This Review is part of a thematic series on the Role of Mitochondria in Cardiovascular Diseases, which includes the following articles:

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Marshall S. Runge, Guest Editor

Defective Mitochondrial Biogenesis
A Hallmark of the High Cardiovascular Risk in the Metabolic Syndrome?

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Abstract—The metabolic syndrome is a group of risk factors of metabolic origin that are accompanied by increased risk for type 2 diabetes mellitus and cardiovascular disease. These risk factors include atherogenic dyslipidemia, elevated blood pressure and plasma glucose, and a prothrombotic and proinflammatory state. The condition is progressive and is exacerbated by physical inactivity, advancing age, hormonal imbalance, and genetic predisposition. The metabolic syndrome is a particularly challenging clinical condition because its complex molecular basis is still largely undefined. Impaired cell metabolism has, however, been suggested as a relevant pathophysiological process underlying several clinical features of the syndrome. In particular, defective oxidative metabolism seems to be involved in visceral fat gain and in the development of insulin resistance in skeletal muscle. This suggests that mitochondrial function may be impaired in the metabolic syndrome and, thus, in the consequent cardiovascular disease. We have recently found that mitochondrial biogenesis and function are enhanced by nitric oxide in various cell types and tissues, including cardiac muscle. Increasing evidence suggests that this mediator acts as a metabolic sensor in cardiomyocytes. This implies that a defective production of nitric oxide might be linked to dysfunction of the cardiomyocyte metabolism. Here we summarize some recent findings and propose a hypothesis for the high cardiovascular risk linked to the metabolic syndrome. (Circ Res. 2007;100:795-806.)

Key Words: nitric oxide • mitochondrial biogenesis • peroxisome proliferator-activated receptor-γ coactivator 1α • cardiomyocytes • obesity

The metabolic syndrome is a term applied to a pattern of metabolic risk factors occurring concurrently; these include atherogenic dyslipidemia, elevated blood pressure, elevated plasma glucose, and a prothrombotic and proinflammatory state.1,2 The dominant underlying risk factors appear to be abdominal obesity and insulin resistance. With aging and increasing obesity, the incidence and severity of these risk factors of metabolic and cardiovascular origin increase dramatically, as does the risk of atherosclerotic cardiovascular disease and its complications.3–6 If atherosclerotic cardiovascular disease does develop, cardiovascular complications—cardiac arrhythmias, heart failure, and thrombotic
episodes—often ensue. Many individuals affected by the metabolic syndrome eventually develop type 2 diabetes mellitus. Once diabetes develops, a host of complications, including renal failure and diabetic cardiomyopathy and neuropathies, can be observed; and when atherosclerotic cardiovascular disease and diabetes exist concomitantly, the risk of subsequent cardiovascular morbidity is very high.5

The multiple aspects of the metabolic syndrome have suggested the coexistence of complex, multiorgan pathophysiological events, with the development of specific organ diseases characteristically linked to each other.2–6 At the cellular level, various pathophysiological events responsible for the different clinical manifestations have also been hypothesized.2–6 Among these, impaired cell metabolism has been suggested as a key pathophysiological process. In particular, defective oxidative metabolism seems to be involved in visceral fat gain and in the development of insulin resistance in fat and skeletal muscle. This suggests that mitochondrial function may be impaired in the metabolic syndrome and, thus, in the linked cardiovascular diseases. Recently, we have reported that mitochondrial biogenesis and function are increased by nitric oxide (NO) in various cell types and tissues, including cardiac muscle.7,8

The purpose of this review is to give a brief overview of NO and mitochondrial biogenesis in relation to cardiac energy metabolism and to suggest new insights into how alterations in this metabolic pathway may lead to the development of cardiovascular disease linked to the metabolic syndrome.

The Metabolic Syndrome and Atherosclerotic Vascular Disease

In 2001, the National Cholesterol Education Program (NCEP) Expert Panel on Detection, Evaluation, and Treatment of High Blood Cholesterol in Adults (Adult Treatment Panel III [ATP III]) suggested a clinical definition for the metabolic syndrome that includes blood pressure, waist circumference, high-density lipoprotein (HDL) cholesterol, and triglyceride and fasting plasma glucose levels.3 According to Third National Health and Nutrition Examination Survey (NHANES III) data, the age-adjusted prevalence for the NCEP-defined metabolic syndrome in the US population is 23.7%.9 The high prevalence of the metabolic syndrome has significant public health implications because of the 2-fold increased risk of prevalent coronary heart disease,10 3- to 4-fold increased risk of mortality attributable to coronary heart disease,11 and a 6-fold risk of developing type 2 diabetes mellitus.12

