Insulin Resistance and Cardiovascular Disease

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Cardiovascular risk factors cluster in obese individuals. Insulin resistance emerges as a common pathogenetic denominator underlying the risk factor cluster. Defects in nonesterified fatty acids metabolism have been implicated in the abnormal lipid and glucose metabolism which characterize the cluster. Other evidence also leads to the adipocyte as an important contributor to the risk factor cluster and cardiovascular complications through effects not only on fatty acids but also on leptin, plasminogen activator inhibitor-1, and angiotensinogen, to name a few. Fatty acids are elevated among abdominally obese individuals, are more resistant to suppression by insulin, and may contribute to hypertension. Fatty acids may affect blood pressure by inhibiting endothelial nitric oxide synthase activity and impairing endothelium-dependent vasodilation. Fatty acids increase $\alpha_{1}$-adrenoceptor-mediated vascular reactivity and enhance the proliferation and migration of cultured vascular smooth-muscle cells. Several effects of fatty acids are mediated through oxidative stress. Fatty acids can also interact with other facets of cluster, including increased angiotensin II, to accentuate oxidative stress. Oxidative stress, in turn, is implicated in the pathogenesis of insulin resistance, hypertension, vascular remodeling, and vascular complications. A clearer delineation of the key reactive oxygen signaling pathways and the impact of various interventions on these pathways could facilitate a rationale approach to antioxidant therapy and improved outcomes among the rapidly growing number of high-risk, insulin-resistant, obese individuals. Am J Hypertens 2001;14:116S–125S © 2001 American Journal of Hypertension, Ltd.

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Epidemiologic data indicate that insulin resistance is a risk factor for cardiovascular disease. Insulin resistance has been implicated as a central pathogenetic feature of the cardiovascular risk factor cluster that includes hypertension, impaired glucose tolerance, and Type 2 diabetes mellitus, hyperinsulinemia, dyslipidemias, and hemostatic disorders. Although much of the cardiovascular risk associated with insulin resistance is mediated by these established risk factors, a component of the excess cardiovascular disease associated with insulin resistance appears to be independent of them. The fact that risk factors cluster suggests a common pathogenetic denominator underlying their association. A better understanding of the fundamental mechanisms linking insulin resistance to cardiovascular risk could provide a foundation for more efficient and effective interventional strategies.

Insulin resistance can occur independently of overweight and obesity, but the two are linked. Obesity is a major and growing public health burden. Although the surge of interest in obesity is relatively recent, many health risks were noted by insurance companies more than 100 years ago. Studies have shown that abdominal obesity is associated with insulin resistance, hypertension, dyslipidemia, Type 2 diabetes, and premature cardiovascular morbidity and mortality. As with insulin resistance, a component of the excess cardiovascular risk associated with obesity is independent of other classical risk factors.

Despite the well-known health risks of excess body weight, the epidemic of overweight and obesity has grown to include 55% of American women and 63% of American men and continues to blossom. The costs of obesity in the United States were estimated at between $46 and $68 billion in 1990. Recent estimates suggest that obesity accounts for more than 5% of direct medical costs and up to 10% of the indirect costs from reduced work force productivity. Much of the obesity-related health expenses result from the excess diabetes, hypertension and heart disease. Obesity-related illnesses account for approximately 300,000 deaths annually in the United States.

Insulin resistance emerges as an important variable linking obesity to disease risks and outcomes. The preva-
The Adipocyte in Obesity-Associated Hypertension
And CV Risk Factor Clustering

The Renin-Angiotensin System

Obesity is associated with higher plasma angiotensinogen, plasma renin activity, tissue-specific angiotensin-converting enzyme (ACE), and plasma aldosterone levels. The renin-angiotensin-aldosterone system may mediate some of the relationship between body mass index, current blood pressure, and future risk of hypertension. Weight loss lowers renin, aldosterone, and blood pressure in a dose-dependent manner.

