Metabolic Syndrome

A Comprehensive Perspective Based on Interactions Between Obesity, Diabetes, and Inflammation

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The original description of the metabolic syndrome by Reaven[1] consisted of obesity, insulin resistance, hypertension, impaired glucose tolerance or diabetes, hyperinsulinemia and dyslipidemia characterized by elevated triglyceride, and low HDL concentrations. All of the features described above are risk factors for atherosclerosis, and thus, metabolic syndrome constituted a significant risk for coronary heart disease[2–5] (Table). The features of obesity/overweight and insulin resistance also provided a significant risk for developing type 2 diabetes.[5,6] The risks for coronary heart disease and diabetes with metabolic syndrome are greater than those for simple obesity alone, and therefore, an understanding of the pathogenesis and through it, a rational approach to its therapy are of prime importance.

As our understanding of the action of insulin evolves to comprehensively include the recent discoveries,[7] we can better see that insulin resistance is the basis of most if not all of the features of this syndrome. The original conceptualization of this syndrome was on the basis of resistance to the metabolic actions of insulin. Thus, hyperinsulinemia, glucose intolerance, type 2 diabetes, hypertriglyceridemia, and low HDL concentrations could be accounted for by resistance to the actions of insulin on carbohydrate and lipid metabolism. Although the features described above would to some extent explain the atherogenesis, Reaven has maintained that hyperinsulinemia itself contributes to atherogenicity, and thus, insulin is atherogenic, leading to the coronary heart disease and cerebrovascular disease associated with this syndrome.

Obesity probably leads to hypertension through (1) increased vascular tone created by a reduced bioavailability of NO because of increased oxidative stress,[8] (2) increased asymmetric dimethylarginine (ADMA) concentrations,[9] (3) increased sympathetic tone,[10] and (4) increased expression of angiotensinogen by adipose tissue leading to an activation of the renin-angiotensin system.[11] The last of these factors requires further critical investigation.

Metabolic syndrome is characterized by a low HDL in association with an elevated triglyceride concentration. This is believed to be a result of an increased triglyceride load in the HDL particle that is acted on by hepatic lipase, which hydrolyzes the triglyceride. The loss of the triglyceride results in a small HDL particle that is filtered by the kidney, resulting in a decrease in apolipoprotein (apo) A and HDL concentrations. Apart from an increase in the loss of apoA, there are data demonstrating that insulin may promote apoA gene transcription.[12] Therefore, insulin resistance states may be associated with diminished apoA biosynthesis.[13]

An increase in plasma free fatty acid (FFA) concentrations plays a key role in the pathogenesis of insulin resistance through specific actions that block insulin signal transduction. An increase in plasma FFA concentrations in normal subjects to levels comparable to those in the obese also results in the induction of oxidative stress, inflammation, and subnormal vascular reactivity, in addition to causing insulin resistance.[14] Because resistance to insulin also results in the relative nonsuppression of adipocyte hormone–sensitive lipase, there is further enhancement of lipolysis and increase in FFA concentration. Thus, there occurs a vicious circle of lipolysis, increased FFA, insulin resistance, and inflammation.

Several new features have been added to the syndrome over time. These include elevated plasminogen activator inhibitor-1 (PAI-1) concentrations and now, elevated C-reactive protein (CRP) concentrations. These features were added on the basis that they were frequently found in association with the metabolic syndrome, and there has hitherto been no rational explanation as to why they actually occur. These features are probably related to both insulin resistance and obesity. The relationship of inflammation to obesity and insulin resistance needs to be explained.[15]

Novel Nonmetabolic Actions of Insulin

These issues are readily explained by the recent observations that insulin is an antiinflammatory hormone and that macronutrient intake is proinflammatory. Insulin has been shown to suppress several proinflammatory transcription factors, such as nuclear factor (NF)-κB, Egr-1, and activating protein-1 (AP-1) and the corresponding genes regulated by them, which mediate inflammation.[16,17] An impairment of the action of

Classic Biological Effects of Insulin and Classic Metabolic Syndrome Based on Resistance to the Metabolic Effects of Insulin

|	Normal Insulin Action	Insulin-Resistant State
Carbohydrates	↓ Hepatic glucose production	Hyperglycemia
	↑ Glucose utilization	Hyperinsulinemia
	↑ Glycogenesis	
Lipids	↓ Lipolysis	↑ Lipolysis
	↓ FFA and glycerol	↑ FFA and glycerol
	↑ Lipogenesis	↑ Hepatic triglyceride and apoB synthesis
	↑ HDL	Hypertriglyceridemia
	↓ Triglycerides	↓ HDL
		↑ Small dense LDL
Proteins	↓ Gluconeogenesis	↑ Gluconeogenesis
	↓ Amino acids	↑ Protein catabolism
	↑ Protein synthesis	↓ Protein synthesis
Purines	↑ Uric acid clearance	Hyperuricemia
	↓ Uric acid formation	

insulin because of insulin resistance would thus result in the activation of these proinflammatory transcription factors and an increase in the expression of the corresponding genes.

Insulin has been shown to suppress NF-κB binding activity, reactive oxygen species (ROS) generation, and p47[phox] expression and to increase IκB expression in mononuclear cells (MNCs) as well as to suppress plasma concentrations of intercellular adhesion molecule-1 and monocyte chemotactic protein-1.[16] In addition, insulin suppresses AP-1 and Egr-1, 2 proinflammatory transcription factors and their respective genes, matrix metalloproteinase-9, tissue factor (TF), and PAI-1.[17–19] Thus, insulin has a comprehensive antiinflammatory effect and in addition has an antioxidant effect, as reflected in the suppression of ROS generation and p47[phox] expression (Figure 1).[16,20]