The underlying pathophysiology of the metabolic syndrome is a subject of debate. Initial studies in this area suggested that insulin resistance has a primary role.13–16 However, more recent investigations show that visceral adiposity is a significant independent predictor of the insulin sensitivity,17–21 impaired glucose tolerance,22 elevated blood pressure,23–25 and dyslipidemia19,26–32 seen in the metabolic syndrome. Furthermore, intraabdominal fat is metabolically active as a source of free fatty acids33,34 and adipokines, such as adiponectin,35,36 and of inflammatory mediators such as tumor necrosis factor-α (TNF-α)34,37,38 and plasminogen activator inhibitor type 1 (PAI-1).39,40

A recent study has examined the relation of insulin sensitivity and intraabdominal adipose tissue and subcutaneous fat areas with the NCEP ATP III criteria for the metabolic syndrome in a nondiabetic population.41 The metabolic syndrome was defined according to the NCEP ATP III as the presence of 3 or more of the following clinical criteria: blood pressure of ≥130/85 mm Hg; waist circumference of >102 cm in men and >88 cm in women; HDL cholesterol of <1.036 mmol/L (40 mg/dL) in men and <1.295 mmol/L (50 mg/dL) in women; triacylglycerol of ≥1.695 mmol/L (150 mg/dL); and fasting plasma glucose levels ≥6.1 mmol/L (110 mg/dL).

In this study, performed on a large number of apparently healthy men and women of varying age, the authors showed that both insulin resistance and central adiposity are significant correlates of the metabolic syndrome.3 The intraabdominal fat area was independently associated with all of the metabolic syndrome criteria, whereas insulin sensitivity was independently associated with the criteria for HDL cholesterol, triacylglycerol, and fasting plasma glucose levels. In contrast, the subcutaneous fat area was independently correlated with waist circumference after adjusting for visceral fat area and insulin sensitivity, but not with other metabolic syndrome criteria. The results of this study, therefore, suggest that accumulation of intraabdominal adipose tissue is an important determinant of the metabolic syndrome. Furthermore, the significant association of visceral adiposity with all the features of the metabolic syndrome, including increased risk of cardiovascular disease, was in part independent of the effect of insulin resistance and abdominal subcutaneous fat, suggesting an important role for visceral adiposity.

Visceral adiposity, but not abdominal subcutaneous fat, is independently associated with insulin resistance27; lower HDL cholesterol levels27,28; higher apolipoprotein B38,39 and triglyceride levels27,28; smaller low-density lipoprotein particles28–31; aortic stiffness42; coronary artery calcification43; and higher blood pressure.23–25,32 Furthermore, a reduction in visceral fat by weight loss or surgical removal is associated with increases in insulin sensitivity44,45 and HDL cholesterol46 and decreases in triglycerides46 and blood pressure.24,46 Thus, visceral adiposity, independently from insulin resistance, may have a significant pathophysiological role in the development of the metabolic syndrome and its sequelae. In this context, in 2004, a group of experts was convened by the International Diabetes Federation to attempt to establish a unified definition for the metabolic syndrome and to highlight areas where more research into the syndrome is needed. A major issue for the International Diabetes Federation consensus consultation was the importance of obesity as a risk factor for several diseases including type 2 diabetes mellitus, cardiovascular disease, hypertension, gallstone disease, and certain cancers.6,47 Interestingly, the amount of obesity associated with increased risk differs among populations.48 At present, the ATP III update5 and the International Diabetes Federation report6 largely harmonize the clinical diagnosis of the syndrome. From the above reports, it clearly emerges that the risk for atherosclerotic cardiovascular disease associated
Is There a Defect in Oxidative Metabolism in the Metabolic Syndrome?

