnson L, Mander AP, Jones LR, Emmett PM, Jebb SA : Energy-dense, low-fiber, high-fat dietary pattern is associated with increased fatness in childhood. *Am J Clin Nutr* 87 : 846-854, 2008
  24. USDA : Composition of Foods Raw, Processed, Prepared. USDA National Nutrient Database for Standard Reference, Release 21 Available from : [http://www.nal.usda.gov/fnic/foodcomp/Data/SR21/sr21\\_doc.pdf](http://www.nal.usda.gov/fnic/foodcomp/Data/SR21/sr21_doc.pdf), 2008
  25. Kien CL, Bunn JY, Ugrasbul F : Increasing dietary palmitic acid decreases fat oxidation and daily energy expenditure. *Am J Clin Nutr* 82 : 320-326, 2005
  26. Lobo S, Bernlohr DA : Fatty acid transport in adipocytes and the development of insulin resistance. *Novartis Found Symp* 286 : 113-121 ; discussion 121-116, 162-113, 196-203, 2007
  27. Su X, Abumrad NA : Cellular fatty acid uptake : a pathway under construction. *Trends Endocrinol Metab* 20 : 72-77, 2009
  28. Gao Z, Zhang X, Zuberi A, Hwang D, Quon MJ, Lefevre M, Ye J : Inhibition of insulin sensitivity by free fatty acids requires activation of multiple serine kinases in 3T3-L1 adipocytes. *Mol Endocrinol* 18 : 2024-2034, 2004
  29. Haversen L, Danielsson KN, Fogelstrand L, Wiklund O : Induction of proinflammatory cytokines by long-chain saturated fatty acids in human macrophages. *Atherosclerosis* 202 : 382-393, 2009
  30. Subauste AR, Burant CF : Role of FoxO1 in FFA-induced oxidative stress in adipocytes. *Am J Physiol Endocrinol Metab* 293 : E159-164, 2007
  31. Lin Y, Berg AH, Iyengar P, Lam TK, Giacca A, Combs TP, Rajala MW, Du X, Rollman B, Li W, Hawkins M, Barzilai N, Rhodes CJ, Fantus IG, Brownlee M, Scherer PE : The

- hyperglycemia-induced inflammatory response in adipocytes : the role of reactive oxygen species. *J Biol Chem* 280 : 4617-4626, 2005
32. Song MJ, Kim KH, Yoon JM, Kim JB : Activation of Toll-like receptor 4 is associated with insulin resistance in adipocytes. *Biochem Biophys Res Commun* 346 : 739-745, 2006
  33. Suganami T, Tanimoto-Koyama K, Nishida J, Itoh M, Yuan X, Mizuarai S, Kotani H, Yamaoka S, Miyake K, Aoe S, Kamei Y, Ogawa Y : Role of the Toll-like receptor 4/NF-kappaB pathway in saturated fatty acid-induced inflammatory changes in the interaction between adipocytes and macrophages. *Arterioscler Thromb Vasc Biol* 27 : 84-91, 2007
  34. Shi H, Kokoeva MV, Inouye K, Tzameli I, Yin H, Flier JS : TLR4 links innate immunity and fatty acid-induced insulin resistance. *J Clin Invest* 116 : 3015-3025, 2006
  35. Lee JS, Pinnamaneni SK, Eo SJ, Cho IH, Pyo JH, Kim CK, Sinclair AJ, Febbraio MA, Watt MJ : Saturated, but not n-6 polyunsaturated, fatty acids induce insulin resistance : role of intramuscular accumulation of lipid metabolites. *J Appl Physiol* 100 : 1467-1474, 2006
  36. Reynoso R, Salgado LM, Calderon V : High levels of palmitic acid lead to insulin resistance due to changes in the level of phosphorylation of the insulin receptor and insulin receptor substrate-1. *Mol Cell Biochem* 246 : 155-162, 2003
  37. Schenk S, Saberi M, Olefsky JM : Insulin sensitivity : modulation by nutrients and inflammation. *J Clin Invest* 118 : 2992-3002, 2008
  38. Sinha S, Perdomo G, Brown NF, O'Doherty RM : Fatty acid-induced insulin resistance in L6 myotubes is prevented by inhibition of activation and nuclear localization of nuclear factor kappa B. *J Biol Chem* 279 : 41294-41301, 2004
  39. Reyna SM, Ghosh S, Tantiwong P, Meka CS, Eagan P, Jenkinson CP, Cersosimo E, DeFronzo RA, Coletta DK, Sriwijitkamol A, Musi N : Elevated toll-like receptor 4 expression and signaling in muscle from insulin-resistant subjects. *Diabetes* 57 : 2595-2602, 2008
  40. Jue T, Rothman DL, Shulman GI, Tavitian BA, DeFronzo RA, Shulman RG : Direct observation of glycogen synthesis in human muscle with <sup>13</sup>C NMR. *Proc Natl Acad Sci USA* 86 : 4489-4491, 1989
  41. Capeau J : Insulin resistance and steatosis in humans. *Diabetes Metab* 34 : 649-657, 2008
  42. Malhi H, Bronk SF, Werneburg NW, Gores GJ : Free fatty acids induce JNK-dependent hepatocyte lipoapoptosis. *J Biol Chem* 281 : 12093-12101, 2006