Two further pieces of evidence demonstrating the antiinflammatory action of insulin have emerged recently. First, the treatment of type 2 diabetes with insulin for 2 weeks caused a reduction in CRP and monocyte chemotactic protein-1.[21] Second, the treatment of severe hyperglycemia associated with marked increases in inflammatory mediators with insulin resulted in a rapid marked decrease in the concentration of inflammatory mediators.[22] Most recently, in a rat model in which inflammation was induced with endotoxin, insulin suppressed the concentration of these inflammatory mediators, including interleukin (IL)-1β, IL-6, macrophage migration inhibition factor (MIF), and tumor necrosis factor (TNF)-α.[23] Insulin also suppressed the expression of the proinflammatory transcription factor CEBP and cytokines in the liver of these animals. Similar reductions in inflammatory mediators were observed in rats with thermal injury treated with insulin.[24] Finally, insulin has been shown to suppress the increase in cytokine concentration in pigs challenged with endotoxin.[23]

Another novel antiapoptotic effect of insulin has recently been described. In experimental acute myocardial infarction
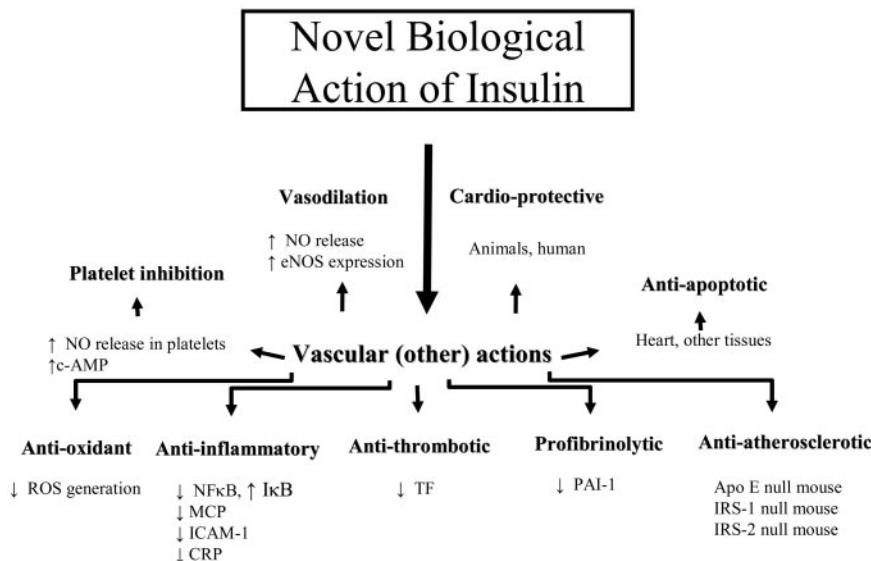
Figure 1. Novel biological effects of insulin targeted at endothelial cells, platelets, and leukocytes resulting in vasodilation, antiaggregatory effects on platelets, antiinflammatory effects, and other related effects.
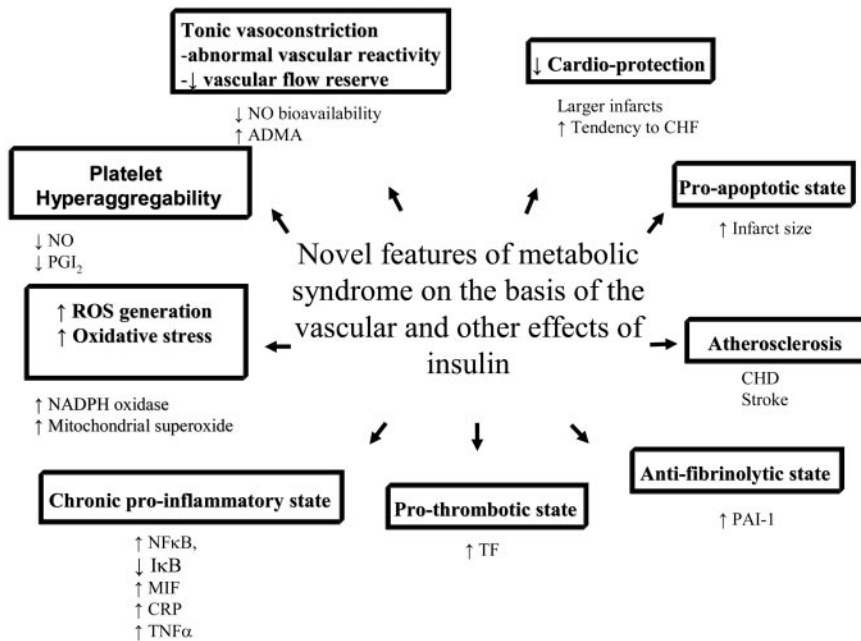
Figure 2. Extension of metabolic syndrome on the basis of resistance to the novel actions of insulin.

in the rat heart, the addition of insulin to the reperfusion fluid leads to a reduction in infarct size by 50%.[25] More recently, a similar cardioprotective effect of insulin has been shown in human acute myocardial infarction when insulin at a low dose was infused with a thrombolytic agent and heparin.[20] Conversely, the insulin-resistant states of obesity and type 2 diabetes have been shown to be associated with larger infarcts than those observed in nondiabetic subjects. Further work is required to establish this feature as an integral component of the metabolic syndrome. It should also be mentioned that insulin administration suppresses atherogenesis in the apoE-null mouse.[26] Conversely, interference with insulin signal transduction, as in the IRS-2–null mouse, results in atherosclerosis.[27] The IRS-1–null mouse also has a tendency toward atherosclerosis. It is relevant that a mutation of IRS-1 (Arginine at 792) leads to abnormal vascular reactivity, a decrease in endothelial nitric oxide synthase (NOS) expression in endothelial cells, and an increased incidence of coronary heart disease.[28]

Consistent with the antiinflammatory effects of insulin, insulin sensitizers of the thiazolidinedione class, troglitazone[29,30] and rosiglitazone,[31] have been shown to exert an antiinflammatory effect in addition to their glucose-lowering effect in patients with diabetes. Troglitazone has been shown to suppress the development of diabetes in patients at high risk of developing this condition.[32] Trials are under way to determine whether rosiglitazone and pioglitazone prevent both type 2 diabetes and atherosclerotic complications. Positive results from those trials would support the concept that inflammatory mechanisms underlie the pathogenesis of both insulin resistance and atherosclerosis. It is of interest in this regard that metformin causes a reduction in the plasma concentrations of MIF in obese subjects.[33] The obese have elevated plasma concentrations of this cytokine and an increase in the expression of this cytokine in MNCs.[33] Although there is evidence that thiazolidinediones exert a direct antiinflammatory effect on macrophages in vitro, it is possible that in vivo, their effect could be through insulin sensitization.