A recent study in subjects who were nondiabetic, insulin-resistant and at high risk for diabetes (“prediabetes”), or had type 2 diabetes mellitus, has demonstrated that prediabetic and diabetic muscle is characterized by a decreased expression of genes involved in oxidative phosphorylation, many of which are regulated by nuclear respiratory factor (NRF)-dependent transcription. Furthermore, in both prediabetic and diabetic subjects, there is a significant reduction in the expression of peroxisome proliferator-activated receptor-γ (PPAR-γ) coactivator 1α (PGC-1α), which is an inducible coregulator of nuclear receptors such as NRF involved in the control of mitochondrial biogenesis and functions (Figure 1). These data indicate that decreased PGC-1α expression may be responsible for the reduced expression of metabolic and mitochondrial genes regulated by NRF and may contribute to the metabolic disturbances characteristic of insulin resistance, diabetes mellitus, and, perhaps, obesity. In contrast, there is evidence to indicate that the expression of genes and gene targets involved in mitochondrial biogenesis and function is increased in the heart in type 1 diabetes. Whereas the healthy adult mammalian heart generates ATP via both carbohydrate utilization and mitochondrial fatty acid oxidation pathways, the diabetic heart derives most of its ATP from fatty acid oxidation, with an activated cardiac PPAR-α/PGC-1α system. This metabolic shift may initially be adaptive to maintain the required rate of ATP production. However, uncontrolled high-level fatty acid import and oxidation may eventually become maladaptive, leading to pathological mitochondrial and myocardial remodeling. Indeed, dysfunction has been described in heart mitochondria from insulin-resistant and diabetic animals, and this is associated with reduced transcriptional activity of the mitochondria.

In both obesity and common forms of type 2 diabetes mellitus, glucose oxidation and storage are reduced, in parallel with reduced activity of the tricarboxylic acid cycle, β oxidation, and electron transport enzymes, specifically complex I, with reductions in mitochondrial area and number. A potential role for dysregulation of oxidative metabolism gene expression in diabetes mellitus is suggested by studies in animals and humans. In streptozotocin-induced diabetic mice, the expression of oxidative phosphorylation genes has been reported to be decreased. Similarly, the expression of multiple energy metabolism genes is altered in poorly controlled type 2 diabetes mellitus in humans. In both studies, some differences were partly reversed by insulin, suggesting that differential regulation in diabetes may, at least in part, reflect secondary changes, perhaps caused by decreases in NRF-1 expression or transcriptional activity. However, although less pronounced, alterations in oxidative phosphorylation genes were also observed in insulin-resistant nondiabetics. Accordingly, the expression of 2 electron chain subunits (NADH dehydrogenase 1 and ATP5C1) is reduced in insulin-resistant nondiabetic Pima Indians, and the expression of ATP synthase subunit F is reduced in the insulin-resistant normoglycemic ob/ob mouse. A similar reduction in the expression of oxidative phosphorylation genes has been shown in white subjects with impaired glucose tolerance and type 2 diabetes mellitus. These results suggest that the metabolic syndrome is accompanied by a deficit of mitochondrial biogenesis and function.

Figure 1. Sympathetic nervous system activity, physical exercise, and calorie restriction induce cGMP production either directly or through an increase in eNOS levels in white adipose tissue and other mouse tissues. Mitochondrial genes involved in mitochondrial biogenesis, including PGC-1α, NRF-1, and Tfam, are upregulated as a consequence, leading to increased mitochondrial biogenesis. Inhibition of eNOS and PGC-1α expression and activity by cytokines and stimulation of eNOS and PGC-1α by leptin are also shown. Adapted from Nisoli E, Carra MO. Nitric oxide and mitochondrial biogenesis. J Cell Sci. 2006;119:2855–2862.
mass, whereas the expression of each mitochondrial transcript correlated negatively.65 These results strengthen the possibility that mitochondrial biogenesis and functions are defective in visceral fat of patients affected by the metabolic syndrome.

It has been demonstrated that leptin, the deficiency of which is linked either to massive obesity (ob/ob mice) or very marked insulin resistance (lipodystrophy),66,67 induces the oxidation of fatty acids,72 it is quite likely that its increase was responsible for the loss of fat through “internal combustion.” Although the action of leptin on mitochondrial biogenesis is controversial,69 profound morphological and molecular changes in mitochondria of white fat cells (which store energy in the form of fat) have recently been described in a model of adenovirus-induced hyperleptinemia.70 These include shrinkage, loss of fat, and loss of shape of adipocytes, with extensive infoldings of the cell membrane. The modified cells (postadipocytes) contained a profusion of mitochondria that differed in size and appearance from the much larger mitochondria of brown adipocytes, which are mitochondria-rich cells specialized for thermogenesis. PGC-1α mRNA rose from the very low levels normally present in white adipose tissue (WAT) to those of BAT. PGC-1α is involved in mitochondrial biogenesis; thus its increase might be causally linked with the abundance of mitochondria in postadipocytes. Indeed, when PGC-1α was not increased, as in fat cells of adenovirus-leptin-treated PPAR-null mice, the hyperleptinemia failed to induce adipocyte fat loss.71 Because forced expression of PGC-1α in human fat cells enhances their oxidation of fatty acids,72 it is quite likely that its increase was responsible for the loss of fat through “internal combustion.” The abundance of mitochondria, induced by the high PGC-1α levels, would increase the machinery required for enhanced oxidation, whereas increased uncoupling protein-1 and -2, the expression of which is enriched in the thermogenically active BAT, would dissipate the unneeded energy as heat. Although this combination of events was reminiscent of hyperthyroidism, triiodothyronine levels were not increased in the plasma of the leptinized rats.70

