

# Fat Metabolism and Diabetes: 2003 American Diabetes Association Postgraduate Course

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**A**t the American Diabetes Association Postgraduate Course in New York, New York, on 10 January 2003, David E. Kelley (Pittsburgh, PA) discussed the relationship between fat metabolism and diabetes. Over the past few decades, he noted, the prevalence of obesity has doubled in adults and has quadrupled in teenagers, with more than half of U.S. adults now being overweight or obese. This is a manifestation of a much more longstanding trend. Data from military recruitment records, for example, show that the average weight, adjusted for height, has been increasing over the past 150 years. Sixty-one percent of the prevalence of diabetes can be attributed to obesity. There is a strong correlation between BMI and body fat. One of the correlates of insulin resistance is the blood fatty acid (FA) level. In 1963, Randle (1) initially suggested that FAs compete with glucose for metabolism. Kelley showed that, in a euglycemic clamp, when comparing subjects with and without lipid infusion, the former have marked resistance to muscle glucose uptake, with increased glycolysis and decreased glycogen formation—"precisely the profile we get in type 2 diabetes."

FA levels are strong predictors of muscle insulin resistance (2). Muscle fat content is increased in obesity and more so in type 2 diabetes. Electron microscopy shows decreased mitochondrial size in muscle from individuals with type 2 dia-

betes. Fats may not directly mediate insulin resistance, Kelly noted, but FA-derived muscle diacylglycerol (DAG) and fatty acyl CoA lead to increased intramuscular ceramide, which may directly affect muscle glucose uptake and metabolism. FA oxidation is greater and storage is lower in lean than in obese persons, but Kelley described an "inefficient use of fat" in persons with obesity: the normal insulin-induced increase in FA storage is attenuated and fails to improve with weight loss (3). He noted that while muscle triglyceride content is also increased in trained athletes, their fat oxidative capacity is increased, as opposed to the situation in obese individuals. When obese sedentary individuals combine weight loss with a fitness program, oxidative capacity increases, mitochondrial size increases, and the cytokine tumor necrosis factor (TNF)- $\alpha$  decreases. In addition, circulating free FAs (FFAs) decrease and skeletal muscle fat utilization capacity increases.

There are important differences between fat depots. Subfascial adipose tissue within muscle bundles is a predictor of insulin resistance, just as visceral fat (although it comprises only 10% of fat mass) shows greater correlation with insulin resistance than abdominal subcutaneous (SC) fat. There may be direct portal delivery of FAs from visceral fat depots, potentially driving hepatic triglyceride synthesis and glucose production and de-

creasing insulin extraction. Visceral adipocytes may also metabolize cortisol differently from SC adipocytes, further leading to resistance to insulin action. Fat shows low density on computed tomography scanning, and the liver-spleen density ratio can be used to quantitate intrahepatic fat, which correlates with the visceral fat mass. Fatty liver, then, is a predictor of insulin resistance, although, based on Kelley's studies, it does not correlate as strongly as visceral fat. Persons with diabetes and fatty liver have higher triglyceride, larger VLDL, and smaller LDL and HDL particles, as well as lower mass of HDL, while those without fatty liver do not show this "diabetic dyslipidemia" pattern. Kelley pointed out that some individuals may show deficiency of SC fat instead of excess of visceral fat as causes of insulin resistance, noting the marked insulin resistance seen in animals lacking SC fat (further discussed by Reitman below). Small fat cells may be more insulin sensitive and may prevent against fat accumulation in muscle and liver, and fat cell size is a predictor of insulin resistance in Pima Indians (4).

Kelley concluded by mentioning the numerous adipocyte secretions, which include adiponectin, adipisin, estrogen, angiotensin II, angiotensinogen, leptin, plasminogen activator inhibitor (PAI)-1, agouti protein, resistin, bone morphogenic protein (BMP), IGF-1 and various IGF binding proteins, TNF- $\alpha$ , interleukins (ILs), transforming growth factor (TGF)- $\beta$ , and fibroblast growth factor (FGF), as well as FAs themselves. Obesity correlates with inflammatory markers, including C-reactive protein (CRP), TNF- $\alpha$ , and IL-6. Leptin has effects on peripheral tissues, decreasing muscle malonyl CoA and increasing fat oxidation. Adiponectin, which originates in adipocytes but shows lower levels with greater degrees of obesity and with overfeeding, is associated with increased insulin sensitivity and increases with thiazolidinedione (TZD) administration.