Obesity and Inflammation

The data given above explain why an insulin-resistant state may be proinflammatory (Figure 2). They do not, however, explain the origin of insulin resistance itself. Mutations of the genes involved in insulin signal transduction provide one approach to the study of this issue in humans and in mice with specific gene knockouts. Such lesions are of interest, but they are too infrequent to provide us with a basis for the understanding of the pathogenesis of insulin resistance at large in humans. Thus, some of the recent observations on the interference of insulin signal transduction by inflammatory mechanisms are of great interest, because obesity is a proinflammatory state.

Even if we accept that inflammatory mechanisms are involved in the pathogenesis of interference with insulin signal transduction and of insulin resistance itself, how does inflammation arise? Over the past decade, obesity has been associated with inflammation. This association was first proposed in the landmark article by Hotamisligil et al[34] in which TNF-α was shown to be constitutively expressed by adipose tissue, to be hyperexpressed in obesity, and to mediate insulin resistance in the major animal models of obesity. This seminal article also demonstrated that the neutralization of TNF-α with soluble TNF-α receptors resulted in the restoration of insulin sensitivity. Thus, the proinflammatory cytokine TNF-α was the mediator of insulin resistance. Although the infusion of soluble TNF-α receptors in the human has not reproduced the results observed in mice,[35] the article by Hotamisligil et al laid the foundation of the concept that inflammatory mechanisms may have a role to play in the pathogenesis of insulin resistance. More data have now accumulated to reinforce the concept that obesity is an inflammatory state in the human: plasma concentrations of TNF-α, IL-6, CRP, MIF, and other inflammatory mediators have been shown to be increased in the obese.[33,36–40] Adipose tissue has been shown to express most of these proinflammatory mediators. It has also been shown that macrophages

residing in the adipose tissue may also be a source of proinflammatory factors and that they also may modulate the secretory activity of adipocytes.[41] Tissue macrophages are derived from monocytes in blood. Recently, the mononuclear cells of the obese, of which monocytes are a fraction, have also been shown to be in an inflammatory state, expressing increased amounts of proinflammatory cytokines and related factors.[42] In addition, these cells have been shown to have a significantly increased binding of NF-κB, the key proinflammatory transcription factor, and an increase in the intranuclear expression of p65 (Rel A), the major protein component of NF-κB. These cells also express diminished amounts of IκBβ, the inhibitor of NF-κB. Clearly, therefore, evidence of inflammation exist in various cells and in plasma in obesity.

In addition to TNF-α and IL-6, the major adipocyte cytokines, 2 other important proteins, leptin and adiponectin, need mention. Although leptin is known for its function as a satiety signal that inhibits feeding, it has additional roles as a regulator of sexual function and as an immune modulator. It is proinflammatory and platelet proaggregatory.[43–45] Thus, its elevated concentrations may contribute to the proinflammatory state of obesity and to atherogenesis in the long term. Conversely, adiponectin, secreted in abundance by adipocytes in normal subjects, is antiinflammatory and potentially antiatherogenic. In contrast to leptin, its concentration falls with weight gain and in obesity.[46,47] It has been suggested that a low adiponectin may be a marker for atherosclerosis and coronary heart disease.[48]

Insulin Resistance: An Inflammatory Hypothesis

Then, what is it in the inflammatory state that results in the causation of insulin resistance? The first of these potential mechanisms was described by Hotamisligil et al.[49] They demonstrated that TNF-α induced serine phosphorylation of IRS-1, which in turn caused the serine phosphorylation of the insulin receptor. This prevented the normal tyrosine phosphorylation of the insulin receptor and thus interfered with insulin signal transduction. IL-6 and TNF-α have recently been shown to induce SOCS-3,[50,51] a protein that was hitherto thought to interfere with cytokine signal transduction but that is now also known to interfere with tyrosine phosphorylation of the insulin receptor and IRS-1 and to cause ubiquitination and proteosomal degradation of IRS-1.[52] This, in turn, reduces the activation of Akt (protein kinase B), which normally causes the translocation of the insulin-responsive glucose transporter, Glut-4, to the plasma membrane. It also induces the phosphorylation of the enzyme NOS and its activation to generate NO.[53] A newly described protein, TRB3, has also been shown to interfere with the activation of Akt and thus to interfere with the action of insulin[54]; however, the association of TRB3 with inflammatory mechanisms has not hitherto been demonstrated.

There are recent data that Akt2, a key protein involved in insulin signal transduction, which mediates the phosphorylation and activation of endothelial NOS and NO secretion, also prevents the mobilization of Rac-1 to the cell membrane, thus preventing superoxide generation. Superoxide generation is dependent on the translocation of essential elements of NADPH oxidase, such as p47[phox], from the cytosol to the membrane. This is mediated by Rac.[55] In the absence of Akt2, therefore, there will be an increase in the translocation of Rac-1 to the membrane, greater formation of NADPH oxidase complex, and increased superoxide generation and oxidative stress. It has been shown that Akt-2–null mice develop insulin resistance and mild hyperglycemia in association with hyperinsulinemia.[56]

