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Review

The Perfect Storm: Obesity, Adipocyte Dysfunction, and Metabolic Consequences

Sarah de Ferranti[1*] and Dariush Mozaffarian[2]

BACKGROUND: As the prevalence of adiposity soars in both developed and developing nations, appreciation of the close links between obesity and disease increases. The strong relationships between excess adipose tissue and poor health outcomes, including cardiovascular disease, diabetes, and cancer, mandate elucidation of the complex cellular, hormonal, and molecular pathophysiology whereby adiposity initiates and maintains adverse health effects.

CONTENT: In this report we review adipocyte metabolism and function in the context of energy imbalance and postprandial nutrient excess, including adipocyte hypertrophy and hyperplasia, adipocyte dysfunction, and other systemic consequences. We also discuss implications for laboratory evaluation and clinical care, including the role of lifestyle modifications. Chronic energy imbalance produces adipocyte hypertrophy and hyperplasia, endoplasmic reticulum stress, and mitochondrial dysfunction. These processes lead to increased intracellular and systemic release of adipokines, free fatty acids, and inflammatory mediators that cause adipocyte dysfunction and induce adverse effects in the liver, pancreatic β-cells, and skeletal muscle as well as the heart and vascular beds. Several specialized laboratory tests can quantify these processes and predict clinical risk, but translation to the clinical setting is premature. Current and future pharmacologic interventions may target these pathways; modest changes in diet, physical activity, weight, and smoking are likely to have the greatest impact.

SUMMARY: Adipocyte endoplasmic reticulum and mitochondrial stress, and associated changes in circulating adipokines, free fatty acids, and inflammatory mediators, are central to adverse health effects of adiposity. Future investigation should focus on these pathways and on reversing the adverse lifestyle behaviors that are the fundamental causes of adiposity.

Obesity

Although understanding of the role of genetics in obesity is increasing *(1)*, the rapid rise in prevalence of overweight and obesity throughout the world demonstrates that environmental changes are the major determinants of this epidemic, with genetic factors likely modifying individual susceptibility to these environmental factors. The etiology of obesity is multifactorial. However, the root cause is energy imbalance: more calories consumed than expended. Putative behavioral and environmental determinants of energy imbalance include factors that increase caloric consumption, including increased portion size; consumption of sugar-sweetened beverages, refined carbohydrates, and meals outside the home; advertising that promotes overconsumption of such foods; and factors that promote a lifestyle with reduced daily energy expenditure, such as increased television watching and environments at home, school, and work that encourage less walking, less physical education or activity, and more sedentary tasks. Other determinants of energy imbalance may include decreased sleep *(2)*, infectious agents such as adenovirus-36 *(3)*, consumption of trans fat *(4)*, perinatal exposures *(5)*, and differences in macronutrient quality (e.g., lower vs higher glycemic-load carbohydrates) that might alter metabolism or appetite *(6)*. Energy imbalance leads to storage of excess energy in adipocytes, which exhibit both hypertrophy and hyperplasia. The processes of adipose hypertrophy and hyperplasia are associated with intracellular abnormalities of adipocyte function, particularly endoplasmic reticulum and mitochondrial stress. Resulting intracellular and systemic consequences include adipocyte insulin resistance; production of adipokines, free fatty acids, and inflammatory mediators; and promotion of systemic dysfunction that leads to clinical man-

