Pathogenic Role of Inflammatory Cytokines in Obesity: From Insulin Resistance to Diabetes mellitus

André Marette

Department of Anatomy-Physiology and Lipid Research Unit, Laval University Hospital Research Center, Ste-Foy, Québec, Canada

Introduction

It is now well established that an inflammatory component contributes to the pathogenesis of obesity-linked diabetes and cardiovascular diseases. First proposed by Hotamisligil et al. [1] more than a decade ago, there is now convincing experimental evidence for the existence of an inflammatory link between obesity and the occurrence of the insulin resistance dyslipidemic syndrome commonly known as the ‘metabolic syndrome’. Indeed, several molecules that are best known for their role in immune and inflammatory cells are now considered as key modulators of energy metabolism in insulin target tissues and are secreted by fat cells in the expanded adipose tissue of obese subjects. These include proinflammatory cytokines, e.g. tumor necrosis factor-α (TNFα), interleukin (IL)-6, IL-1, and interferon-γ, as well as adipose-specific cytokines or ‘adipokines’ such as leptin and resistin. These inflammatory mediators exert their actions via a complex interplay of signal transduction mechanisms that we are just beginning to fully appreciate. Several molecular targets have been proposed to mediate the insulin-resistant effects of cytokines in insulin target tissues. The principal goal of this chapter is to briefly review current knowledge on the mechanisms by which cytokines promote insulin resistance in obesity. Particular emphasis will be placed on inducible nitric oxide (NO) synthase (iNOS), an inducible isoform of the NO synthase (NOS) family that is overexpressed in the skeletal muscle and adipose tissues of several animal models of obesity.
Cytokines as Inflammatory Mediators of Insulin Resistance

Cytokines have been suggested to be involved in the development of an abnormal glucose metabolism in some diseased states such as cancer, trauma, and sepsis [2–4]. Administration of the cytokines, TNF-α and IL-1β, to experimental animals has been reported to mimic the metabolic response to sepsis which involves both an increase in glucose production and an accelerated rate of peripheral glucose clearance [5–7]. However, sepsis is also associated with a state of insulin resistance, as evidenced by diminished glucose tolerance, hyperinsulinemia, and impaired insulin action on peripheral glucose disposal [8, 9]. Accordingly, in vivo treatment with endotoxins or cytokines have been found to reduce insulin-stimulated glucose uptake by skeletal muscles [7, 10], providing convincing evidence of their implication in the muscle insulin resistance associated with acute systemic inflammation.

Is Obesity-Linked Diabetes an Inflammatory Condition?

Whereas a link between inflammation and atherosclerosis has long been established [for review see, 11], more recent evidence strongly suggests that obesity, insulin resistance and type-2 diabetes are also associated with a cytokine-mediated acute-phase or stress response [12–15]. The presence of a chronic inflammatory state is in line with the finding of increased plasma levels of IL-6 and TNF-α [13] and overexpression of TNF-α in adipose tissues of obese humans and animals [1, 16, 17].

Hotamisligil et al. [1] first provided evidence that TNF-α plays a major role in the development of insulin resistance for glucose utilization by skeletal muscle and fat cells of obese insulin-resistant animals. The enlarged adipose tissues of obese Zucker rats were found to overexpress TNF-α with a corresponding elevation of the cytokine, both locally and systemically [1]. Elevated expression of TNF-α has also been reported in the adipose tissue and skeletal muscle of insulin-resistant subjects [16, 18, 19]. The skeletal muscle of obese subjects is infiltrated with fat and this may further contribute to local overproduction of TNF-α in the former tissue. Neutralization of TNF-α in obese Zucker rats with a TNF receptor-immunoglobulin fusion protein, administered parenterally or via gene transfer, resulted in increased insulin sensitivity, further suggesting the involvement of abnormal TNF-α production in the insulin resistance of obesity [1, 20]. The role of TNF-α in obesity-induced insulin resistance was further confirmed by generating obese mice (induced by a high-fat diet or by gold-thioglucose injection) with no functional copy of the TNF-α gene or by targeted mutation of p55 and p75 TNF-α receptors (TNFR) in genetically obese ob/ob mice [21, 22]. Mice lacking TNF-α or TNFR,
developed obesity like their wild-type counterparts, but showed improved insulin sensitivity, as revealed by insulin and glucose tolerance tests. These results clearly demonstrated that the genetic absence of TNF action can reduce the development of insulin resistance associated with both experimental and genetic obesity. However, the lack of complete normalization of insulin sensitivity in mice lacking TNF-α or TNFR further indicates that factors other than TNF-α (e.g. other cytokines, resistin, etc.) are involved in the pathogenesis of obesity-linked insulin resistance.