Endothelial Nitric Oxide Synthase—Null Mice: A Model of the Metabolic Syndrome?

Endothelial nitric oxide synthase (eNOS) deficiency in null-mutant mice has been found to be associated with a clustering of cardiovascular risk factors that mirror those present in patients with the metabolic syndrome.77–79 eNOS−/− mice are hypertensive, insulin resistant, and have dyslipidemia.74–76 Furthermore, they have been reported to have elevated plasma levels of leptin, uric acid, and fibrinogen and to become more glucose intolerant than control mice when challenged with a metabolic stress, such as a high-fat diet.73 The augmented leptin plasma levels in eNOS−/− mice suggest that body weight and/or relative adipose tissue content may be raised in the knockout mice, resulting from the fact that fat accumulation increases adipose synthesis and release. We have shown that in eNOS−/− mice, there is a marked accumulation of visceral fat (Figure 2) and body weight is increased.7 We also found that oxygen consumption rates—an indicator of metabolic rate—are decreased in these animals, suggesting that brown fat–dependent thermogenesis is impaired.7 In genetic models of obesity, defective energy expenditure is involved in body weight gain. Because 8-week-old eNOS−/− mice showed similar food consumption but weighed more than wild-type mice, their increased body weight could be accounted for by higher feed efficiency (ie, weight gain/food intake) caused by defective energy expenditure. Indeed, histological analysis indicated that eNOS−/− BAT was functionally inactive, and exposure of these animals to cold did not result in the well-known mitochondrial process that is usually observed in wild-type animals. In addition, deletion of eNOS was sufficient to reduce the number and function of mitochondria even in tissues that have a basal level of neuronal, and possibly inducible NOS expression, such as the brain, liver and heart.7 These findings led us to suggest a critical role for eNOS in mitochondrial biogenesis.

Our group and others have observed that NO generation is triggered by many of the stimuli initiating BAT differentiation, including cold exposure and physical exercise. Cold exposure triggers eNOS, inducible NOS, and PGC-1α expression,77–79 through activation of β3-adrenergic receptors and increases in intracellular cAMP and Ca2+, all of which stimulate NO production in brown adipocytes.77 eNOS can be activated through increases in cytosolic Ca2+ concentrations and phosphorylation by various protein kinases, including AMP-activated protein kinase, suggesting that different mitochondrialogenic stimuli might act, at least in part, through the generation of NO.

Both in vivo and in vitro evidence has indicated that NO, either exogenously applied or generated by eNOS, plays a significant role in brown adipocyte differentiation, proliferation, and thermogenesis78,80–82 Systemic inhibition of NOS with NG-nitro-L-arginine caused defective adaptive thermogenesis in rodents.83,84 Interestingly, mitochondrial conversion of 3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyl tetrazolium bromide (MTT) to formazan, a method usually used for the indirect measurement of cell proliferation on the basis of mitochondrial dehydrogenase activity, was reduced by only 30% to 40% in spite of the complete inhibition of brown fat cell proliferation after chronic treatment with NO do-
nors.76 These results suggest that NO may promote mitochondrial biogenesis in brown fat cells even while inhibiting cell proliferation.

