doi: 10.1111/j.1467-789X.2007.00402.x

Complications of Obesity

Insulin resistance: central and peripheral mechanisms. The 2007 Stock Conference Report

J. Ye[1] and T. Kraegen[2]

[1]Pennington Biomedical Research Center, Louisiana State University, Baton Rouge, LA, USA; [2]Garvan Institute of Medical Research, Sydney, Australia

Received 20 June 2007; accepted 21 June 2007

Address for correspondence: Dr J. Ye, Pennington Biomedical Research Center, 6400 Perkins Rd, Baton Rouge, LA 70808, USA. E-mail: yej@pbrc.edu

Summary

Insulin resistance in peripheral tissues and in the brain are fundamental to the development of obesity and its associated type 2 diabetes. Multiple mechanisms contribute to the development of insulin resistance. The 2007 Stock Conference focused on the role of fatty acids and other lipid signals in initiating insulin resistance and the consequences of central insulin resistance to the regulation of energy balance and peripheral metabolism.

Keywords: Free fatty acids, inflammation, signalling pathways.

obesity reviews (2008) **9**, 30–34

The 2007 Stock Conference 'Insulin Resistance: Central and Peripheral Mechanisms' was held in Bangkok, Thailand in 29–31 March, 2007. The meeting was attended by 12 speakers and 12 attendants. Five sessions were included in the 3-day meeting. On the first day, two sessions were held with 'Insulin Resistance in Obesity: Role of Lipids' in the morning and 'Fatty Acid Utilization: A Useful Therapeutic Target in Insulin Resistance State?' in the afternoon.

Insulin resistance is a key characteristic of obesity and the metabolic syndrome and is a prelude to type 2 diabetes as well as other metabolic problems. Regarding role of lipids in insulin resistance, Dr Ted Kraegen (Conference Co-chair, Garvan Institute, Australia) reviewed the current status of the 'lipid overload hypothesis' of insulin resistance in muscle (1), and current attempts to develop therapies targeting muscle and liver to prevent cytosolic lipid accumulation and ameliorate insulin resistance, and the relationship of the lipid overload hypothesis to other recently enunciated theories of insulin resistance. Insulin resistance and lipid accumulation, particularly in liver, occur within a few days of commencing a high-fat diet in rats and may well precede other abnormalities associated with ongoing obesity. The presentation reinforced the concept of lipid overload in the cause of insulin resistance and pointed out the importance of various lipid metabolites such as long-chain fatty acyl CoAs, diacylglycerol (DAG) and ceramides in the impairment of insulin signalling (2,3). Dissecting out precise mechanisms of lipid action and designing optimal therapeutic strategies are important challenges for the next few years.

Dr Jason Kim (Penn State College of Medicine, USA) presented more evidence from transgenic mice on the deleterious effects of intracellular lipid accumulation on insulin signalling and glucose metabolism. Reduction of lipid uptake with deletion of hormone-sensitive lipase (HSL), fatty acid transporter protein 1 (FATP1), or fatty acid translocase (CD36) leads to prevention of diet-induced increase in intramuscular lipid and protection of mice from insulin resistance (4). Blockage of DAG signalling in protein kinase C (PKC)-θ knockout was demonstrated to protect mice from lipid infusion-induced muscle insulin resistance (5). Increased energy expenditure protects mice from diet-induced insulin resistance in ApoD knockout mice. The role of inflammation in insulin resistance in skeletal muscle and liver was observed in mice with modulation of cytokines such as interleukine (IL)-6 and IL-10 (MCK-IL10). Muscle-specific over-expression of IL-10 in MCK-IL10 mice increases insulin signalling and prevents diet-induced insulin resistance. Lastly, he discussed the roles of altered myocardial metabolism and insulin resistance in diabetic heart

failure. Mice with heart over-expression of peroxisome proliferator-activated receptor alpha (PPARα)or on high-fat diet develop cardiac phenotypes of diabetic heart together with insulin resistance. The evidence from transgenic mice further supports the 'lipid overload hypothesis' as the cause of insulin resistance in skeletal muscle, liver and heart.