**Mediator(s) of Cytokine-Induced Insulin Resistance**

Insulin increases glucose disposal in skeletal muscle, heart and fat cells by a complex signaling cascade that eventually leads to the mobilization of glucose transporter-4 (GLUT4) proteins from intracellular storage vesicles to the cell surface membrane (fig. 1). Following binding of insulin to its receptor α-subunits, autophosphorylation of the transmembrane β-subunits occurs, leading to intrinsic activation of the receptor tyrosine kinase activity. The activated insulin receptor (IR) increases the tyrosine phosphorylation of IR substrates 1 and 2 (IRS-1, IRS-2), leading to activation of phosphatidylinositol

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**Fig. 1.** Cytokine-induced mediators of insulin resistance in obesity. Obesity is associated with increased release and action of inflammatory cytokines that leads to the activation of multiple mediators of insulin resistance in insulin target cells. Cytokines have been reported to activate c-Jun N-terminal kinase (JNK), suppressors of cytokine signaling (SOCS), the mammalian target of rapamycin (mTOR), inducible nitric oxide synthase (iNOS), lipid mediators (ceramides (CER) and gangliosides (GM)) and IκB kinase (IKK). These mediators may all impair insulin action by interfering with insulin receptor signaling through enhancing serine phosphorylation of IRS-1 or by inhibiting downstream targets such as PI 3-kinase, Akt or GLUT-4 translocation to the cell surface.

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(PI) 3-kinase, an essential element for insulin stimulation of GLUT4 translocation [for review see, 23]. The downstream mediators of PI 3-kinase in the insulin-stimulated signaling cascade leading to GLUT4 translocation are still not fully understood. There is evidence for both the serine/threonine kinase Akt/PKB and atypical protein kinase C (PKCζ) to be implicated.

TNF-α and other proinflammatory cytokines (IL-1, IL-6, interferon-γ) have been shown to inhibit insulin-mediated tyrosine phosphorylation of the IR and IRS-1 [4]. It is currently believed that TNF-α interferes with insulin signaling by increasing serine phosphorylation of IRS-1 which then functions as an inhibitor of the IR kinase activity [24, 25]. This leads to defective activation of downstream insulin signaling to PI 3-kinase, and reduced translocation of GLUT4 to the cell surface (fig. 1). Direct inhibition of PI 3-kinase or of further downstream insulin-activated kinases such as Akt/PKB and atypical PKCs by cytokine-induced pathways may also contribute to impair insulin action. In the next sections we shall discuss the potential role of various factors and signaling pathways in mediating cytokine-induced insulin resistance in acute inflammatory settings as well as obesity-linked diabetes.

One of the most challenging tasks is to identify the intracellular mediator(s) of cytokine-induced insulin resistance. Cytokines activate a multitude of second-messenger pathways (fig. 1) and several of those may be regarded as potential candidates for mediating insulin resistance. Generation of intracellular lipid species has been proposed to mediate cytokine-induced insulin resistance. It has been reported that TNF-α inhibits insulin action by activating sphingomyelinase and promoting the release of the bioactive sphingolipid metabolite ceramide either through enhancing increases IRS-1 serine phosphorylation [25] or by interfering with further downstream targets of insulin such as PKB/Akt [26, 27]. Another class of lipids that has recently been proposed to mediate the insulin-resistant effect of cytokines are gangliosides, a major component of lipid rafts in the plasma membrane. The expression of the ganglioside GM3 is increased by TNF-α and pharmacological depletion of GM3 in fat cells reversed TNF-α-induced serine phosphorylation of IRS-1 [28]. Moreover, GM3 mRNA levels are elevated in the adipose tissue of obese Zucker rats and ob/ob mice, two models of TNF-α overexpression [28].