Cross-cultural studies show a strong positive correlation of $r = 0.90$, $P < .001$, between the mean body mass index and average plasma angiotensinogen concentrations of various populations. In normotensive rats, fasting reduces angiotensinogen production by adipocytes, whereas refeeding has the opposite effects. The decline of blood pressure with fasting and the increase with refeeding correlates strongly with the changes in adipocyte angiotensinogen. Angiotensinogen gene expression is enhanced by high fat diets, fatty acids, and insulin. The nutritional regulation of angiotensinogen gene expression may have important implications for weight-related changes of blood pressure. In transgenic mice, blood pressure rises 8 mm Hg for each additional copy of the angiotensinogen gene expressed, whereas overexpression of angiotensin-converting enzyme is not associated
with higher blood pressure. Adipocytes produce angiotensinogen as well as cathepsins D and G and angiotensin-converting enzyme, which are capable of generating angiotensin II independently of renin. Evidence suggests an expanded adipocyte mass generates more angiotensinogen, which may provide the substrate for high blood pressure.

Treatment of obese hypertensives with an angiotensin-converting enzyme inhibitor improves insulin’s nonsterified fatty acid (NEFA) lowering action and decreases blood pressure. The reduced rate of NEFA turnover during a euglycemic clamp in these patients on an angiotensin-converting enzyme inhibitor therapy correlated with the reduction of arterial blood pressure (Fig. 2). These data suggest that increased activity of the renin-angiotensin system in obesity contributes to impairment of insulin’s effects on NEFA in adipocytes which may lead to elevated blood pressure.

Obesity is associated with high aldosterone levels. Unmodified fatty acids inhibit the biosynthesis of aldosterone by isolated adrenal zona glomerulosa cells in culture. In contrast, addition of fatty acids in cocultures of zona glomerulosa and hepatocytes increases aldosterone biosynthesis. These observations raise the possibility that abdominal obesity may raise aldosterone by increasing the delivery of fatty acids to the liver, through the portal circulation. The liver converts the fatty acids to stimuli of adrenal aldosterone production. Given the evidence that aldosterone contributes to sodium retention and cardiac, vascular, and renal pathology, these data provide another plausible mechanism by which excess adiposity may raise blood pressure and adversely affect cardiovascular outcomes.

Angiotensin infusions produce a sustained blood pressure elevation. The magnitude of the pressor response is a direct function of sodium balance. Angiotensin and aldosterone are both implicated in clinically significant cardiac, vascular, and renal disease. However, an activated renin-angiotensin system is not always pathologic. Cardiovascular and renal diseases do not emerge as significant health concerns of unacculturated people consuming very low salt diets, despite intense, lifelong activation of the renin-angiotensin-aldosterone axis. The cross-cultural studies suggest that angiotensin and aldosterone alone are probably not sufficient to explain cardiovascular and renal pathology.

There are several possible explanations for the apparent paradox that angiotensin and aldosterone appear to play a major role in the cardiovascular disease of acculturated but not in unacculturated people on very low-salt diets. First, aldosterone alone is generally a weak mitogen. However, angiotensin significantly enhances cellular responses to a number of other growth factors. Thus, angiotensin may contribute more to cardiovascular remodeling in subjects with the risk factor cluster who probably have higher levels of other mitogens related to platelet activation, hyperinsulinemia, oxidized low-density lipoprotein (LDL), and fatty acids. Second, obesity may increase tissue angiotensin-converting enzyme and raise angiotensin concentrations locally. Third, angiotensin converting enzyme participates in the degradation of Ang 1-7, which has effects opposing those of Ang II. Higher levels of angiotensin-converting enzyme could serve as one explanation for the lower values of Ang 1-7 in hypertensives than in normotensives.

Nonesterified Fatty Acids (NEFA)

Abdominal obesity is linked to increased NEFA concentrations and turnover, which are resistant to suppression by insulin. NEFA may contribute to metabolic aspects of the risk factor cluster by reducing hepatic insulin uptake and increasing hepatic glucose output, very LDL synthesis, and apolipoprotein B production. These changes of lipid metabolism underlie the greater proportion of small, dense LDL-cholesterol particles which characterize patients with the cluster. NEFA decrease skeletal muscle glucose use and impair insulin-mediated glucose disposal. NEFA activate apoptotic pathways in pancreatic β cells which may contribute to Type 2 diabetes mellitus in genetically predisposed individuals.