To investigate this issue, we studied the mitochondrial biogenesis in primary cultures of mouse brown and white fat cells and found that treatment with NO donors increased the mitochondrial DNA (mtDNA) content in these cells.7 We also found that NO-stimulated mitochondrial biogenesis occurred through activation of PGC-1α in BAT and cardiac and skeletal muscle. Furthermore, we demonstrated that the mitochondrial biogenesis depends on cGMP. Similar results were obtained in mouse white-fat 3T3-L1 and human monocytic U937 cell lines, revealing that the NO-dependent mitochondrial biogenesis was not restricted to brown adipocytes and their differentiation processes. To investigate the role of endogenous NO, we stably transfected HeLa cells with eNOS and found that its induction was sufficient to initiate mitochondrial biogenesis in the transfected cells.7

Interestingly, key signaling molecules mediating the metabolic actions of insulin, including the insulin receptor tyrosine kinase, phosphatidylinositol 3-kinase, and Akt,85 are also necessary for insulin to stimulate production of NO and vasodilation in human vascular endothelium.86 In particular, insulin induces eNOS phosphorylation at Ser1179 through activation of Akt with an increased production of NO.87 Thus, one physiological role of the eNOS-derived NO in vascular endothelium may be to mediate the action of insulin in coupling hemodynamic and metabolic homeostasis.

eNOS is known to be present in murine myocardium, not only in the microvascular and arterial endothelium88 but also in the cardiac myocytes.90 Studies in eNOS−/− mice subjected to myocardial infarction have demonstrated the role of eNOS in limiting left ventricular dilatation, dysfunction, and hypertrophy, possibly by limiting the hypertrophic response to myocardial infarction.90 The eNOS deficiency was associated with increased mortality after myocardial infarction.90

**Mitochondrial Biogenesis Is Impaired in Obesity**

Obese subjects are deficient in energy in the form of ATP.91,92 Inadequate energy supply in the body results in increased appetite. Although the mechanism of such signaling is not yet known, there is evidence that it exists.93–95 Treatment of rats with the metabolic inhibitors 2,5-anhydro-d-mannitol or/and methyl palmitate reduces the ATP levels in liver cells. Decreasing ATP concentrations in this way has been shown to lead to changes in the eating habits of the animals, including the frequency of eating, the amount of food consumed at a single eating session, and the total amount of food eaten. These experiments indicate that there is an integrated metabolic control of food intake, with liver ATP levels acting as a major sensor of energy status in the body. In obese people, the levels of hepatic ATP are decreased. Moreover, a decreased exercise capacity, together with high fatigability, has been linked to the low ATP levels in skeletal muscle of obese subjects.96 This in context, genetically heterogeneous rats can be separated into 2 groups according to their aerobic treadmill-running capacity, with low-capacity runners showing higher visceral adiposity, blood pressure, insulin-resistance, plasma triglycerides, and free fatty acids as compared with the high-capacity runners.97 Intriguingly, mitochondrial dysfunction with a defective oxidative metabolism and a low mitochondrial gene expression were evident in low-capacity runners,97 suggesting that visceral obesity and related disorders are linked to defective mitochondrial biogenesis and oxidative metabolism with a decreased ATP production. Recent studies in obese rodents have supported this hypothesis, not only at the molecular and biochemical but also at the morphological level.98,99 In our experiments in 3 models of obesity, we found that eNOS expression was markedly reduced in WAT, BAT, and soleus muscle of ob/ob mice, falfa rats, and high-fat diet–induced obese mice (DIO mice).100 Moreover, mtDNA, respiratory protein (such as COX IV and Cyt c) levels, PGC-1α, NRF-1, and mtDNA transcription factor A (Tfam) gene expression were decreased in parallel, as well as oxygen consumption and ATP production.100 Changes in mitochondrial morphology were also observed in tissues from obese animals; these paralleled the functional deficits of mitochondria, suggesting an increase of the fission events in mitochondrial dynamics. The possibility that altered mitochondrial dynamics plays a role in the pathophysiology of mitochondrial dysfunction linked to obesity is supported by the observation that the expression of mitofusin 2, which is involved in the mitochondrial fusion events, was reduced in skeletal muscle of both eNOS−/− mice (E.N. and C. Tonello, unpublished results, 2006) and obese falfa rats.101

As mentioned above, large-scale gene-expression analyses in obese animals65,102,103 and humans49,64 have demonstrated that many genes encoding mitochondrial proteins are negatively correlated with body mass,104 providing further evidence that mitochondrial dysfunction is significant in the pathophysiology of obesity. Additionally, mice with genetic deletion of NEIL1 DNA glycosylate, an enzyme involved in base excision repair of ring-fragmented purines and some pyrimidines, have increased mtDNA damage and deletions and develop severe obesity with dyslipidemia, fatty liver disease, and a tendency toward hyperinsulinemia.105