Dr Scott Summers (University of Utah, USA) then went into some details concerning the molecular mechanisms which putitatively link lipid metabolites to impaired insulin signalling. He pointed out that metabolic fates of lipids are critical for their biological effects on insulin sensitivity. Fatty acids may be shunted into one of the three competing pathways: B-oxidation, glycerolipid formation and sphingolipid formation. His work highlights the importance of the sphingolipid ceramide, a precursor of all complex sphingolipids, in inhibiting insulin signalling at the Akt/PKB step (6). He introduced the concept that different fatty acids are likely to induce insulin resistance by different mechanisms depending on their influence on activating ceramide synthesis. In particular, saturated fatty acids (also inflammatory cytokines and glucocorticoids) can increase ceramide synthesis, and his elegant studies point to inhibition of enzymes required for ceramide synthesis as novel therapeutic targets effective in protecting against insulin resistance in obese rodents such as the Zucker Diabetic Fatty rat. Debate will go on concerning the importance of ceramides in human obesity and other insulin resistant states, but there are certainly data in support.

The symposium is timely in that there are emerging a number of interesting new prospects for enhancing the 'burning off' of fat in insulin-resistant states, but we need to consider the likelihood that they will indeed be therapeutically effective in enhancing insulin action. A 'pharmaceutical industry' perspective was presented by Dr Brendan Leighton (AstraZeneca, UK) who considered the fatty acid oxidation pathway as a therapeutic target for insulin resistance. He explored the idea that energy partitioning, particularly of lipids, plays a key role in the failure of a majority of obese subjects to maintain weight loss and associated insulin sensitivity. If this is correct, then it defines a role for agents which can partition the fate of lipids towards oxidation. He differentiated pharmacological agents on a 'push' versus 'pull' basis; a lot of interest is now centred on 'push' agents which enhance the ability of muscle to oxidise lipids and contrast with 'pull' agents which uncouple or lessen the degree of energy production from lipid oxidation. Relative interest in the pharmaceutical industry among targets can be gauged in part from the patent literature; e.g. there is a lot of interest in AMP-activated protein kinase (AMPK) activators and acetyl-CoA carboxylase (ACC) inhibitors, with increasing interest in newer possible targets such as DGAT-1 inhibitors.

Dr Ellen Blaak (Maastricht University, Netherlands) presented a valuable 'human' perspective to the physiologic mechanisms responsible for channelling a 'lipid overload' to muscle. Reasons which lead to a lipid overload in muscle may differ in humans and rodents, although the consequences of the overload for insulin action may well be similar. In the human context, the 'flexibility' of adipose tissue to trap dietary fatty acids and regulate lipolysis may be important contributors to muscle lipid accumulation. In addition she discussed whether an impaired switch between carbohydrate and fat oxidation in muscle may be another contributor. She presented evidence for this metabolic inflexibility in pre-diabetes, suggesting that it is an early factor in progression to type 2 diabetes. She discussed whether the disturbed muscle fat metabolism and lipid accumulation could at least be partially reversed by means of nutritional or pharmacological intervention in parallel with improvements in insulin sensitivity. For example one study suggested that weight loss in pre-diabetic men could lead to enhanced muscle oxidative capacity.

Dr Michael Roden (Hanusch Hospital, Austria) addressed the links between impaired mitochondrial function and insulin resistance in humans, reviewing the literature in this regard. He presented his recent work involving lipid infusions demonstrating that short-term changes in plasma insulin and fatty acids could influence ATP synthase flux in resting skeletal muscle. While free fatty acid (FFA) elevation did not seem to affect basal, it markedly inhibited insulin-stimulated ATP synthase flux in parallel with reduction in muscle glucose transport/phosphorylation (7). He also presented later studies of well-controlled non-obese type 2 diabetes subjects which found impaired ATP synthetic flux prior to intramyocellular lipid (IMCL) accumulation or impaired glucose transport or metabolism (8). Based on his findings, he suggested that decreased mitochondrial phosphorylation can be either a primary (inherited) or a secondary (because of environmental and lifestyle factors) abnormality in insulin-resistant states. Abnormalities in muscle ATP synthesis could lead to a vicious cycle in which mitochondrial dysfunction, elevation of IMCLs, impaired lipid oxidation and insulin resistance amplify each other.