Macronutrients and the Origin of Inflammation

If, indeed, obesity is a proinflammatory state and inflammatory mechanisms interfere with insulin signal transduction, what is the origin of this proinflammatory state? The answer to this question comes primarily from recent observations demonstrating that macronutrient intake may induce oxidative stress and inflammatory responses. Thus, a 75-g glucose challenge has been shown to induce an increase in superoxide generation by leukocytes by 140% over the basal levels in addition to increasing p47[phox] expression, a subunit of NADPH oxidase, the enzyme that converts molecular O_2 to superoxide radical.[57] Equicaloric amounts of cream (fat) intake result in similar amounts of oxidative stress.[58] Glucose intake also results in comprehensive inflammation, as reflected in an increase in intranuclear NF-κB binding, a decrease in IκB expression, and an increase in IKKα and IKKβ, the 2 kinases that phosphorylate IκBα and IκBβ and result in their ubiquitination and proteosomal degradation.[59] Glucose intake also causes an increase in 2 other proinflammatory transcription factors: AP-1 and Egr-1.[60] AP-1 regulates the transcription of matrix metalloproteinases, whereas Egr-1 modulates the transcription of TF and PAI-1. Thus, glucose intake increases the expression of matrix metalloproteinases 2 and 9 as well as that of TF and PAI-1.

A mixed meal from a fast food chain was also shown to induce the activation of NF-κB, a reduction in IκBα, and an increase in IKKα and IKKβ along with an increase in superoxide radical generation by MNCs.[61] It is also of interest that the intravenous infusion of triglyceride with heparin in normal subjects with an elevation of FFA concentration to a level comparable to that found in the obese results in an inflammatory response.[14] All genes that are stimulated by acute nutritional intake have also been shown to be activated in the basal state of obese subjects such that the concentrations of these gene products are elevated in the obese. Consistent with this, a reduction in macronutrient intake in the obese (1000 kcal/d for 4 weeks) has been shown to reduce both oxidative stress and inflammatory mediators.[8] Similarly, a 48-hour fast has been shown to reduce ROS generation by more than 50% in normal subjects; the expression of p47[phox] was also reduced.[62] Clearly, macronutrient intake is a major regulator of oxidative stress. It is relevant that the superoxide radical generated during oxidative stress is an activator of at least 2 major proinflammatory transcription factors, NF-κB and AP-1. NF-κB regulates the transcriptional activity of at least 125 genes, most of which are proinflammatory.[63–66] Thus, it is not surprising that obesity is a proinflammatory condition. Indeed, the MNC in the obese is in a proinflammatory state, expressing an excess of a series of proinflammatory genes in addition to having increased NF-κB binding and p65 expression and decreased IκBβ protein.[42] In addition
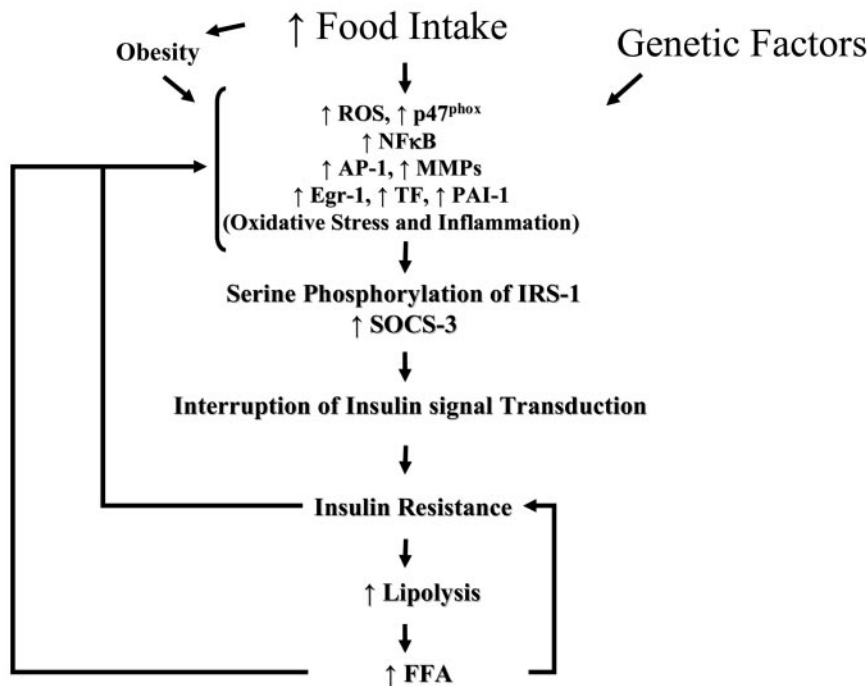
Figure 3. Pathogenesis of metabolic syndrome: inflammation hypothesis.

to obesity and increased macronutrient intake, there may also be genetic and other environmental factors that may induce the activation of inflammatory mechanisms and the induction of oxidative stress. These genetic and other environmental factors may be relevant in those ethnic groups in whom metabolic syndrome has been shown to occur in the absence of obesity. In these groups, migration to western countries such as the United States and the United Kingdom results in increased adiposity with a sedentary lifestyle, which results in the phenotype of the metabolic syndrome, against an appropriate genetic background.

When considering macronutrient-induced inflammation, it can be argued that the foods that are being consumed now were being consumed always; so why is their proinflammatory effect suddenly becoming relevant? The reason is that the amounts of food that are being consumed are far greater than before; furthermore, larger portions of the average diet consist of fast foods and do not contain sufficient fiber, fruit, and vegetables. This combination results in the inability of the endogenously secreted insulin in response to the meal intake to suppress the inflammation generated by the meal. It is of interest in this regard that a 900-kcal American Heart Association step 2 diet–based meal rich in fruit and fiber does not cause significant oxidative stress or inflammation, in contrast to the effect of an isocaloric fast food meal.[67]