[1] Department of Cardiology, Children's Hospital Boston, Boston, MA; [2] Division of Cardiovascular Medicine, Harvard Medical School, and Departments of Epidemiology and Nutrition, Harvard School of Public Health, Boston, MA.
* Address correspondence to this author at: Department of Cardiology, Children's Hospital Boston, 300 Longwood Avenue, Boston, MA 02115. Fax 617 730-0600; e-mail Sarah.deferranti@cardio.chboston.org.
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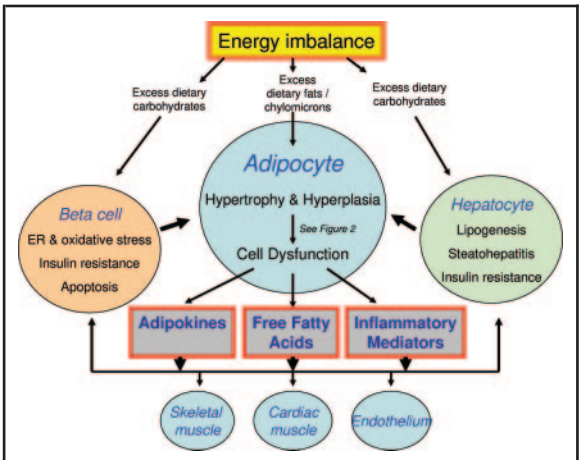

Fig. 1. The consequences of energy imbalance.

Excess postprandial lipids and glucose circulate through the blood stream and are taken up by the pancreas, the liver, and adipose tissue. The adipocyte stores triglycerides in the lipid droplet, leading to adipocyte hypertrophy. These exposures in excess lead to cellular dysfunction, manifested as abnormalities in adipokines, increased circulating free fatty acids, and a proinflammatory state. These in turn affect skeletal muscle (lipid accumulation, peripheral insulin resistance), cardiac muscle (lipid deposition), and endothelial dysfunction. Exposing the β cell to excess nutrients promotes insulin resistance; exposing the hepatocytes to excess fats and carbohydrates leads to steatohepatitis and insulin resistance.

ifestations and sequelae of obesity. Ongoing research is addressing laboratory implications and potential effects of pharmacologic and lifestyle interventions for diagnosis and treatment of obesity-associated disease.

ADIPOSE HYPERTROPHY AND HYPERPLASIA

Chronic imbalance of calories consumed vs expended causes increased storage of the excess energy in the form of adipocyte intracellular triglyceride stores. The increase in fat mass manifests as both increased intracellular lipids and greater adipocyte size (hypertrophy) and increased numbers of adipocytes (hyperplasia) (Fig. 1). Adipocyte hypertrophy, evident in both overweight and type 2 diabetes patients *(7)*, was originally considered the sole route whereby adipose tissue mass increased in adults. However, adipocyte hyperplasia (adipogenesis) is now known to contribute to the increased adipose tissue mass of obesity. Animal studies suggest that hyperplasia occurs in 2 steps: an increase in numbers of preadipocytes and differentiation of preadipocytes into mature (adipokine-secreting) adipocytes. The factors regulating adipose hypertrophy and hyperplasia are not clearly understood, but circulating insulin and glucocorticoid concentrations both appear to stimulate preadipocyte differentiation *(8)*. In vitro studies suggest that factors released locally by hypertrophied adipocytes, such as tumor necrosis factor (TNF)[3]-α and insulin growth factor (IGF)-1, stimulate hyperplasia in a paracrine fashion *(8)*. Growth hormone and thyroxine also appear to play roles in this process, although growth hormone seems to have conflicting effects in vitro vs in vivo, perhaps owing to different adipose deposition sites *(8)*. Various transcription factors influence the differentiation of preadipocytes; peroxisome proliferator-activated receptor-γ (PPAR-γ) is one of the important nuclear receptors *(9)* that stimulates adipocyte hyperplasia *(10)* and may lead to redistribution (decrease) of adipocyte size *(11)*. Some animal experiments suggest that adipocyte hyperplasia may occur later than hypertrophy and be associated with greater severity and less reversibility of metabolic consequences *(12, 13)*, but these potential differences are not well established, and further research is needed to elucidate the relative importance of adipocyte hypertrophy vs hyperplasia in humans.