Individuals with type 2 diabetes show a decrease in the potentiation of insulin

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**Abbreviations:** ADA, American Diabetes Association; AMPK, AMP-activated protein kinase; CCK, cholecystokinin; CNS, central nervous system; CRP, C-reactive protein; DAG, diacylglycerol; DPP, dipeptidyl peptidase; FA, fatty acid; FFA, free FA; G3P, glycerol-3-phosphate; G6Pase, glucose-6 phosphatase; GIP, glucose-dependent insulinotropic peptide; GLP, glucagon-like peptide; HGP, hepatic glucose production; IGT, impaired glucose tolerance; IL, interleukin; IRS, insulin receptor substrate; PAI, plasminogen activator inhibitor; PEP, phosphoenol pyruvate; PKC, protein kinase C; PPAR, peroxisome proliferator-activated receptor; SC, subcutaneous; TNF, tumor necrosis factor; TZD, thiazolidinedione; WAT, white adipose tissue.

A table elsewhere in this issue shows conventional and Système International (SI) units and conversion factors for many substances.

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secretion by oral rather than parenteral glucose loading, a phenomenon known as the "incretin effect." Daniel J. Drucker (Toronto, ON) reviewed incretin physiology, discussed data from experimental models related to their roles, and presented data related to the effect of glucagon-like peptide (GLP)-1 in diabetes. Glucose-dependent insulinotropic peptide (GIP) was the first identified incretin; GLP-1 was later identified and found to be present in the proglucagon gene. Both GIP and GLP-1 are small peptide insulin secretagogues, secreted from the duodenum and distal bowel, respectively, that have short half-lives. GLP-1, in contrast to GIP, also decreases glucagon secretion, gastric emptying, and food intake and promotes  $\beta$ -cell growth. In addition, persons with type 2 diabetes, as well as first-degree relatives, appear to be resistant to GIP, so that GLP-1 offers greater therapeutic promise. The alanine at position 2 of GLP-1 is rapidly clipped by the ubiquitous enzyme dipeptidyl peptidase (DPP) IV, leading to an  $\sim 90$ -s half-life. There is evidence of low GLP-1 in persons with impaired glucose tolerance (IGT) and of a further decrease in individuals with type 2 diabetes, suggesting GLP-1 might play a role in the treatment of the disease. Incretins offer the potential to control fasting and postprandial insulin release in a glucose-dependent manner, making them promising agents for treating type 2 diabetes. GLP-1 also promotes satiety and inhibits food intake in experimental animals and humans, potentially operating through central, vagal, or direct gastric-emptying effects (the effects on gastric emptying also tend to lower blood glucose). Type 2 diabetes is associated with decreased islet cell growth (5); therefore, the effects of GLP-1 on  $\beta$ -cell proliferation and inhibition of  $\beta$ -cell apoptosis may be particularly important.

Several GLP-1 analogs and inhibitors of DPP-IV are being studied. Continuous SC GLP-1 infusion lowers blood glucose and HbA<sub>1c</sub>, increases insulin sensitivity, and leads to modest weight loss (6). The analog exendin-4, when administered by twice-daily SC injection, lowers fasting and postprandial blood glucose in type 2 diabetes and is effective in combination with sulfonylureas and insulin, without causing weight gain (it is, however, associated with some nausea and vomiting at high doses and with hypoglycemia in combination treatment). NN2211 has a

longer half-life, which allows a single daily injection. Longer-acting compounds (including an albumin-linked form) are being studied to examine the potential of once-monthly dosing. Inhibition of DPP-IV may be another effective approach to increase GLP-1. This would allow oral treatment, but may be less potent or less likely to decrease appetite. The enzyme also degrades cytokines and vasoactive peptides, with potential for adverse effects by its inhibition.