Activation of serine kinases by TNF-α and other inflammatory cytokines have also been suggested to promote IRS-1 serine phosphorylation and insulin resistance in insulin target cells. Yuan et al. [29] recently showed that high-dose aspirin improved insulin action in obese rodents through inhibition of the proinflammatory pathway IκB kinase (IKK)/NF-κB. The IKK/NF-κB pathway is activated in insulin target tissues of obese insulin-resistant animals and high-dose aspirin/salicylates improve insulin sensitivity and glucose tolerance in such animals [29]. Furthermore, studies in heterozygous Ikkβ+/- mice showed that a 50% decrease in the Ikkβ gene improved insulin sensitivity and glucose homeostasis in both dietary and genetic models of obesity [29]. These findings have rejuvenated a 100-year-old concept that salicylates exert
glucose-lowering action and should thus be reconsidered for the treatment of type-2 diabetic subjects. Studies using low doses of salsalate, a less irritating head-to-tail dimer of salicylate, are currently underway to determine the efficacy of this drug in alleviating insulin resistance and improving glucose control (Shoelson, S, personal commun.)

Inflammatory cytokines also activate the mitogen-activated protein (MAP) kinase pathway in several cell types. There is evidence for TNF-α activation of the classical MAP kinase pathways such as p42/44 (ERK-1/2), p38 and the c-jun N-terminal kinase (JNK1), but the latter has received much more attention in the last 3 years. Indeed, JNK1 was reported to interact with IRS-1 and increase its phosphorylation on serine 307 [30]. Phosphorylation of serine 307 by JNK1 disrupts the interaction between the catalytic domain of the IR and the PTB domain of IRS-1, resulting in reduced insulin stimulation of downstream effectors such as PI 3-kinase [31]. The role of the JNK pathway in obesity and insulin resistance has also been demonstrated in vivo. JNK activity is elevated in tissues of dietary and genetic models of obesity. Moreover, JNK1 but not JNK2 gene invalidation in mice protects from high-fat-induced obesity and insulin resistance, apparently by reducing IRS-1 phosphorylation on serine 307 [32].

Other insulin-sensitive kinases, such as the mammalian target of rapamycin (mTOR) can also phosphorylate IRS-1 on serine residues and terminate insulin signaling. It has been shown that mTOR activation by insulin/amino acids inhibits insulin action on glucose transport through increased IRS-1 serine/threonine phosphorylation and accelerated deactivation of PI 3-kinase activity [33]. The mTOR pathway was also reported to participate in the degradation of IRS-1 during prolonged hyperinsulinemia [33–35]. mTOR has also recently been suggested to mediate the insulin-resistant effect of TNF-α [36]. mTOR may thus represent an efficient feedback regulatory mechanism of insulin signaling, which, in inflammatory conditions such as obesity-linked diabetes, may override the normal activation of insulin signaling and thus promote insulin resistance.

iNOS in Obesity-Linked Insulin Resistance and Type-2 Diabetes

Proinflammatory cytokines may also cause insulin resistance by transcriptional mechanisms. Previous studies have shown that cytokines induce expression of iNOS in several cell types. In macrophages, sustained production of NO enables cytotoxic activity against invading microorganisms. Although this high-output NO pathway probably evolved to protect the host from infection, there is also evidence that it can cause deleterious effects to other normal host cells which confers to iNOS the protective/destructive duality inherent in every other major component of the immune response.
For example, the marked NO production that is consequent to iNOS expression in many tissues in endotoxic shock is believed to cause hypotension, organ injury and dysfunction [for review see, 37]. Unlike the small amounts of NO that are formed by constitutive members of the NOS family, eNOS and nNOS, activation of iNOS and production of excessive amounts of NO by cytokines and endotoxins may promote insulin resistance in inflammatory settings (fig. 2). We first tackled this question in 1997 and work carried out in my laboratory since then has confirmed that insulin resistance represents another deleterious effect of iNOS induction during systemic inflammation. Thus, we have reported that administration of the endotoxin lipopolysaccharide (LPS), a model of acute systemic inflammation associated with release of proinflammatory cytokines, induces iNOS in muscle, liver, and adipose tissues [10, 38]. Cytokines and LPS also induce iNOS expression in cultured muscle and adipose cells [10, 38, 39] thus indicating that iNOS expression in adipose and muscle tissues of LPS-treated animals is not limited to resident or circulating macrophages. LPS- and cytokine-induced iNOS induction in muscle was associated with marked insulin resistance [10, 39]. Importantly, the insulin-resistant effects of cytokine/LPS in muscle cells in vitro could be abrogated by iNOS inhibition with L-NAME [39].