Session 3 was held in the second day in the morning with a focus on insulin action in the central nervous system. Dr Darleen Sandoval (University of Cincinnati, USA) made a presentation for Dr Steve Woods about regulation of energy balance by insulin. In the scenario presented, brain insulin resistance was seen as another factor in the metabolic dysfunction associated with a high-fat diet (9). Insulin is transported into the brain from the blood by a receptor-mediated process. Neurones express insulin receptors and neurones in certain areas of the brain, especially the hypothalamic arcuate nucleus (ARC), respond to elevated insulin by initiating a catabolic response including reduction in food intake and increase in energy expenditure (10). This process is sufficient to maintain a stable body weight.

When body weight is reduced, insulin level is decreased, and then catabolic signal will be reduced until body weight returns to the normal level. Conversely, if weight is gained, the increase in insulin signal acts to limit food intake in the ARC until the weight is lost. A high-fat diet renders the ARC relatively insensitive to insulin (11). This brain insulin resistance occurs before body weight gain and tends to promote weight gain. Interestingly, men are relatively more sensitive to insulin's action in the brain than women, suggesting that the regulation of body fat in the two genders is fundamentally different. It was suggested that strategies for overcoming brain insulin resistance will be useful to control body weight.

Dr Yasuhiko Minokoshi (National Institute for Physiological Sciences, Japan) reported on central and peripheral regulation of energy metabolism by leptin's action on the AMPK system. As a background, he discussed his studies showing the bidirectional nature of AMPK regulation peripherally and centrally; thus increased AMPK activity plays an important role in the regulation of peripheral metabolism by adipocytokines such as leptin and adiponectin, whereas hypothalamic AMPK activity is inhibited by anorexigenic hormones such as leptin and insulin, high glucose and refeeding. Leptin decreases AMPK activity in the paraventricular (PVH) and arcuate hypothalamus preferentially and thus inhibits food intake. He then went on to discuss newer work showing that increased expression of constitutively active AMPK in the PVH of mice could lead to dramatic increases in food intake and body weight. Interestingly this appeared to alter food preference, favouring carbohydrate vs. fat intake. Evidence furthermore suggested that this was related to increased fatty acid oxidation in the PVH and chemically blocking PVH fatty acid oxidation reversed the food preference. Dr Minokoshi then went on to describe cell-based studies examining signalling pathways linking leptin to AMPK; these demonstrate the reciprocal actions of leptin in stimulating the alpha subunit of AMPK in C2C12 muscle cells. While inhibiting this subunit in a neuronal cell line, leptin increased fatty acid oxidation in C2C12 muscle cell. Thus AMPK is differently regulated by leptin in central and peripheral tissues.

Dr Masato Kasuga (Kobe University, Japan) continued on the theme of central effects which can influence peripheral metabolism, and his novel work has provided a biochemical mechanism for the poorly understood manner in which the brain could influence liver glucose metabolism. The presentation suggests that insulin can reduce hepatic glucose production (HGP) through an action in the brain. He first presented data showing a liver transcription factor, namely signal transducer and activator of transcription-3 (STAT3) play a key role in regulation of HGP and is required in the liver for the nerve-dependent action of insulin (12). A deficiency in STAT3 results in insulin resistance with up-regulation of hepatic gluconeogenic genes, correctable by restoration of STAT3 expression. Liver-specific expression of a constitutively active form of STAT3 markedly reduced blood glucose, plasma insulin concentrations and hepatic gluconeogenic gene expression in diabetic mice. Interestingly he and his colleagues have demonstrated that insulin activates STAT3 in the liver through an action in the brain, and intracerebral ventricular infusion of insulin was unable to suppress HGP in the liver-specific STAT3 deficient mice or IL-6 knockout mice (13). These data indicate the importance of IL-6-STAT3 signalling in the liver for the suppression of HGP by insulin.