Stephen C. Woods (Cincinnati, OH) discussed central nervous system (CNS) signals that influence appetite and body weight. Metabolic signals are important because of the need to balance energy intake with expenditure. The average energy intake exceeds 900,000 cal/year; thus, the average weight gain of the U.S. population of one pound per year averages out to "around a potato chip a day," suggesting a remarkably well-balanced system. The gastrointestinal tract generates satiety signals, fat cells generate adiposity signals including leptin, and insulin also acts in the hypothalamus, with many CNS signals having potent effects on energy regulation.

Meal initiation is not controlled by energy deficit; instead, hunger, to a large extent, is based on learned associations. Satiety controls meal size, mediated by peptides secreted by the gastrointestinal tract, such as cholecystokinin (CCK), bombesins, glucagon, enterostatin, apolipoprotein A4, somatostatin, amylin, peptide YY, and GLP-1. In experimental models, administration of CCK reduces meal size but increases meal frequency and, thus, does not result in weight loss (7).

Humeral regulation of adiposity involves signals proportionate to adipocyte tissue mass. Insulin is such a signal, with levels increasing in persons with obesity (8). Leptin is secreted directly from adipocytes and also shows levels increased by obesity. As CNS insulin (9) or leptin (10) levels increase, there is a decrease in food intake and weight loss. Similarly, decreased CNS action of either insulin or leptin increases food intake. It is, however, difficult to devise approaches to increase the CNS insulin or leptin signals, so direct use of these agents has not led to clinical therapy. Insulin and leptin act in the hypothalamus to stimulate neurons, such as the one that makes  $\alpha$ -melanocyte-stimulating hormone (MSH), leading to

catabolic effect, and to inhibit neurons that tend to increase body weight. Obesity develops in mice lacking leptin (*ob/ob*), the leptin receptor (*db/db*), the CNS insulin receptor, or the  $\alpha$ -MSH receptor. Such abnormalities may account for 5% of severe human obesity (11). Woods suggested a potential approach that combines subthreshold CNS insulin and CCK, which might potentiate each other. He also noted that insulin correlates with visceral fat and that leptin correlates with SC fat, perhaps explaining the lower predictive value of leptin for the metabolic syndrome. CNS leptin administration is more effective in female animal models, and insulin is more effective in male animal models. These effects, thus, are potentially related to sex differences in fat distribution.

Michael P. Czech (Worcester, MA) reviewed the biochemical aspects of TZD action. Muscle is a major component of body mass and accounts for the disposal of most ingested glucose. In addition, it mediates the change of fuel disposition between FAs and glucose. FAs from adipocytes affect glucose disposition in muscle, inducing the "muscle sensor" to spare glucose during fasting and to utilize glucose after meals. FA oxidation in muscle leads to inhibition of muscle glucose utilization. During fasting, glucose derived from hepatic gluconeogenesis is derived from amino acids originating from muscle. In liver, FA oxidation stimulates gluconeogenesis and therefore provides glucose. This glucose-FA cycle is amplified in obesity, in part via increased FA flux from the adipocyte, which in turn increases hepatic gluconeogenesis. In the nondiabetic individual, this is compensated by increased insulin secretion. TZDs appear to inhibit adipocyte FFA release, but they also show distal effects when FFA levels are increased with triglyceride-heparin infusion.

Muscle glucose metabolism is controlled at the levels of uptake via the transporter GLUT4, of glycogen formation and degradation, of lactate formation, and of further metabolism to form CO<sub>2</sub>. Both insulin and exercise (or muscle contraction) stimulate GLUT4. FAs and cytokines such as TNF- $\alpha$  inhibit the process of muscle glucose metabolism. TZDs both increase the stimulatory effects of insulin and exercise and block the inhibitory effects of FAs and cytokines. Membrane vesicles containing GLUT4 in the perinuclear re-

gion travel via microtubules to the plasma membrane by two sets of molecular motors, kinesin-1 heavy chain (KIF5B), which brings GLUT4 to the plasma membrane, and dynein, which returns GLUT4 to the perinuclear region. In the plasma membrane region, myosin-actin units appear to be involved in the process.