The increase in superoxide radical generation also results in diminished bioavailability of NO, because NO binds to superoxide radical to form peroxynitrite.[68] In addition to the fact that Akt is inhibited because of insulin resistance, and thus NOS is also inhibited, the reduction in NO bioavailability can result in a marked reduction in NO action. Furthermore, TNF-α suppresses the expression of NOS. These factors result in abnormalities in endothelium-mediated vasodilatation and vascular reactivity.[69] Interestingly, the abnormalities in vascular reactivity in the obese insulin-resistant population can be repro-

duced acutely by a 900-kcal fast food meal, just as the proinflammatory changes in obesity can be reproduced by a similar meal.[61] It is noteworthy that in obesity, the plasma concentrations of ADMA are elevated and that it inhibits NOS activity, thus reducing the synthesis and secretion of NO.[70] It is of interest that rosiglitazone suppresses plasma ADMA concentrations while improving the impaired vascular reactivity in the obese and type 2 diabetes.[71]

Although the initial work on macronutrient intake with glucose, cream, and a fast food meal shows a proinflammatory effect associated with oxidative stress, data are now emerging to demonstrate that some macronutrients may be "safe" and noninflammatory. Thus, a 900-calorie breakfast rich in fruit and fiber does not cause oxidative stress or inflammation. The intake of vitamin E before glucose challenge also suppresses oxidative stress and inflammation. Similarly, alcohol and orange juice given in equicaloric amounts do not cause oxidative stress or inflammation. Because orange juice is rich in flavonoids and vitamin C, it is possible that the presence of macronutrients in food may alter or suppress oxidative stress or inflammation. Furthermore, there are data to show that vitamin E administration to patients with insulin resistance reduces cytokine production by MNCs.[72]

Conclusion: Metabolic Syndrome: Inflammation Hypothesis

In conclusion, the proinflammatory state of obesity and metabolic syndrome originates with excessive caloric intake and is probably a result of overnutrition in a majority of patients in the United States. The proinflammatory state induces insulin resistance, leading to clinical and biochemical manifestations of the metabolic syndrome. This resistance to insulin action promotes inflammation further through an increase in FFA concentration and interference with the antiinflammatory effect of insulin. Although these factors

may be the most important factor in a majority of patients with metabolic syndrome, it is possible that other factors, such as genetic factors, may also contribute to the inflammatory stress in metabolic syndrome (Figure 3). These factors may be important in ethnic groups like Asian Indians, who may have increased amounts of upper abdominal fat despite a normal body mass index.[73] Because excessive nutritional intake probably accounts for the inflammation at least in obesity-associated metabolic syndrome, the most rational way to suppress such inflammation is through caloric restriction. The other lifestyle change that affects inflammation is exercise. Exercise results in a fall in the indices of inflammation, such as plasma CRP concentration.[74] The mechanism underlying this effect of exercise is not known; however, it is noteworthy that lifestyle change is a very effective way to reduce the rate of development of diabetes in a prediabetic population, as shown by the diabetes prevention study.[75,76] Both a reduction in macronutrient intake and exercise cause a reduction in inflammation.