Consistent with our observations in the metabolically active tissues of obese rodents, a 3.7-fold decrease of eNOS expression in total abdominal WAT of male DIO mice and an inverse correlation between the skeletal muscle eNOS content and the percentage body fat and body mass index in young adult women have been described.106,107 In this context, dietary supplementation with L-arginine, which increases serum NO concentration and PGC-1α expression in WAT, has been shown to reduce fat mass in Zucker Diabetic Fatty (ZDF) rats 4 to 10 weeks after the initiation of treatment.108

**Inflammation and Obesity**

The proinflammatory cytokine TNF-α is overproduced in the adipose as well as muscle tissues of obese subjects.65,103,109,110 We have shown that TNF-α markedly decreased both eNOS expression and mitochondrial biogenesis in cultured fat and muscle cells. Downregulation of eNOS seems to be the major molecular mechanism by which TNF-α affects mitochondrial biogenesis in vitro models, because its effects on mitochondria can be reversed by treatment with the NO donors.
(Z)-1-[N-(2-aminoethyl)-N-(2-aminoethyl)amino]diazen-1-ium-1,2-diolate (DETA-N0) and S-nitroso-N-acetylpenicillamine (SNAP). Furthermore, mitochondrial biogenesis in WAT, BAT, and muscle was at least partially restored in obese mice with defective TNF-α signaling (ie, obese mice with genetic deletion of TNF-α receptors, either p55 or p75 or both). This partial recovery of mitochondrial biogenesis in WAT and BAT of both ob/ob p55−/− and ob/ob p55−/−p75−/− mice was accompanied by a full restoration of eNOS expression in these tissues. These results suggest that TNF-α plays a relevant role in decreasing eNOS expression and mitochondrial biogenesis in metabolically active tissues of obese animals. The fact that ob/ob p55−/− p75−/− mice behaved similarly to the ob/ob p55−/− mice in terms of mitochondrial parameters suggests that p55 is the most relevant receptor in mediating the effects of TNF-α.

In different animal models of obesity, it has been shown that the production of TNF-α is increased in adipose tissue and that it induces insulin resistance. Other active substances were subsequently found to be produced in fat; these include leptin, interleukin (IL)-6, resistin, monocyte chemoattractant protein-1, PAI-1, angiotensin, visfatin, retinol-binding protein-4, serum amyloid A. Whereas leptin and adiponectin are true adipokines that appear to be produced exclusively by adipocytes, TNF-α, IL-6, monocyte chemoattractant protein-1, visfatin, and PAI-1 are also expressed at high levels in activated macrophages and/or other cells. Thus, obesity has emerged over the last few years as a chronic, low-grade inflammation. This inflammatory state is evident in adipose tissue, liver, and skeletal muscle. Inflammation is also closely linked to the pathogenesis of atherosclerosis, suggesting that inflammation might be a common factor that links obesity to many of its pathological sequelae. Indeed, increasing evidence indicates the existence of a link among mitochondrial dysfunction, increased TNF-α serum levels, and cardiovascular disease. This evidence, together with that described in the following section, strongly suggests that defects in mitochondrial number and/or function are causally linked to the cardiovascular alterations found in obesity and the metabolic syndrome.

Potential mechanisms for activation of inflammation in adipose tissue are intensely investigated and can be summarized as follows. Nutrients such as lipids and cytokines such as TNF-α and IL-1β can trigger inflammatory kinases, including c-Jun N-terminal kinase (JNK) and IκB kinase/nuclear factor κB (IKK/NF-κB) (Figure 3) through classical receptor-mediated mechanisms that have been well characterized. These kinases have been reported to exert powerful effects on gene expression, including promoting further expression of inflammatory genes through activation of activator protein-1 complexes. NF-κB activity has also been implicated in the pathogenesis of atherosclerosis. Cellular stresses, including reactive oxygen species (ROS) and endoplasmic reticulum (ER) stress, also activate JNK and IKK, both of which then regulate the production of various cytokines, including TNF-α. ROS has been implicated in the development of obesity-induced insulin resistance because systemic markers of oxidative stress increase with adiposity. Furthermore, under pathophysiological conditions, such as inflammation, hyperglycemia, hyperlipidemia, and hypoxia, all of which are present in obesity, ROS and NO may react avidly. Consequently, the half-life of the bioactive NO is reduced and reactive nitrogen species, particularly dinitrogen trioxide and peroxynitrite (ONOO−), are generated with significant damage to cellular components up to cell death. Lipid accumulation also activates the unfolded protein response to increase ER stress in fat and liver. Hyperactivation of JNK by ER stress has been reported to result in serine phosphorylation of insulin receptor substrate-1. Furthermore, ER stress has been shown to activate NF-κB. These observations suggest that obesity may be a chronic stimulus for ER stress, which itself may be a central mechanism underlying peripheral insulin resistance. Once activated, the processes may be self-perpetuating through a positive-feedback loop created by the proinflammatory cytokines produced (Figure 3).
These findings suggest that the inadequate utilization of body energy by obese subjects an “emergency” signal difficult to ignore, namely that “more energy is needed to survive.” As a consequence, obese subjects increase their food intake and restrict physical activity to maintain energy supply to the body. Thus, although overeating and a sedentary lifestyle lead to fat accumulation, they might not be the only pathophysiological bases of obesity. In fact, they may be the result of an already “obese metabolism,” which would force individuals to over-eat and preserve energy. This hypothesis may be supported by the observation that, whereas, in normal-weight animals, a moderate calorie restriction enhances mitochondrial oxidative capacity by increasing eNOS expression and mitochondrial biogenesis; in obese subjects, a restrictive diet lowers further an already defective lipid oxidation in different tissues, probably because of the lack of the compensatory NO-related mitochondrial biogenesis. These findings suggest that a decrease in inflammatory processes and increase in mitochondrial mass in metabolically active tissues might provide a way of managing obesity and related disorders, including insulin resistance, type 2 diabetes mellitus, and some forms of cardiovascular disease.