Induction of iNOS expression in insulin target tissues may also be relevant to the pathophysiology of insulin resistance in metabolic diseases associated with lower-grade but chronic inflammatory settings. Such a role for iNOS has already been proposed in the pathogenesis of atherosclerosis. Indeed, iNOS expression was found to be induced in atherosclerotic plaques where it could trigger or promote a local inflammatory response through oxidation and peroxidation of lipids [40–42]. To evaluate the potential role of iNOS induction in
the insulin resistance associated with obesity, we have recently tested whether iNOS is induced in tissues of obese animals fed high-fat and if this was associated with the development of insulin resistance. We found that iNOS expression is increased in muscle and fat of both dietary (high-fat feeding) and genetic models (ob/ob mice and Zucker diabetic rats) of obesity [17]. To gain genetic evidence for a role of iNOS in obesity-linked insulin resistance, we also generated obese mice that had a selective disruption of the iNOS gene (iNOS^−/−). Whereas both wild-type and iNOS^−/− mice developed obesity on the high-fat diet, obese iNOS^−/− mice exhibited improved glucose tolerance, normal insulin sensitivity in vivo and normal insulin-stimulated glucose uptake in isolated muscle [17]. iNOS induction in obese wild-type mice was associated with impairments in PI 3-kinase and Akt activation by insulin in muscle. These defects were fully prevented in obese iNOS^−/− mice demonstrating the key role of iNOS in promoting defective insulin signaling in muscle of obese mice.

Elegant studies by Shimabukuro et al. [43, 44] also suggest that iNOS induction in pancreatic β-cells may be involved in the development of impaired insulin secretion in the later stage of obesity-linked type-2 diabetes. Indeed, they showed that iNOS is induced in the pancreatic β-cells of obese Zucker diabetic fatty rats and proposed that elevated NO production may cause β-cell apoptosis/necrosis and impaired insulin secretion in this model [43, 44]. Further work by the same group also showed that iNOS expression is increased in cardiac muscle of Zucker diabetic fatty rats [45], which led to the proposal that cardiac dysfunction in obesity may be consequent to iNOS-mediated apoptosis. Together with our recent studies, the work by Shimabukuro et al. strongly suggests that iNOS is implicated in both the occurrence of insulin resistance and β-cell failure in the evolution of type-2 diabetes in obesity. Moreover, the overexpression of iNOS in the hearts of obese diabetic rats and the proposed role of iNOS in the vascular dysfunction of atherosclerosis further suggest that iNOS is also implicated in the development of cardiovascular diseases in obesity.

**iNOS Inhibitors as Anti-Diabetic Agents**

The ever-increasing incidence of obesity-related type-2 diabetes in developed countries calls for novel and potent treatments against insulin resistance and impaired β-cell function. Since there is now convincing evidence for the pathogenic role of iNOS in both of these defects, it is critical to develop and test iNOS inhibitors for their ability to prevent and/or reduce insulin resistance and impaired insulin secretion. Different classes of compounds known to inhibit iNOS activity, iNOS expression, or the bioavailability of NO and NO-derived nitrogen species have recently been developed. I will focus on two iNOS activity inhibitors that we have recently tested in our laboratory, namely 1400 W and aminoguananinie.
N-(3-(Aminomethyl)benzyl)acetamidine (1400 W) is a competitive and tightly selective iNOS inhibitor that was first described in 1997 [46]. It exhibits a 250- and 5,000-fold selectivity for iNOS over nNOS and eNOS, respectively [46, 47]. Chronic treatment (2 mg/kg/day for 10 days) of obese diabetic db/db mice with 1400 W significantly reduced fasting hyperglycemia and tended to lower fasting hyperinsulinemia [48]. We have also observed in preliminary studies that 1400 W improves whole-body insulin sensitivity in these obese diabetic mice [Dallaire and Marette, unpublished data]. Very recently, we have also tested the potential beneficial effects of another iNOS inhibitor, aminoguanidine, on muscle insulin sensitivity of LPS-challenged rats. Aminoguanidine is not as selective as 1400 W for iNOS (it exhibits a 10- and 6-fold selectivity for iNOS over nNOS and eNOS, respectively) but it has the advantage of being already used for its ability to reduce protein glycation in diabetes. Thus, its lack of specificity is counterbalanced by its duality of action which enhances its clinical relevance. We found that AGN pretreatment prevents insulin resistance for glucose transport in skeletal muscle of LPS-challenged rats [Kapur and Marette, unpublished data]. These results suggest that iNOS inhibitors may represent promising pharmacological agents for the treatment of insulin resistance in obesity and other inflammatory disorders in which iNOS induction is pathogenic but not critical for health (i.e. non-infectious diseases). However, it is important to consider possible adverse effects of uncontrolled NO inhibition on other physiological systems. Thus, iNOS plays a protective role in the development of transplant arteriosclerosis [49] and is also critical for optimal wound healing [50] which calls into question the use of iNOS inhibitory agents to treat diseases such as atherosclerosis and diabetes. It is therefore important to find the therapeutic window of safe iNOS inhibition. Thus, although our initial studies are very promising, more work is needed before iNOS inhibitors can be safely used to treat inflammatory-linked metabolic disorders.