In addition to adverse effects on lipid and carbohydrate metabolism, NEFA increase plasminogen activator inhibitor-1 (PAI-1), a prothrombotic factor which is elevated in subjects with the risk factor cluster. Aldosterone also increases production of PAI-1. PAI-1 is implicated in cardiovascular disease mainly through its capacity to inhibit the activation of tissue plasminogen activator, a critical component of the fibrinolytic system. However, PAI-1 is more directly implicated in renal pathology.

Raising plasma NEFA in minipigs to ~2 mM with an Intralipid and heparin infusion elevates vascular resistance.
and raises blood pressure by nearly 30 mm Hg.\textsuperscript{62} Raising plasma NEFA with Intralipid and heparin to similar levels in obese humans also increases blood pressure,\textsuperscript{63} although the magnitude of the pressor response was much smaller in humans. Infusion of oleic acid into the portal circulation of normal rats elicits blood pressure and is blocked by $\alpha_1$-receptor antagonists but not by angiotensin-1 receptor blockade.\textsuperscript{64} Infusion of the same amount of oleic acid systemically elicits a much smaller rise of blood pressure. These data suggest that portal oleic acid infusions produce a neurogenically mediated pressor effect in rats.\textsuperscript{64} The experimental data in rats are consistent with observations in obese humans.

Abdominal obesity is strongly related to elevated blood pressure.\textsuperscript{1,65} The visceral fat mass is active with rapid lipolysis and reesterification of triglycerides.\textsuperscript{54} Abdominally obese individuals probably have increased delivery of fatty acids, first to the portal circulation and liver, then systemically.\textsuperscript{64} Increased delivery of fatty acids to the portal circulation could have a significant role in the metabolic risk associated with the cluster. The findings in normal rats suggest that increased portal fatty acids levels may contribute to obesity-related hypertension, which has neurogenic components in both animals and man.\textsuperscript{66–68}

Even within the normotensive range, abdominally obese subjects have higher blood pressures than individuals with gluteofemoral obesity, and these differences are correlated directly to measures of insulin resistance.\textsuperscript{65} Subjects with resistance to insulin-mediated glucose disposal are also resistant to insulin’s NEFA lowering actions.\textsuperscript{54,69,70} Moreover, resistance to insulin’s effects on fatty acids during a euglycemic clamp coincides with higher plasma NEFA over 24 h and a higher plasma NEFA nadir after a mixed meal.\textsuperscript{69,71,72} Obese hypertensives have a severe defect of insulin’s capacity to suppress NEFA. NEFA were suppressed in upper body obese normotensives by ~50% and turnover by about 30% when plasma insulin was raised by as little as 5 $\mu$U/mL.\textsuperscript{54} Obese hypertensives did not suppress NEFA concentration and turnover by 50% even when plasma insulin was raised ~100 $\mu$U/mL (Fig. 3).\textsuperscript{70}

Blood pressure appears more closely related to abnormalities of insulin’s actions on NEFA than on glucose. NEFA concentration and turnover during the clamp correlated directly with blood pressure (Fig. 3).\textsuperscript{70} These correlations persisted when lean normotensives were excluded and after controlling for hyperinsulinemia and insulin-mediated glucose disposal. This finding was confirmed in another group of volunteers, in which NEFA measured at minute 15 of an insulin tolerance test correlated with blood pressure independently of the changes in glucose during the same test.\textsuperscript{70}

Patients with familial combined hyperlipidemia have a genetic syndrome which may provide clues on the link between fatty acids and hypertension. Patients with familial combined hyperlipidemia have plasma NEFA of $\sim 1.5 \pm 0.5$ mM [standard deviation (SD)] after a high-fat test meal.\textsuperscript{57} These are close to the NEFA levels which caused vasoconstriction and a pressor response in minipigs.\textsuperscript{52} Hypertriglyceridemic family members of proband cases with familial combined hyperlipidemia have a tendency to higher fasting insulin ($P = .002$) and NEFA ($P = .09$) as well as higher systolic blood pressure ($P = .02$) than family members with normal triglycerides.\textsuperscript{57} Patients with familial combined hyperlipidemia comprise about 1 to 2% of the general population but about 12% of hypertensive patients.\textsuperscript{73} These observations suggest that the abnormality in NEFA metabolism may link this genetic syndrome to the lipid and blood pressure abnormalities. This notion is strengthened by data from the Paris Prospective Study demonstrating that fasting and postoral
glucose plasma NEFA were positively and independently related to the development of hypertension.74