Are There Pathophysiological Consequences of Defective NO-Induced Mitochondrial Biogenesis in the Heart of Obese Subjects?

Although there are clinical reports of cardiomyopathy of obesity that can be reversed by weight loss, cardiac dysfunction, arrhythmias, cardiomyopathy, and congestive heart failure are at present attributed by most clinicians to atherosclerotic consequences of obesity, including coronary artery disease and hypertension. Nevertheless, increasing evidence indicates that lipotoxic heart disease exists both alone and in concert with these well-recognized causes of heart disease and that it impairs the ability of the myocardium to compensate for them. However, the details of the mechanism involved require further investigation because, in fact, nonadipocytes have a very limited capacity to store excess fat. If they are exposed to high levels of plasma lipids, as usually occurs in obesity, they may undergo steatosis and loss of function, and ultimately fatty acid-induced “lipoapoptosis” may occur. In ZDF rats, a model of obesity secondary to genetic unresponsiveness to leptin, as the animals become increasingly obese, triacylglycerol accumulates rapidly in the heart and in other nonadipose tissues. This is the consequence not only of elevated plasma lipids but also of increased expression in nonadipose tissues of lipogenic enzymes such as glycerol-phosphate acyltransferase coupled with decreased expression of acyl-CoA oxidase and carnitine palmitoyltransferase-1 and their transcription factor, PPAR-α. These changes are associated with a progressive increase in ceramide content and increased apoptosis, resulting in myocyte dysfunction or death, termed “lipotoxicity.” Significant reduction in fractional area shortening is observed at 20 weeks of age of ZDF rats, indicating a clinically significant reduction in cardiac contraction. As previously noted, some other studies have shown that the insulin-resistant and diabetic heart exhibit a markedly increased capacity for fatty acid oxidation, associated with an activation of PPAR-α and its target genes involved in this process at least in the short term. Thus, one can suggest that in the ZDF rat the expression of PPAR-α and its target genes begins to fall in the latter stages of disease.

A further pathophysiological mechanism of cardiomyopathy in obesity may originate from defects in eNOS-dependent signaling, because eNOS expression is decreased in the heart of obese rodents (our unpublished results). Indeed, the accumulation of triacylglycerol and consequent reduction in cardiac contraction may also be attributable to a reduced β-oxidation of fatty acids in cardiomyocytes resulting from impaired mitochondrial biogenesis and function. Evidence for a link between mitochondrial respiratory dysfunction and heart failure is compelling. The cardiomyopathic phenotype of humans with mitochondrial genome defects underscores the importance of high-capacity mitochondrial ATP production for normal striated-muscle function. Mutations in both nuclear- and mitochondrial-encoded genes account for heritable respiratory chain defects. Respiratory chain defects typically present as a multisystem dysfunction, disproportionately affecting organs with a high demand for ATP, such as the heart, skeletal muscle, and the central nervous system. Cardiomyopathy may develop during childhood or at later ages.