References

1. Reaven GM. Banting lecture 1988. Role of insulin resistance in human disease. *Diabetes*. 1988;37:1595–1607.
2. Pyorala K. Relationship of glucose tolerance and plasma insulin to the incidence of coronary heart disease: results from two population studies in Finland. *Diabetes Care*. 1979;2:131–141.
3. Ninomiya JK, L'Italien G, Criqui MH, Whyte JL, Gamst A, Chen RS. Association of the metabolic syndrome with history of myocardial infarction and stroke in the Third National Health and Nutrition Examination Survey. *Circulation*. 2004;109:42–46.
4. Lakka HM, Laaksonen DE, Lakka TA, Niskanen LK, Kumpusalo E, Tuomilehto J, Salonen JT. The metabolic syndrome and total and cardiovascular disease mortality in middle-aged men. *JAMA*. 2002;288:2709–2716.
5. Festa A, D'Agostino R Jr, Howard G, Mykkanen L, Tracy RP, Haffner SM. Chronic subclinical inflammation as part of the insulin resistance syndrome: the Insulin Resistance Atherosclerosis Study (IRAS). *Circulation*. 2000;102:42–47.
6. Klein BE, Klein R, Lee KE. Components of the metabolic syndrome and risk of cardiovascular disease and diabetes in Beaver Dam. *Diabetes Care*. 2002;25:1790–1794.
7. Dandona P, Aljada A, Mohanty P. The anti-inflammatory and potential anti-atherogenic effect of insulin: a new paradigm. *Diabetologia*. 2002;45:924–930.
8. Dandona P, Mohanty P, Ghanim H, Aljada A, Browne R, Hamouda W, Prabhala A, Afzal A, Garg R. The suppressive effect of dietary restriction and weight loss in the obese on the generation of reactive oxygen species by leukocytes, lipid peroxidation, and protein carbonylation. *J Clin Endocrinol Metab*. 2001;86:355–362.
9. Lin KY, Ito A, Asagami T, Tsao PS, Adimoolam S, Kimoto M, Tsuji H, Reaven GM, Cooke JP. Impaired nitric oxide synthase pathway in diabetes mellitus: role of asymmetric dimethylarginine and dimethylarginine dimethylaminohydrolase. *Circulation*. 2002;106:987–992.
10. Esler M, Rumantir M, Kaye D, Lambert G. The sympathetic neurobiology of essential hypertension: disparate influences of obesity, stress, and noradrenaline transporter dysfunction? *Am J Hypertens*. 2001;14:139S–146S.
11. Giacchetti G, Faloia E, Sardu C, Camilloni MA, Mariniello B, Gatti C, Garrapa GG, Guerrieri M, Mantero F. Gene expression of angiotensinogen in adipose tissue of obese patients. *Int J Obes Relat Metab Disord*. 2000;24(suppl 2):S142–S143.
12. Groenendijk M, Cantor RM, Blom NH, Rotter JI, de Bruin TW, Dallinga-Thie GM. Association of plasma lipids and apolipoproteins with the insulin response element in the apoC-III promoter region in familial combined hyperlipidemia. *J Lipid Res*. 1999;40:1036–1044.
13. Mooradian AD, Haas MJ, Wong NC. Transcriptional control of apolipoprotein A-I gene expression in diabetes. *Diabetes*. 2004;53:513–520.
14. Tripathy D, Mohanty P, Dhindsa S, Syed T, Ghanim H, Aljada A, Dandona P. Elevation of free fatty acids induces inflammation and impairs vascular reactivity in healthy subjects. *Diabetes*. 2003;52:2882–2887.
15. Dandona P, Aljada A, Bandyopadhyay A. Inflammation: the link between insulin resistance, obesity and diabetes. *Trends Immunol*. 2004;25:4–7.
16. Dandona P, Aljada A, Mohanty P, Ghanim H, Hamouda W, Assian E, Ahmad S. Insulin inhibits intranuclear nuclear factor kappaB and stimulates IkappaB in mononuclear cells in obese subjects: evidence for an anti-inflammatory effect? *J Clin Endocrinol Metab*. 2001;86:3257–3265.
17. Aljada A, Ghanim H, Mohanty P, Kapur N, Dandona P. Insulin inhibits the pro-inflammatory transcription factor early growth response gene-1 (Egr)-1 expression in mononuclear cells (MNC) and reduces plasma tissue factor (TF) and plasminogen activator inhibitor-1 (PAI-1) concentrations. *J Clin Endocrinol Metab*. 2002;87:1419–1422.
18. Ghanim H, Mohanty P, Aljada A, Chowhan S, Tripathy D, Dandona P. Insulin reduces the pro-inflammatory transcription factor, activation protein-1 (AP-1), in mononuclear cells (MNC) and plasma matrix metalloproteinase-9 (MMP-9) concentration. *Diabetes*. 2001;50(suppl 2):A408.
19. Dandona P, Aljada A, Mohanty P, Ghanim H, Bandyopadhyay A, Chaudhuri A. Insulin suppresses plasma concentration of vascular endothelial growth factor and matrix metalloproteinase-9. *Diabetes Care*. 2003;26:3310–3314.
20. Chaudhuri A, Janicke D, Wilson MF, Tripathy D, Garg R, Bandyopadhyay A, Calieri J, Hoffmeyer D, Syed T, Ghanim H, Aljada A, Dandona P. Anti-inflammatory and profibrinolytic effect of insulin in acute ST-segment–elevation myocardial infarction. *Circulation*. 2004;109:849–854.
21. Takebayashi K, Aso Y, Inukai T. Initiation of insulin therapy reduces serum concentrations of high-sensitivity C-reactive protein in patients with type 2 diabetes. *Metabolism*. 2004;53:693–699.
22. Stentz FB, Umpierrez GE, Cuervo R, Kitabchi AE. Proinflammatory cytokines, markers of cardiovascular risks, oxidative stress, and lipid peroxidation in patients with hyperglycemic crises. *Diabetes*. 2004;53:2079–2086.
23. Jeschke MG, Klein D, Bolder U, Einspanier R. Insulin attenuates the systemic inflammatory response in endotoxemic rats. *Endocrinology*. 2004;145:4084–4093.
24. Jeschke MG, Einspanier R, Klein D, Jauch KW. Insulin attenuates the systemic inflammatory response to thermal trauma. *Mol Med*. 2002;8:443–450.
25. Jonassen AK, Sack MN, Mjos OD, Yellon DM. Myocardial protection by insulin at reperfusion requires early administration and is mediated via Akt and p70s6 kinase cell-survival signaling. *Circ Res*. 2001;89:1191–1198.
26. Shamir R, Shehadeh N, Rosenblat M, Eshach-Adiv O, Coleman R, Kaplan M, Hamoud S, Lischinsky S, Hayek T. Oral insulin supplementation attenuates atherosclerosis progression in apolipoprotein E–deficient mice. *Arterioscler Thromb Vasc Biol*. 2003;23:104–110.
27. Kubota T, Kubota N, Moroi M, Terauchi Y, Kobayashi T, Kamata K, Suzuki R, Tobe K, Namiki A, Aizawa S, Nagai R, Kadowaki T, Yamaguchi T. Lack of insulin receptor substrate-2 causes progressive neointima formation in response to vessel injury. *Circulation*. 2003;107:3073–3080.
28. Federici M, Pandolfi A, De Filippis EA, Pellegrini G, Menghini R, Lauro D, Cardellini M, Romano M, Sesti G, Lauro R, Consoli A. G972R IRS-1 variant impairs insulin regulation of endothelial nitric oxide synthase in cultured human endothelial cells. *Circulation*. 2004;109:399–405.
29. Ghanim H, Garg R, Aljada A, Mohanty P, Kumbkarni Y, Assian E, Hamouda W, Dandona P. Suppression of nuclear factor-kappaB and stimulation of inhibitor kappaB by troglitazone: evidence for an anti-inflammatory effect and a potential antiatherosclerotic effect in the obese. *J Clin Endocrinol Metab*. 2001;86:1306–1312.
30. Aljada A, Garg R, Ghanim H, Mohanty P, Hamouda W, Assian E, Dandona P. Nuclear factor-kappaB suppressive and inhibitor-kappaB stimulatory effects of troglitazone in obese patients with type 2 diabetes: evidence of an antiinflammatory action? *J Clin Endocrinol Metab*. 2001;86:3250–3256.
31. Mohanty P, Aljada A, Ghanim H, Hofmeyer D, Tripathy D, Syed T, Al-Haddad W, Dhindsa S, Dandona P. Evidence for a potent antiinflammatory effect of rosiglitazone. *J Clin Endocrinol Metab*. 2004;89:2728–2735.
32. Buchanan TA, Xiang AH, Peters RK, Kjos SL, Marroquin A, Goico J, Ochoa C, Tan S, Berkowitz K, Hodis HN, Azen SP. Preservation of pancreatic beta-cell function and prevention of type 2 diabetes by pharmacological treatment of insulin resistance in high-risk Hispanic women. *Diabetes*. 2002;51:2796–2803.
33. Dandona P, Aljada A, Ghanim H, Mohanty P, Tripathy C, Hofmeyer D, Chaudhuri A. Increased plasma concentration of macrophage migration

inhibitory factor (MIF) and MIF mRNA in mononuclear cells in the obese and the suppressive action of metformin. *J Clin Endocrinol Metab*. 2004;89:5043–5047.