The phosphatidylinositol (PI) 3-kinase signaling pathway mediates the insulin signal, activating pyruvate dehydrogenase kinase-1, which phosphorylates further protein kinases, including Akt 1, 2, and 3; serum- and glucocorticoid-regulated kinase (SGK)-1 and -2, and protein kinase C (PKC)- $\delta$  and - $\zeta$ . A mouse model not expressing Akt2 has glucose intolerance with partially decreased muscle glucose disposal in response to insulin (12). Selective inhibition of expression of both Akt1 and -2 with interference RNA technology (13) decreases even in response to maximal insulin dosages. The ability of muscle contraction to stimulate glucose transport is not, however, decreased in animals not expressing Akt. Muscle contraction affects a different protein kinase, AMP-activated protein kinase (AMPK), which is increased by high AMP or low ATP levels. AMPK phosphorylates downstream targets, thereby leading to less activity of energy-dependent pathways, while activating FA  $\beta$ -cell oxidation to increase ATP synthesis. AMPK itself is phosphorylated by AMPK. AMPK phosphorylates acyl CoA carboxylase, leading to increased FA  $\beta$ -cell oxidation. 5-Aminoimidazole-4-carboxamide 1- $\beta$ -D-ribofuranoside (AICAR), an adenosine analog that mimics AMP, activates AMPK and has effects similar to insulin in increasing glucose transport. AMPK has three subunits. Its enzymatic activity can be blocked with a variety of mutations, showing that AMPK is required for hypoxia and for approximately half of contraction-mediated glucose uptake, but not for insulin-mediated glucose uptake. There may be a second pathway of contraction-mediated glucose transport, perhaps a calcium-sensitive pathway.

FAs and cytokines also modulate their negative effects through PKC- $\theta$  (14), c-Jun NH<sub>2</sub> terminal kinase (JNK)-1 (15), and the inhibitor of nerve factor  $\kappa$ B (I $\kappa$ B) (16). Each of these cause serine phosphorylation of insulin receptor substrate (IRS)-1, which disrupts insulin signaling, while TZD improvement of FA-mediated insulin resistance in muscle may involve

reversal of the serine phosphorylation. FA release from adipocytes via hormone-sensitive lipase is attenuated by reesterification, which involves FA-CoA synthesis with subsequent reesterification by glycerol-3-phosphate (G3P) acyltransferase, utilizing G3P formed from glycerol via glycerol kinase or from pyruvate via phosphoenol pyruvate (PEP) carboxykinase, all three enzymatic steps activated by TZDs. An important question is whether it would be possible to increase FA oxidation rather than use TZD-promoted reesterification to decrease FA release without increasing adipocyte triglyceride stores.

Meridith A. Hawkins (Bronx, NY) discussed the control of hepatic glucose metabolism. Hepatic glucose production (HGP) is suppressed directly by glucose, insulin, and adiponectin and indirectly via FA suppression by insulin. HGP is increased by cortisol, glucagon, growth hormone, catecholamines, FAs, and resistin. Glucose elevation from 90 to 180 mg/dl in persons without diabetes suppresses glucose production by half, an effect similar to that of a four- to fivefold elevation in plasma insulin. The higher portal glucose levels following meals may particularly be effective in suppressing HGP. Using glucose and insulin clamps, individuals with type 2 diabetes show no suppression of glucose production by hyperglycemia. Glucokinase activity is decreased and glucose-6 phosphatase (G6Pase) is increased in diabetes. The effects, perhaps, could be in response to chronic hyperglycemia, which would suggest that intracellular G6P levels do not increase, but mediate the lack of response to hyperglycemia, with suppression of HGP by hyperglycemia being seen in well-controlled individuals with type 2 diabetes. Hawkins noted that activation of hepatic glucokinase with small catalytic amounts of fructose partially restores the regulation of HGP by hyperglycemia in persons with type 2 diabetes, although this effect is not enhanced in normal persons whose glucokinase activity is presumably adequate. FAs increase G6Pase and inhibit formation of G6P from PEP. The increase in FFA levels in diabetes may be another mechanism of the adverse effect of hyperglycemia. Indeed, elevating FFAs with lipid infusion in nondiabetic individuals adversely affects the response to hyperglycemia, while lowering FFA levels with nicotinic acid in persons with type 2 diabetes improves the response. In-

sulin sensitivity increases over 3 weeks in individuals with type 2 diabetes treated with pioglitazone, in conjunction with doubling of adiponectin levels. However, only modest changes in FFA levels are observed during this timeframe, which suggests that there may be another mechanism of TZD action.