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Discussion

Dr. Basu: Type-2 diabetes has been associated with reduced nitric oxide availability, at least in the endothelial nitric oxide production, so clearly it is not just nitric oxide availability that is causing or contributory to insulin resistance. So what do you speculate is the mechanism by which inducible nitric oxide synthase (iNOS) knockout mice will have increased insulin sensitivity? Clearly it is not due to nitric oxide molecule production as such. Did these iNOS knockout mice have immune deficiencies or ability to fight infections? Because as we know iNOS is important for immune resistance against infections.

Dr. Marette: I will start with the second question because the answer will be shorter. These mice of course had a relative immune deficit so we have to maintain them in a micro-isolator. Actually Nathan [1], our collaborator on this, wrote a beautiful review many years ago showing that actually iNOS can sometimes play a detrimental role and sometimes play a beneficial role in several infectious diseases. So it is not that iNOS only plays a good role by limiting infection, sometimes it is even detrimental to the host so we don’t take any chances, we maintain these mice in the micro-isolator. So under our conditions we did not see any problems because we were maintaining the mice in a controlled environment. With regard to the first question, the nitric oxide field, especially as it relates to insulin, is a very complex one and there is considerable debate about the role of nitric oxide in insulin action. iNOS, like every other molecule of the immune system, plays a dual role; it has a double-edged role: it is not good to have too little but it is not good to have too much either. We believe that when there is too much and sustained production of nitric oxide, i.e. in the micromolar range, then you start having problems like reactions of nitric oxide with radical molecules such as superoxide. It is well known now in the field that nitric oxide reacts with superoxide to make peroxinitrite. I have data that I did not present today showing that if you add peroxinitrite to cells in culture you immediately cause insulin resistance. There is also an increased peroxinitrite production in the adipose tissue and skeletal muscle of septic animals, as well as in obese animals; these are unpublished data. So what we believe is that not nitric oxide per se but some reactive nitrogen species or reactive oxygen species are increased with nitric oxide and are responsible for insulin resistance. In fact it has been shown that when you induce iNOS and markedly increase nitric oxide production, it causes a feedback inhibition on the other nitric oxide synthase isoforms such as endothelial nitric oxide synthase (eNOS) [2]. So not only will you get insulin resistance at the level of the myocyte but you will also downregulate the eNOS isoform in the endothelium, thereby limiting blood flow and of course the ability of the muscle to use substrates. So it is a vicious circle. It is a complex mechanism. But if we simply look at the nitric oxide molecule per se this is wrong, I think we should look at all the other players involved, and unfortunately I didn’t have time today to go into this more deeply.

Dr. Mace: You mentioned that, in your model, treatment with a peroxisome proliferator-activated receptor (PPAR)-γ agonist decreased iNOS activity. Did you try a PPAR-α or δ agonist?

Dr. Marette: No, for now we only tested the PPAR-γ agonist but we are currently investigating the effect of PPAR-α and δ, but I cannot comment on this now because these studies are ongoing.

Dr. Sitges-Serra: If I understood correctly, you said in the first part of your talk that iNOS expression is associated with insulin resistance, and in the second part that some drugs do in fact inhibit iNOS expression, but you didn’t show that some drugs in fact improve insulin resistance. The reason for the question is that we learned from some studies that pointing at single targets (i.e. cytokines) is usually something that doesn’t work. So perhaps targeting just for iNOS expression won’t work either in
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insulin resistance. Do you have any proof that a single drug would both inhibit iNOS expression and improve insulin resistance?