34. Hotamisligil GS, Shargill NS, Spiegelman BM. Adipose expression of tumor necrosis factor-alpha: direct role in obesity-linked insulin resistance. *Science*. 1993;259:87–91.

35. Ofei F, Hurel S, Newkirk J, Sopwith M, Taylor R. Effects of an engineered human anti–TNF-alpha antibody (CDP571) on insulin sensitivity and glycemic control in patients with NIDDM. *Diabetes*. 1996;45:881–885.

36. Kern PA, Ranganathan S, Li C, Wood L, Ranganathan G. Adipose tissue tumor necrosis factor and interleukin-6 expression in human obesity and insulin resistance. *Am J Physiol*. 2001;280:E745–E751.

37. Dandona P, Weinstock R, Thusu K, Abdel-Rahman E, Aljada A, Wadden T. Tumor necrosis factor-alpha in sera of obese patients: fall with weight loss. *J Clin Endocrinol Metab*. 1998;83:2907–2910.

38. Vozarova B, Weyer C, Hanson K, Tataranni PA, Bogardus C, Pratley RE. Circulating interleukin-6 in relation to adiposity, insulin action, and insulin secretion. *Obes Res*. 2001;9:414–417.

39. Wakabayashi I. Age-related change in relationship between body-mass index, serum sialic acid, and atherogenic risk factors. *J Atheroscler Thromb*. 1998;5:60–65.

40. Pradhan AD, Manson JE, Rifai N, Buring JE, Ridker PM. C-reactive protein, interleukin 6, and risk of developing type 2 diabetes mellitus. *JAMA*. 2001;286:327–334.

41. Xu H, Barnes GT, Yang Q, Tan G, Yang D, Chou CJ, Sole J, Nichols A, Ross JS, Tartaglia LA, Chen H. Chronic inflammation in fat plays a crucial role in the development of obesity-related insulin resistance. *J Clin Invest*. 2003;112:1821–1830.

42. Ghanim H, Aljada A, Hofmeyer D, Tufail S, Mohanty P, Dandona P. The circulating mononuclear cells in the obese are in a pro-inflammatory state. *Circulation*. 2004;110:1564–1571.

43. La Cava A, Alviggi C, Matarese G. Unraveling the multiple roles of leptin in inflammation and autoimmunity. *J Mol Med*. 2004;82:4–11.

44. Huang L, Li C. Leptin: a multifunctional hormone. *Cell Res*. 2000;10:81–92.

45. Nakata M, Yada T, Soejima N, Maruyama I. Leptin promotes aggregation of human platelets via the long form of its receptor. *Diabetes*. 1999;48:426–429.

46. Kubota N, Terauchi Y, Yamauchi T, Kubota T, Moroi M, Matsui J, Eto K, Yamashita T, Kamon J, Satoh H, Yano W, Froguel P, Nagai R, Kimura S, Kadowaki T, Noda T. Disruption of adiponectin causes insulin resistance and neointimal formation. *J Biol Chem*. 2002;277:25863–25866.

47. Ukkola O, Santaniemi M. Adiponectin: a link between excess adiposity and associated comorbidities? *J Mol Med*. 2002;80:696–702.

48. Pischon T, Girman CJ, Hotamisligil GS, Rifai N, Hu FB, Rimm EB. Plasma adiponectin levels and risk of myocardial infarction in men. *JAMA*. 2004;291:1730–1737.

49. Hotamisligil GS, Peraldi P, Budavari A, Ellis R, White MF, Spiegelman BM. IRS-1–mediated inhibition of insulin receptor tyrosine kinase activity in TNF-alpha– and obesity-induced insulin resistance. *Science*. 1996;271:665–668.

50. Senn JJ, Klover PJ, Nowak IA, Zimmers TA, Koniaris LG, Furlanetto RW, Mooney RA. Suppressor of cytokine signaling-3 (SOCS-3), a potential mediator of interleukin-6–dependent insulin resistance in hepatocytes. *J Biol Chem*. 2003;278:13740–13746.

51. Emanuelli B, Peraldi P, Filloux C, Chavey C, Freidinger K, Hilton DJ, Hotamisligil GS, Van Obberghen E. SOCS-3 inhibits insulin signaling and is up-regulated in response to tumor necrosis factor-alpha in the adipose tissue of obese mice. *J Biol Chem*. 2001;276:47944–47949.

52. Rui L, Yuan M, Frantz D, Shoelson S, White MF. SOCS-1 and SOCS-3 block insulin signaling by ubiquitin-mediated degradation of IRS1 and IRS2. *J Biol Chem*. 2002;277:42394–42398.

53. Dimmeler S, Fleming I, Fisslthaler B, Hermann C, Busse R, Zeiher AM. Activation of nitric oxide synthase in endothelial cells by Akt-dependent phosphorylation. *Nature*. 1999;399:601–605.

54. Du K, Herzig S, Kulkarni RN, Montminy M. TRB3: a tribbles homolog that inhibits Akt/PKB activation by insulin in liver. *Science*. 2003;300:1574–1577.

55. Ozaki M, Haga S, Zhang HQ, Irani K, Suzuki S. Inhibition of hypoxia/reoxygenation-induced oxidative stress in HGF-stimulated antiapoptotic signaling: role of PI3-K and Akt kinase upon rac1. *Cell Death Differ*. 2003;10:508–515.

56. Cho H, Mu J, Kim JK, Thorvaldsen JL, Chu Q, Crenshaw EB III, Kaestner KH, Bartolomei MS, Shulman GI, Birnbaum MJ. Insulin

57. resistance and a diabetes mellitus–like syndrome in mice lacking the protein kinase Akt2 (PKB beta). *Science*. 2001;292:1728–1731.