One potential mechanism by which fatty acids could raise blood pressure is through the induction of oxidative stress.75,76 The notion that oxidant stress contributes to hypertension is supported by a recent study in normotensive Sprague-Dawley rats.72 Addition of the glutathione synthase inhibitor buthionine sulfoximine to drinking water for four weeks reduced tissue glutathione ~70%, decreased urinary nitrate excretion, a marker for nitric oxide, by over 60%, and increased systolic blood pressure (BP) ~75 mm Hg. Addition of vitamins C and E attenuated the rise of BP by ~50% and corrected the defect in urinary nitrate excretion without significantly altering tissue glutathione content.

The DASH study indirectly supports a role for antioxidants in BP control.78 The DASH combination diet, which was high in fruits, vegetables, and low-fat dairy products, lowered systolic blood pressure by 11.4 mm Hg in hypertensives but only 3.5 mm Hg on normotensives. The diet that was high in fruits and vegetables but relatively Ca2+- deficient lowered systolic BP by 7.2 mm Hg in the hypertensive subset but only 0.8 mm Hg in normotensives. Thus, much of the benefit of the DASH diet occurred in hypertensive patients and resulted from the increased consumption of fruits and vegetables, which are high in antioxidants and K+. The Trial of Hypertension Prevention demonstrated that diets supplemented with K+, Ca2+, and Mg2+ had minimal (~1 mm Hg) effects on blood pressure.79 Based on this evidence, it is tempting to speculate that much of the BP reduction in hypertensive patients consuming the high fruit and vegetable diet was related to increased antioxidant intake. The DASH sodium study shows that although Na+ restriction is beneficial, much of the BP effect of DASH is Na+ independent.80

Signaling Mechanisms by Which NEFA May Affect the Risk Factor Cluster

Nonesterified fatty acids have cellular actions which could affect vascular biology including effects on membrane fluidity and ion transport, e.g., Na+ /K+ ATPase, Na+ and K+ channels, and Ca2+ currents.81 NEFA enhance α1 adrenoceptor–mediated vascular reactivity through a cyclo-oxygenase sensitive mechanism.82,83 In cultured vascular smooth-muscle cells, oleic and linoleic acids raise 6-keto-PGF1α through protein kinase C– and extracellular signal-regulated kinase (ERK)–independent pathways.83 These observations suggest that fatty acids may exert cardiovascular actions through effects on eicosanoids. Of note, antioxidants (flavonoids) can alter eicosanoid signaling.84

Protein Kinase C

Cis-unsaturated NEFA, including oleic and linoleic acids, directly activate the typical and atypical isoforms of PKC by a diglyceride-independent mechanism.85,86 Activation of PKC is involved in regulation of vascular tone87 and vascular smooth-muscle cell growth,88 and may contribute to impaired endothelial function,89 decreased microvessel formation,90 and metabolic aspects of the risk factor cluster.91 The ζ isoform of PKC, which is nutritionally regulated,92 elevated in insulin-resistant diabetics independently of hyperglycemia,93 activated by cis-unsaturated NEFA including oleic acid,94 and linked to mitogenic responses,95,96 may contribute to the risk factor cluster and complications.