Session 4 addressed the systemic influence of adipose tissue on insulin sensitivity. Dr Mark Febbraio (Baker Heart Research Institute, Australia) reviewed data on lipid-induced inflammation in macrophages for over-nutrition-associated insulin resistance. His data suggest that lipid is able to activate inflammatory signalling pathways [c-Jun N-terminal kinase (JNK), and inhibitor KappaB kinase (IKK)] through multiple pathways, such as endoplasmic reticulum (ER) stress, Toll like 4 receptor and CD36. CD36 is believed to transport the fatty acid into cells and thus promotes biosynthesis of ceramide and DAG. Ceramides is identified as the major deleterious lipid species in the activation of inflammatory signalling. Treatment of cells with ceramide synthetic analogues recapitulates the lipid induced signalling events. Moreover, treatment of cells with myriocin, a ceramide synthetic inhibitor, does not affect lipid-induced DAG accumulation, but prevents inflammatory signalling. Thus there is consistency with Dr Summer's conclusion that ceramide is a major lipid derivative leading to insulin resistance.

Dr Jianping Ye (Co-chair, Louisiana State University, USA) described a novel mechanism for the development of chronic inflammation in obesity. He first proposed a signalling pathway for inflammation-induced insulin resistance with a focuse on IKK-NFκB activities. IRS-1 and PPARg were shown as the targets of the IKK-NFkB pathway for inflammation-induced insulin resistance (14,15). He then presented data about the cause of chronic inflammation in obesity. His lab finds that hypoxia occurs in adipose tissue in obese mice. The hypoxia is supported by a reduction in the interstitial partial oxygen pressure (pO_2), an increase in the hypoxia probe signal and elevation in expression of the hypoxia response genes. By analysis of cause–effect relationships of adipose hypoxia and inflammatory response, he concluded that adipose hypoxia induces chronic inflammation in the adipose tissue in obesity. Data about inhibition of adiponectin expression by hypoxia were also presented. A reduction in adipose tissue blood flow (ml per min per 100 g tissue) was proposed to be involved in the development of adipose hypoxia. Additionally, an increase in adipocyte size may also contribute to the interstitial hypoxia in obesity.

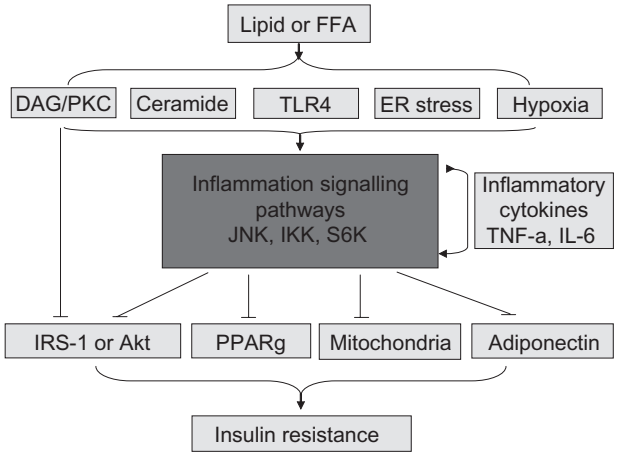

Figure 1 The signalling pathways for lipid-induced insulin resistance. The lipids or FFAs are able to activate inflammatory signals (JNK, IKK and S6K) through multiple pathways which include production of diglycerides and ceramide, activation of TLR4 and ER stress, and induction of hypoxia. These result in activation of signalling pathways for inflammatory or stress responses that is characterized by activation of PKC, JNK, IKK and S6K. This in turn leads to increased expression of inflammatory cytokines that enhance the inflammatory or stress pathways in a positive feedback. The activation of PKC, JNK, IKK and S6K is able to block insulin signalling pathway through functional inhibition of several targets such as IRS-1, PPARγ, adiponectin and mitochondrial function. FFA, free fatty acid; DAG, diacylglycerol; PKC, protein kinase C; TLR4, Toll like receptor 4; ER, endoplamic reticulum; JNK, c-Jun N-terminal kinase; IKK, inhibitor kappaB kinase; IRS-1, insulin receptor substrate 1; PPARγ, peroxisome proliferator-activated receptor gamma.