Marc C. Reitman (Rahway, NJ) discussed the lipodystrophies, reviewing clinical studies and analysis of mouse models. White adipose tissue (WAT) plays a role in energy storage and produces FFAs as well as messenger molecules, including leptin, adiponectin, TNF- $\alpha$ , IL-6, resistin, angiotensinogen, and PAI-1. Obesity and overweight are common and are associated with development of insulin resistance, dyslipidemia, and hypertension, but, importantly, deficiency of adipose tissue is often associated with many of these same complications. Human lipodystrophies are a heterogeneous group of diseases; they may be generalized, partial, or localized with redistribution of fat; and they may have genetic, autoimmune, or drug-related causes, as seen with HIV medications. In general, the metabolic severity of the lipodystrophies correlates with the degree of fat loss. Severe lipodystrophy, the near-complete lack of adipose tissue, is associated with severe insulin resistance, diabetes, acanthosis, features of polycystic ovary syndrome, hirsutism, hypertriglyceridemia, hepatomegaly, and hepatic steatosis, often progressing to cirrhosis. Leptin levels are increased, hyperphagia is common, and there is often muscle hypertrophy. The insulin resistance may correlate with increased muscle and liver fat.

Acquired generalized or partial lipodystrophy was first described by the British endocrinologist Robert D. Lawrence (17). It is inflammatory and autoimmune in origin, occurs more commonly in females, has onset in childhood or adulthood, and is associated with panniculitis, with fat loss beginning in the legs and progressing to the upper body. (17). Congenital generalized lipodystrophy (CGL) is associated with either one of two mutations: a recessive mutation in the gene encoding 1-acylglycerol-3-phosphate-O-acyltransferase-2 on chromosome 9q34, which has at least eight described variants and is expressed primarily in WAT, though also in liver and, to a lesser extent, in muscle (18); or a mutation in BSCL2 on chromosome 11q13 in seipin, a 398-

amino acid protein of unknown function, with mRNA widely expressed for this, particularly in brain and testes, but also in WAT (19). Familial partial lipodystrophy (Dunnigan's variety) is associated with deficit in limbs and trunk with excessive facial and neck fat (20), due to a dominantly inherited mutation in lamin A/C that also causes forms of muscular dystrophy and cardiomyopathy (21). Peroxisome proliferator-activated receptor (PPAR)- $\gamma$  mutations may also be associated with partial lipodystrophy. HIV-associated lipodystrophy is seen with effective treatment by virtually any regimen in ~40–50% of patients. It is associated with visceral and cervicodorsal obesity but also with facial peripheral lipodystrophy. Hypertriglyceridemia and insulin resistance are typical.

The primary deficit in lipodystrophy is the lack of adipose tissue. Consequent loss of adipocyte-derived hormones and increased circulating FFAs and triglycerides lead to increased hepatic and muscle deposition, with consequent insulin resistance at these sites. A mouse model of virtually complete absence of adipose tissue (22) is associated with increased triglycerides and FFAs, fatty liver, hyperglycemia and hyperinsulinemia without ketoacidosis, and visceral organomegaly. Transplantation of adipose tissue grafts reverses the hyperinsulinemia, diabetes, and muscle and liver insulin resistance in a dose-dependent fashion (23). Thus, lack of fat causes the metabolic symptoms of lipodystrophy. Interestingly, transplantation of *ob/ob* adipose tissue (which does not produce leptin) does not reverse the diabetes (24). Leptin administration can partially reverse the phenotype, perhaps because of decreased food intake, CNS effects on energy expenditure and insulin sensitivity, or peripheral effects on increasing energy expenditure. The effect of fat transplantation is not due to adiponectin, which does not increase to control levels.