**Dr. Marette:** Your point is well taken and actually I am using that argument against those who are only using drugs that would target only one cytokine, i.e. TNF-α or only IL-1 or IL-6. At least by trying to tackle iNOS you are looking at one biological endpoint. iNOS might not be the only target, but I think it is better than just looking at one cytokine if you try to inhibit one of the biological endpoint responses. Notwithstanding this I agree with you, iNOS is not the only player. Coming back to the first part of your question: in studies using the iNOS inhibitor 1400 W, a very preliminary study with 4 animals showed improved insulin tolerance. We are now running a second study with much more animals. And as far as PPAR-γ is concerned I think there are many studies that have shown that in the type of experiments we did and the dose we used of these PPAR-γ agonists, there is a marked improvement in insulin sensitivity. So we did not measure it in the experiments I have shown you today, but these drugs are really well known to improve insulin sensitivity.

**Dr. Mace:** You mentioned at the beginning of your talk that in high-fat mice fed the high-fat diet you have an increase in all the proinflammatory cytokines in adipose tissue. What is the reason for that?

**Dr. Marette:** I wish I knew the answer to this question, it is still puzzling me. I think now most people in the field agree that obesity is a low-grade chronic inflammatory disorder, and clearly there is more and more evidence coming from several laboratories that the adipose tissue and the adipocytes should be considered not only as cells that have the ability to store and release lipids but also as an endocrine gland that can release many adipokines, some of them being beneficial, some of them unfortunately being detrimental to insulin sensitivity. But the question is still why a fat cell that increases in size would start to release cytokines, and every time I put that question to other specialists in the field, they don't know the answer. There is one path that we could follow. Makowski et al. [3] in Boston have shown that in mice lacking AP2, a lipid-binding protein, the effect of high-fat feeding to increase TNF-α expression was reduced, suggesting that the ability of a fat cell to take up the free fatty acid appears to be linked to its ability to produce TNF-α. So perhaps we can think like this, if an adipose cell is getting bigger it is because it takes more fatty acids and there must be some elements within the genes encoding cytokines that is driven by these fatty acids. This is an oversimplified view, I think it is much more complex than that, but it is certainly the key to understanding why a fat-loaded adipose cell secretes more TNF-α or more cytokines in general. It is the best answer I can give you for now.

**Dr. Biolo:** About 10 years ago Baron [4] published a study showing that people with type-2 diabetes, people with hypertension, obese people, are characterized by an impaired insulin action on stimulation of blood flow. Do you think that insulin resistance is characterized by a chronic activation of iNOS, which may explain those old results?

**Dr. Marette:** I think it could contribute to these results because there is some published literature showing that iNOS induction in endothelial cells can actually reduce eNOS activity [2]. Now the problem of course is that iNOS also produces nitric oxide but in that case the hemodynamic effect of nitric oxide would be uncontrolled as compared to the eNOS stimulation which is tightly controlled by insulin and other vasodilators. So the idea behind this is that nitric oxide released locally at the level of skeletal muscle will create insulin resistance in the myocytes but the lack of ability of eNOS to release nitric oxide is limited in obese type-2 diabetic animals or humans because the eNOS isoform has been downregulated by iNOS. This is one mechanism and I don't think it explains everything, but it is one possibility. This is how people try to explain the link between having more nitric oxide with iNOS but still an inability to increase blood flow when needed by insulin. Again I would like to stress that the problem might
not be nitric oxide per se, but the derivatives that are produced such as reactive nitrogen species and the reactive oxygen species.

**Dr. Mace:** You mentioned different fat depots that you used to check iNOS activity. Did you see a difference between visceral fat adipose tissue and subcutaneous?

**Dr. Marette:** Until now we have looked at three different depots, the inguinal, retroperitoneal as well as the epidermal, and in all 3 of those we have seen increased iNOS expression in the adipose tissue. But I must be honest with you, we didn’t carefully look at possible regional differences in the amount of iNOS. I don’t think we fairly compared those, so I cannot give you a fair response whether there are regional differences. The only time we actually looked at this carefully was with tissues from obese humans, and when we compared visceral adipose tissue to what would be considered more subcutaneous, we see a greater abundance of iNOS in the visceral depot. But I think we need to do more studies so that I can be more convinced.

**Dr. Mace:** How do you explain the difference that you see between adipose tissue and skeletal muscle in terms of insulin sensitivity?

**Dr. Marette:** I have been thinking about that for a long time, and still the only thing that comes to my mind is that myocytes are probably the cells that produce the highest levels of radicals, not only during exercise but also in the resting state. So that is why I believe that nitric oxide is causing insulin resistance probably by reacting with these radicals and forming very oxidative molecules that will inhibit insulin action. The formation of these secondary metabolites is probably not sufficiently important in adipose tissue to cause a similar defect. But that is speculation until we prove it.

### References