Dr Feng Liu (University of Texas, USA) presented data about a new signalling molecule associated with the adiponectin receptor. The molecular mechanisms by which adiponectin sensitizes insulin action remains largely unknown. AdipoR1 and AdipoR2 have recently been identified as adiponectin receptors, yet signalling events downstream of these receptors remain poorly defined. His lab together with Dr Lily Dong's research team identified APPL1(adaptor protein containing pleckstrin homology domain, phospho-tyrosine binding domain and leucine zipper motif) as a downstream protein for adiponectin receptor (16). APPL1 functions as an adaptor protein mediating adiponectin signalling for activation of AMPK and p38. The new molecule provides a link between adiponectin and insulin signalling pathways.

The final session discussed the current status of our knowledge on the mechanisms and consequences of insulin resistance and the needs for better understanding of insulin resistance in the peripheral and central tissues/organs. The conference reinforced the current widely-accepted view that fatty acids and their derivatives are primary factors leading to insulin resistance. However, the contribution from excess exposure to fatty acids themselves, or to indirect factors such as increased pro-inflammatory cytokines, is a matter of much current debate and ongoing investigation. It is likely that the relative contribution to insulin resistance from these major influences will vary from tissue to tissue, e.g. muscle, liver and adipose tissue. Indeed even within muscle, there is debate on the relative importance of lipids such as diacylglycerols and ceramides in impairing insulin signalling. Some commonality, however, seems to be emerging and, for example, ceramides were implicated in both muscle insulin resistance and in mediating the inflammatory responses induced by fatty acids in macrophages. A focus was on the new signalling pathways by which lipid induces insulin resistance, and multiple signalling pathways are implicated in the mechanism whereby fatty acids and their derivatives inhibit insulin action. These signalling pathways include Toll like 4 receptor, ER stress, CD36-ceramide and adipose hypoxia. All of these pathways are dependent on inflammatory signalling pathways for the translation of lipid signal into insulin resistance (Fig. 1).

Conflict of Interest Statement

No conflict of interest was declared.

References

1. Hegarty BD, Furler SM, Ye J, Cooney GJ, Kraegen EW. The role of intramuscular lipid in insulin resistance. *Acta Physiol Scand* 2003; **178**: 373–383.
2. Kraegen EW, Saha AK, Preston E, Wilks D, Hoy AJ, Cooney GJ, Ruderman NB. Increased malonyl-CoA and diacylglycerol content and reduced AMPK activity accompany insulin resistance induced by glucose infusion in muscle and liver of rats. *Am J Physiol Endocrinol Metab* 2006; **290**: E471–E479.
3. Cleasby ME, Reinten TA, Cooney GJ, James DE, Kraegen EW. Functional studies of Akt isoform specificity in skeletal muscle in vivo; maintained insulin sensitivity despite reduced insulin receptor substrate-1 expression. *Mol Endocrinol* 2007; **21**: 215–228.
4. Kim JK, Gimeno RE, Higashimori T, Kim H-J, Choi H, Punreddy S, Mozell RL, Tan G, Stricker-Krongrad A, Hirsch DJ, Fillmore JJ, Liu Z-X, Dong J, Cline G, Stahl A, Lodish HF, Shulman GI. Inactivation of fatty acid transport protein 1 prevents fat-induced insulin resistance in skeletal muscle. *J Clin Invest* 2004; **113**: 756–763.
5. Kim JK, Fillmore JJ, Sunshine MJ, Albrecht B, Higashimori T, Kim DW, Liu ZX, Soos TJ, Cline GW, O'Brien WR, Littman DR, Shulman GI. PKC-theta knockout mice are protected from fat-induced insulin resistance. *J Clin Invest* 2004; **114**: 823–827.
6. Holland WL, Brozinick JT, Wang LP, Hawkins ED, Sargent KM, Liu Y, Narra K, Hoehn KL, Knotts TA, Siesky A, Nelson DH, Karathanasis SK, Fontenot GK, Birnbaum MJ, Summers SA. Inhibition of ceramide synthesis ameliorates glucocorticoid-, saturated-fat-, and obesity-induced insulin resistance. *Cell Metab* 2007; **5**: 167–179.
7. Brehm A, Krssak M, Schmid AI, Nowotny P, Waldhausl W, Roden M. Increased lipid availability impairs insulin-stimulated ATP synthesis in human skeletal muscle. *Diabetes* 2006; **55**: 136–140.