Divergent hepatic insulin effects are seen in animal models of lipodystrophy. Insulin-induced sterol regulatory element-binding protein-1c levels are increased, which increases lipogenesis, while IRS-2-mediated effects on glucose metabolism are decreased. Liver PPAR- $\gamma$  mRNA is increased in mouse models of hepatic steatosis, and mice lacking PPAR- $\gamma$  with lipodystrophy have decreased liver triglyceride levels and greater hepatic insu-

lin sensitivity, although with lower muscle insulin sensitivity and higher serum triglyceride levels. Hepatic triglyceride clearance presumably mediates this paradoxical PPAR- $\gamma$  effect, and in this model, TZDs increase hepatic steatosis. The role of hepatic PPAR- $\gamma$  in humans appears to be different, with marked improvement in 19 insulin-resistant persons with lipodystrophy treated for 6 months with troglitazone (25). Although TZDs may be effective, one must be sure that liver function does not worsen, and metformin, acarbose, and triglyceride-lowering treatment also have potential; insulin and insulin secretagogues are less desirable. Treatment with leptin also appears to be effective, with a recent study of methionyl leptin in nine leptin-deficient patients with lipodystrophy markedly improving triglyceride and glucose levels. (26). Reitman suggested that currently leptin is "the best way to go."

Philipp E. Scherer (Bronx, NY) extended the previous discussions of the adipocyte as both a target and a source of cytokines, acute-phase reactants, and hormones, in discussions at both the ADA Postgraduate Course and the Niagara Falls conference (27). In the course of differentiation of adipocytes, a number of mediators are produced, including the proinflammatory mitogen-activated protein (MAP) kinase P38. Diabetes leads to a low-level inflammatory adipocyte response with induction of serum amyloid A. Pituitary homeobox 3, a homologue of CRP produced by adipocytes, is another inflammatory mediator produced in diabetes. "Why," Scherer asked, "is an inflamed fat cell a bad fat cell?"

The adipocyte-specific secretory protein adiponectin is deficient in states of insulin resistance in humans (28) and in animal models (29). The 3q27 locus implicated in syndrome X may play a role in adiponectin production (30). Adiponectin resembles complement factor C1q and TNF- $\alpha$ . It circulates in serum as hexameric and higher-order complexes, with high levels in female models because of high-molecular weight complexes, which appear to be a circulating precursor pool. Prolactin and estrogen decrease adiponectin, despite the higher levels in females. Insulin administration activates adiponectin by returning it to the hexameric form, with a serum reductase producing the trimer, which then dissociates into the active moiety. Transgenic mice over-

expressing adiponectin show increased hepatic insulin sensitivity. Body weight and body fat are increased in these animals, with proliferation of dorsal and also retro-orbital fat, leading to exophthalmos, which is of interest in view of the increase in adiponectin levels in Graves' disease, suggesting a relationship to ophthalmopathy. Adiponectin-null mice display high-fat/high-sugar-induced insulin resistance and show decreased triglyceride clearance (31). Thus, it appears that a potential mechanism of the interrelationship between inflammation and insulin resistance is in the suppression of adiponectin secretion by adipocytes.

Although produced in adipocytes, levels are greater with lower adipose tissue mass. Adiponectin shows an inverse relationship with fasting insulin and a positive correlation with insulin sensitivity (32). In a rhesus monkey model of progression to diabetes, adiponectin levels decrease as insulin resistance occurs (33). Euglycemic clamp studies show increased peripheral glucose uptake and, particularly, decreased HGP with adiponectin administration. In hepatocyte culture, adiponectin greatly increases insulin sensitivity. Adiponectin acts on AMPK to increase glucose utilization and FA oxidation, and AICAR mimics the effect of adiponectin.

Most adiponectin is derived from visceral fat, and TZD (not metformin) treatment increases visceral fat adiponectin (34). Ongoing studies will address the question of whether monitoring adiponectin will allow prediction of which patients will respond to TZD treatment. In mouse studies, the central fat pads are the prime source of adiponectin, and these sites are the source of the increased adiponectin with TZD treatment. As these fat stores increase in size, their adiponectin production decreases.