57. Mohanty P, Hamouda W, Garg R, Aljada A, Ghanim H, Dandona P. Glucose challenge stimulates reactive oxygen species (ROS) generation by leucocytes. *J Clin Endocrinol Metab*. 2000;85:2970–2973.

58. Mohanty P, Ghanim H, Hamouda W, Aljada A, Garg R, Dandona P. Both lipid and protein intakes stimulate increased generation of reactive oxygen species by polymorphonuclear leukocytes and mononuclear cells. *Am J Clin Nutr*. 2002;75:767–772.

59. Dhindsa S, Tripathy D, Mohanty P, Ghanim H, Syed T, Aljada A, Dandona P. Differential effects of glucose and alcohol on reactive oxygen species generation and intranuclear nuclear factor-kappaB in mononuclear cells. *Metabolism*. 2004;53:330–334.

60. Aljada A, Ghanim H, Mohanty P, Tufail S, Bandyopadhyay A, Dandona P. Glucose intake induces an increase in AP-1 and Egr-1 binding activities and tissue factor and matrix metalloproteinase expressions in mononuclear cells and plasma tissue factor and matrix metalloproteinase concentrations. *Am J Clin Nutr*. 2004;80:51–57.

61. Aljada A, Mohanty P, Ghanim H, Abdo T, Tripathy D, Chaudhuri A, Dandona P. Increase in intranuclear nuclear factor kappaB and decrease in inhibitor kappaB in mononuclear cells after a mixed meal: evidence for a proinflammatory effect. *Am J Clin Nutr*. 2004;79:682–690.

62. Dandona P, Mohanty P, Hamouda W, Ghanim H, Aljada A, Garg R, Kumar V. Inhibitory effect of a two day fast on reactive oxygen species (ROS) generation by leucocytes and plasma ortho-tyrosine and meta-tyrosine concentrations. *J Clin Endocrinol Metab*. 2001;86:2899–2902.

63. Woronicz JD, Gao X, Cao Z, Rothe M, Goeddel DV. IkappaB kinase-beta: NF-kappaB activation and complex formation with IkappaB kinase-alpha and NIK. *Science*. 1997;278:866–869.

64. Wang S, Leonard SS, Castranova V, Vallyathan V, Shi X. The role of superoxide radical in TNF-alpha induced NF-kappaB activation. *Ann Clin Lab Sci*. 1999;29:192–199.

65. Verma IM, Stevenson JK, Schwarz EM, Van Antwerp D, Miyamoto S. Rel/NF-kappa B/I kappa B family: intimate tales of association and dissociation. *Genes Dev*. 1995;9:2723–2735.

66. Velasco M, Diaz-Guerra MJ, Martin-Sanz P, Alvarez A, Bosca L. Rapid up-regulation of IkappaBbeta and abrogation of NF-kappaB activity in peritoneal macrophages stimulated with lipopolysaccharide. *J Biol Chem*. 1997;272:23025–23030.

67. Mohanty P, Daoud N, Ghanim H, Ravishankar S, Szudzik E, Aljada A, Dandona P. Absence of oxidative stress and inflammation following the intake of a 900 kcalorie meal rich in fruit and fiber. *Diabetes*. 2004;53:A405.

68. Koppenol WH, Moreno JJ, Pryor WA, Ischiropoulos H, Beckman JS. Peroxynitrite, a cloaked oxidant formed by nitric oxide and superoxide. *Chem Res Toxicol*. 1992;5:834–842.

69. Fard A, Tuck CH, Donis JA, Sciacca R, Di Tullio MR, Wu HD, Bryant TA, Chen NT, Torres-Tamayo M, Ramasamy R, Berglund L, Ginsberg HN, Homma S, Cannon PJ. Acute elevations of plasma asymmetric dimethylarginine and impaired endothelial function in response to a high-fat meal in patients with type 2 diabetes. *Arterioscler Thromb Vasc Biol*. 2000;20:2039–2044.

70. Chan NN, Chan JC. Asymmetric dimethylarginine (ADMA): a potential link between endothelial dysfunction and cardiovascular diseases in insulin resistance syndrome? *Diabetologia*. 2002;45:1609–1616.

71. Stuhlinger MC, Abbasi F, Chu JW, Lamendola C, McLaughlin TL, Cooke JP, Reaven GM, Tsao PS. Relationship between insulin resistance and an endogenous nitric oxide synthase inhibitor. *JAMA*. 2002;287:1420–1426.

72. Devaraj S, Jialal I. Low-density lipoprotein postsecretory modification, monocyte function, and circulating adhesion molecules in type 2 diabetic patients with and without macrovascular complications: the effect of alpha-tocopherol supplementation. *Circulation*. 2000;102:191–196.

73. Banerji MA, Faridi N, Atluri R, Chaiken RL, Lebovitz HE. Body composition, visceral fat, leptin, and insulin resistance in Asian Indian men. *J Clin Endocrinol Metab*. 1999;84:137–144.

74. Church TS, Barlow CE, Earnest CP, Kampert JB, Priest EL, Blair SN. Associations between cardiorespiratory fitness and C-reactive protein in men. *Arterioscler Thromb Vasc Biol*. 2002;22:1869–1876.

75. Tuomilehto J, Lindstrom J, Eriksson JG, Valle TT, Hamalainen H, Ilanne-Parikka P, Keinanen-Kiukaanniemi S, Laakso M, Louheranta A, Rastas M, Salminen V, Uusitupa M. Prevention of type 2 diabetes mellitus by changes in lifestyle among subjects with impaired glucose tolerance. *N Engl J Med*. 2001;344:1343–1350.

76. Knowler WC, Barrett-Connor E, Fowler SE, Hamman RF, Lachin JM, Walker EA, Nathan DM. Reduction in the incidence of type 2 diabetes with lifestyle intervention or metformin. *N Engl J Med*. 2002;346:393–403.