Glutathione, a key component of antioxidant defenses, reduces PKC activation.97 The typical diet contributes to obesity and insulin resistance with a rise in oxidant stressors, e.g., NEFA, relative to antioxidant defenses. The implications of these facts are highlighted by evidence that glutathione depletion produces severe hypertension in normal rats which is significantly attenuated by vitamins C and E.77

Reactive Oxygen Species (ROS)

Activation of PKC leads to activation of NADPH oxidase,98 and induces the respiratory burst in leukocytes.99 NEFA activate PKC and induce the respiratory burst in white blood cells.100 Activation of PKC induces the generation of ROS in vascular smooth-muscle and mesangial cells.101,102 Oleic acid induces a PKC-dependent production of ROS in vascular smooth-muscle cells.72 ROS are associated with activation of ERK, transcription factors, phospholipase A2, and matrix metalloproteinases, increases in IGF-1 levels, and decreases in IGF binding proteins, DNA synthesis, and endothelial function.102–107 Matrix metalloproteinases are concentrated in the shoulder regions of atherosclerotic plaque,108 which are prone to rupture and initiate cardiovascular events.109 ROS activate matrix metalloproteinases by destabilizing the sulfhydryl bonds which maintain the inactive state.109

Studies indicate an important role for angiotensin in cardiac and vascular remodeling. ROS are critical signaling molecules in the cellular response to angiotensin.110,111 Angiotensin increases ROS through an NADPH oxidase–dependent mechanism,111,112 p22phox, a cytochrome b–like protein, participates in the transfer of oxygen in the generation of ROS.111 These signaling events lead to activation of p38 MAP kinase and early-response genes.110,113 Collectively, the studies demonstrate that ROS represent an important component of the mitogenic signaling response of vascular smooth-muscle cells to angiotensin.

Angiotensin-induced hypertension in rats doubles vascular superoxide (O2–) production.112 The aortic rings of these rats generate more O2– ex vivo than rings from control animals. Pretreatment of the rings with diphenyleneiodonium, which inhibits NADH/NADPH oxidase, reversed the excess O2– production. The blood pressure of hypertensive rats receiving angiotensin fell by 60 mm Hg
with liposomal delivery of superoxide dismutase. $O_2^-$ rapidly associates with nitric oxide to produce peroxynitrite, which is not vasodilatory and may have adverse effects by promoting nitrosylation of proteins.

Oleic acid and angiotensin induced a synergistic mitogenic response in vascular smooth-muscle cells through a PKC- and ERK-dependent pathway. The combination of oleic acid and angiotensin II produced greater activation of PKC and ERK than either agent alone, and a synergistic increase in the production of ROS in cultured VSMC (Fig. 4).

Reactive oxygen species may contribute to insulin resistance. Adipocytes exposed to $H_2O_2$ in vitro manifest impaired lipid synthesis, glycogen synthetase activity, and glucose uptake in response to insulin. Studies done in Type II diabetics also show an inverse relationship between measures of oxidant stress and insulin action. Moreover, antioxidant therapy with vitamin E was associated with improved glucose metabolism in some studies of diabetics. Collectively, these observations implicate a role for oxidant stress in the metabolic components of insulin resistance observed in subjects with the risk factor cluster.

The HOPE Trial Paradox

If ROS contribute to cardiovascular disease, benefits of antioxidant therapy should be evident in subjects at greatest risk, eg, those in the Heart Outcomes Prevention Evaluation (HOPE) Study. However, supplemental vitamin E had no significant benefit. We observed that vitamin E, in a wide range of concentrations of the racemic mixture as well as the specific enantiomers, had no effect on the ROS produced by vascular smooth-muscle cells stimulated with oleic acid and angiotensin (unpublished observation). Thus, vitamin E may not block some important signaling events in the genesis of vascular disease in humans with insulin resistance.

Natural foods contain several antioxidants that collectively could affect signaling processes involved in the pathogenesis of vascular disease. Foods high in antioxidants are associated with less cardiovascular disease, whereas trials of specific antioxidants often are not. Negative studies with a single antioxidant are insufficient to conclude that oxidative stress does not contribute to cardiovascular disease. Antioxidant approaches to cardiovascular disease prevention require a greater understanding of the signaling pathways involved and the effects of specific antioxidants on these processes.

Summary

Obesity and insulin resistance are closely related, and both are linked to the risk factor cluster and premature cardiovascular and renal disease. An increased adipocyte mass may contribute to insulin resistance and risk factor clustering through effects on the renin-angiotensin system,
fatty acids, and other mediators. A better understanding of adipocyte biology and relevant signal transduction processes should facilitate efforts to reduce the costly and disabling complications associated with obesity.

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