Guenther H. Boden (Philadelphia, PA) also spoke at both conferences, reviewing fat modulation of muscle and liver metabolism. "We are hard-wired genomically to protect against starvation," he pointed out, rather than against abundance. Diabetes risk increases with increasing body weight (35). Clearly, obesity causes insulin resistance, fat feeding produces insulin resistance, and weight loss reduces insulin resistance. "There is now solid evidence," Boden stated, "that a major link between obesity and muscle and liver resistance is fatty



acids." FFA levels are increased in most obese individuals, and acute elevations in FFAs cause insulin resistance in muscle and the liver. Using euglycemic-hyperinsulinemic clamps, administration of lipid emulsion with heparin to increase FFA levels causes insulin resistance for ~4 h, after a delay of 2–4 h, in a dose-dependent fashion, in persons with and without diabetes (36). The FFA level correlates inversely with insulin-stimulated peripheral glucose uptake. In contrast, overnight FFA lowering using the drug acipimox increases peripheral glucose uptake in lean and obese individuals without diabetes, as well as in persons with IGT and with diabetes, although the latter groups remain insulin resistant (37). Thus, normalization of FFAs could double insulin sensitivity in type 2 diabetes. However, no available drugs can lower plasma FFAs more than the 15–20% seen with TZDs, and nicotinic acid causes only intermittent FFA lowering followed by a marked rebound increase in levels, perhaps explaining the tendency for worsening glucose tolerance with this agent.

FFAs interfere with the inhibitory effect of insulin on HGP (38). Acutely, insulin modestly decreases gluconeogenesis, but it almost completely suppresses glycogenolysis, which can be counteracted by increasing FFA levels (39). FFAs increase intramyocellular triglyceride over ~2 h (40). Boden noted that FFAs entering muscle are metabolized to long-chain acyl CoA with DAG production, thus increasing PKC, which interferes with insulin signaling by decreasing tyrosine phosphorylation and increasing serine phosphorylation of IRS-1. Muscle biopsy studies show no effect at 2 h but a four- to sixfold increase at 6 h in intramyocellular DAG (41). PKC- $\beta$ 2 and PKC- $\delta$  appear to increase over a similar time course. Interestingly, I $\kappa$ B- $\alpha$  decreases at the same time, a potential linkage between insulin resistance and inflammation, suggesting pro-atherosclerotic effects of FFAs.

Barbara Corkey (Boston, MA) discussed fat modulation of  $\beta$ -cell function. Only the subset of insulin-resistant individuals who are low insulin responders develop diabetes. Although FFAs stimulate insulin secretion, there may also be adverse effects of fat on  $\beta$ -cells. Corkey pointed out that fuel signals in the  $\beta$ -cell are oscillatory. In the normal course of glucose metabolism in the  $\beta$ -cell, the

ATP-to-ADP ratio increases, closing the ATP-sensitive K-channel, which leads to  $\beta$ -cell depolarization and increases cytosolic calcium with subsequent insulin exocytosis. However, glucose phosphorylation uses ATP and produces ADP. Initially, the ATP-to-ADP ratio falls, stimulating glycolysis and oxidative phosphorylation, with ATP levels then increasing, producing oscillations in ATP/ADP, oxygen consumption, cytosolic  $\text{Ca}^{2+}$ , and membrane potential. The oscillations allow a greater range of signals with higher peaks and lower troughs, which may prevent peripheral desensitization to insulin. The loss of oscillatory insulin secretion among diabetic individuals and their first-degree relatives may then contribute to loss of insulin sensitivity.

FFAs may affect insulin signaling in this model by increasing long-chain acyl CoA. Pyruvate entry into the mitochondrion leads to acetyl CoA production via pyruvate dehydrogenase with citrate production. This process leads to increased malonyl CoA, which decreases the activity of carnitine palmitoyltransferase-1, thereby regulating mitochondrial FA uptake. Inhibition of long-chain CoA production by FAs appears to block the stimulatory effect of FAs on insulin secretion. Many of the isoforms of PKC are regulated by long-chain CoA, suggesting this, after glucose and FAs, to be the "third signal that comes from fuel metabolism." Interestingly, GLP-1 acts through cAMP to stimulate insulin secretion, which appears to occur via lipolysis. As Corkey concluded, "The double stimulus to the  $\beta$ -cell of both fat and carbohydrate" causes particularly great stimulation of insulin secretion and therefore might be important to avoid, particularly as acute and chronic FFA responses differ and as long-term exposure to the FA oleate interferes with  $\beta$ -cell response.

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