Several recently developed mouse models have identified potential links between PGC-1α–mediated control of mitochondrial function and the development of heart failure. PGC-1α is abundantly expressed in the heart. As seen previously, PGC-1α activates most genes of mitochondrial function and biogenesis and stimulates both fatty acid oxidation and oxidative respiration in cardiac tissue. Conversely, it has recently been shown that ablation of the PGC-1α gene caused significant deficiencies in cardiac energy reserves and function. In the absence of PGC-1α, the expression of mitochondrial genes in the heart was suppressed, the activities of mitochondrial enzymes were aberrant, and ATP production was blunted. These energetic defects led to an inability to increase contractile function when the hearts were stimulated with adrenergic agents. Mice lacking the PGC-1α transcriptional coactivator develop cardiac dysfunction in response to cardiac duress initiated by constriction of the transverse thoracic aorta, a commonly used model of pressure overload in rodents and other animals. The cardiac dysfunction observed was accompanied by clinical signs of heart failure.

Overexpression of cyclin T/Cdk9, an RNA polymerase kinase, has recently been shown to trigger cardiac hypertrophy. Gene expression–profiling studies demonstrated that Cdk9 suppresses the expression of PGC-1α and its downstream targets. Rescue of PGC-1α expression in cardiac myocytes in culture prevented Cdk9-triggered apoptosis. Despite strong evidence for a link between mitochondrial dysfunction and heart failure in genetic models, the role of altered mitochondrial ATP generation in the pathogenesis of acquired forms of heart failure still requires investigation.

Finally, mature human adipocytes have been reported to release a substance that strongly and acutely suppresses the
contraction of electrically paced adult cardiomyocytes by reducing intracellular Ca\(^{2+}\). This adipocyte-derived negative inotropic activity appeared to be a protein with a molecular mass between 10 and 30 kDa. These findings suggest a direct involvement of adipose tissue in the pathogenesis of myocardium dysfunction.

The evidence summarized above indicates that defects of the NO-induced mitochondrial biogenesis, with decreased PGC-1\(\alpha\) expression, are relevant in the pathophysiology of cardiovascular disease linked to obesity. Further support for this concept comes from the fact that eNOS-dependent synthesis of NO by the vascular endothelium regulates arterial pressure and is defective in human essential hypertension. Endothelium-derived NO also mediates insulin-induced stimulation of the perfusion of skeletal muscle. In insulin-resistant individuals, insulin stimulation of endothelial NO production is impaired and may contribute to defective skeletal muscle glucose uptake. In line with this, NOS inhibitors reduce insulin-stimulated muscle glucose uptake in rats in vivo. Furthermore, eNOS is expressed in the skeletal muscle, and NO donors stimulate glucose transport in isolated rat muscle preparations.

Partial gene deletion of eNOS (eNOS\(^{-/-}\) mice) has been shown to predispose to exaggerated high-fat diet–induced insulin resistance and arterial hypertension, suggesting an important interaction between genetic (eNOS polymorphism) and environmental factors (high-fat diet) in the regulation of vascular NO synthesis and glucose and blood pressure homeostasis. Thus, the development of the metabolic syndrome and cardiovascular disease might be facilitated by the combination of a genetic predisposition (eg, eNOS polymorphisms) together with environmental factors such as a Western-type diet.

The relationship among eNOS deficiency, mitochondrial biogenesis, and cardiovascular disease linked to the metabolic syndrome has been demonstrated in rodents. Whether these findings are applicable to humans is still unknown. However, there is some evidence that hypertension, coronary artery disease, and myocardial infarction might be associated with eNOS gene polymorphism. The resultant impaired NO synthesis could predispose to insulin resistance and the metabolic syndrome.

At present, most clinicians attribute the cardiac defects that occur in obesity to coronary artery disease or hypertension, because these are established diagnostic categories commonly associated with obesity. The evidence reported in this review, however, suggests that lipotoxic heart disease, characterized by defective mitochondrial biogenesis and increased apoptosis, can act both alone and in concert with these well-recognized causes of heart disease, possibly by impairing the ability of the myocardium to compensate for them.

Conclusions

In summary, evidence is emerging to support the concept that alterations in mitochondrial biogenesis and energetics of cardiomyocytes are linked to the development and progression of cardiovascular disease and heart failure in obesity. Accordingly, signaling pathways involved in cardiac ATP generation, including eNOS-dependent NO production and PGC-1\(\alpha\)–modulated expression of genes involved in mtDNA replication and cell respiration, are attractive targets to consider as potential novel therapeutic strategies for the prevention and early treatment of obesity-linked heart diseases.

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Disclosures

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