8. Szendroedi J, Schmid AI, Chmelik M, Toth C, Brehm A, Krssak M, Nowotny P, Wolzt M, Waldhausl W, Roden M. Muscle mitochondrial ATP synthesis and glucose transport/phosphorylation in type 2 diabetes. *PLoS Med* 2007; **4**: e154.
9. Schwartz MW, Woods SC, Porte D Jr, Seeley RJ, Baskin DG. Central nervous system control of food intake. *Nature* 2000; **404**: 661–671.
10. Woods SC, Lotter EC, McKay LD, Porte D Jr. Chronic intracerebroventricular infusion of insulin reduces food intake and body weight of baboons. *Nature* 1979; **282**: 503–505.
11. Clegg DJ, Benoit SC, Reed JA, Woods SC, Dunn-Meynell A, Levin BE. Reduced anorexic effects of insulin in obesity-prone rats fed a moderate-fat diet. *Am J Physiol Regul Integr Comp Physiol* 2005; **288**: R981–R986.
12. Inoue H, Ogawa W, Ozaki M, Haga S, Matsumoto M, Furukawa K, Hashimoto N, Kido Y, Mori T, Sakaue H, Teshigawara K, Jin S, Iguchi H, Hiramatsu R, LeRoith D, Takeda K, Akira S, Kasuga M. Role of STAT-3 in regulation of hepatic gluconeogenic genes and carbohydrate metabolism in vivo. *Nat Med* 2004; **10**: 168–174.
13. Inoue H, Ogawa W, Asakawa A, Okamoto Y, Nishizawa A, Matsumoto M, Teshigawara K, Matsuki Y, Watanabe E, Hiramatsu R, Notohara K, Katayose K, Okamura H, Kahn CR, Noda T, Takeda K, Akira S, Inui A, Kasuga M. Role of hepatic STAT3 in brain-insulin action on hepatic glucose production. *Cell Metab* 2006; **3**: 267–275.
14. Gao Z, Hwang D, Bataille F, Lefevre M, York D, Quon M, Ye J. Serine phosphorylation of insulin receptor substrate 1 (IRS-1) by inhibitor KappaB kinase (IKK) complex. *J Biol Chem* 2002; **277**: 48115–48121.
15. Gao Z, He Q, Peng B, Chiao PJ, Ye J. Regulation of Nuclear Translocation of HDAC3 by I{kappa}B{alpha} Is Required for Tumor Necrosis Factor Inhibition of Peroxisome Proliferator-activated Receptor {gamma} Function. *J Biol Chem* 2006; **281**: 4540–4547.
16. Mao X, Kikani CK, Riojas RA, Langlais P, Wang L, Ramos FJ, Fang Q, Christ-Roberts CY, Hong JY, Kim RY, Liu F, Dong LQ. APPL1 binds to adiponectin receptors and mediates adiponectin signalling and function. *Nat Cell Biol* 2006; **8**: 516–523.