Whether hypertrophy, hyperplasia, or both occur in response to energy imbalance may vary with location of the adipose tissue. For example, women with higher subcutaneous fat mass exhibited both adipocyte hypertrophy and hyperplasia, whereas increased omental fat was primarily due to hypertrophy *(9)*. Drolet et al. suggest that subcutaneous deposition of fat occurs early in obesity, with visceral deposition occurring only after subcutaneous capacity has been reached *(9)*. Some evidence suggests that excess subcutaneous fat may have fewer adverse health effects than excess visceral fat *(14)*. In humans, macrophages were observed more frequently, and expression of inflammatory factor monocyte chemoattractant protein −1 (MCP-1) was greater in omental fat than in subcutaneous fat, and MCP-1 expression correlated with waist circumference and possibly insulin resistance *(15)*. Changes associated with adipocyte hypertrophy appear be the first steps toward adipocyte cellular dysfunction, by means of processes that will be described further in the next sections.

[3] Nonstandard abbreviations: TNF, tumor necrosis factor; IGF, insulin growth factor; PPAR-γ, peroxisome proliferator-activated receptor-gamma; MCP, monocyte chemoattractant protein; ER, endoplasmic reticulum; SREBP, sterol-regulatory element binding proteins; CHOP, C/EBP homologous protein; UPR, unfolded protein response; JNK, c-Jun N-terminal kinases; eIF, eukaryotic initiation factor; MDA, malondialdehyde; sRBP-4, serum retinol binding protein 4; CRP, C-reactive protein.

ADIPOCYTE DYSFUNCTION AND THE ENDOPLASMIC RETICULUM

Excess lipid storage appears to cause functional abnormalities of the endoplasmic reticulum (ER) and mitochondria that are central to the pathophysiologic effects of obesity. The ER is responsible for synthesizing proteins, forming lipid droplets, and sensing and regulating cholesterol; all 3 of these pathways respond to changes in nutritional supply. Following translation of protein from mRNA in the ER, each nascent protein must be folded into its proper functional configuration and packaged in the Golgi apparatus for use in cellular metabolic and regulatory processes. "Chaperone" proteins guide the newly synthesized proteins and are essential for proper synthesis, folding, and packaging. The ER also actively regulates lipid storage, including modulation of fatty acid uptake, storage of fatty acids as triglycerides, and bundling of triglycerides into lipid droplets to serve as energy stores or for phospholipid synthesis. The ER is also involved in cholesterol sensing. For example, the ER releases sterol-regulatory element binding proteins (SREBPs) in response to insulin and low sterol concentrations *(16)*; SREBPs activate cholesterol and lipid synthesis, and have reduced activity in states of insulin resistance.

In animal models of nutrient oversupply and adipocyte hypertrophy, a situation of ER "stress" has been described. ER stress refers to the cellular condition present when ER function is perturbed, so that the proper folding and modification of proteins, lipid droplet creation, and/or cholesterol-sensing is inhibited. Thus, in states of energy imbalance and adiposity, excessive demands on the ER result in dysfunction of protein folding, lipid-droplet creation, and cholesterol sensing. Manifestations of adipocyte ER stress include increased lactate concentrations and production of C/EBP homologous protein (CHOP). In mouse models, CHOP decreases production of adiponectin, and interfering with CHOP mRNA counteracts this decrease in adiponectin *(17)*, providing insight into how intraorganelle dysfunction may be communicated systemically via circulating adipokines (see below). Another cellular manifestation of ER stress is the "unfolded protein response" (UPR). In the setting of excess nutrients and/or inadequate chaperone proteins, abnormally folded proteins aggregate in the cytosol and can interfere with normal cellular functions. The cell responds by altering regulatory pathways to inhibit protein synthesis and increase clearance of the abnormally folded proteins, a process characterized as the UPR (Fig. 2). For example, eukaryotic initiation factor (eIF)2-α is important for initiation and control of ribosomal protein translation from mRNA, and the phosphorylation of eIF2-α inhibits protein translation. Excess of unfolded proteins activates pancreatic endoplasmic reticulum kinase (PERK), which phosphorylates eIF2-α and thereby decreases protein translation *(18)*. Ultimately, if ER and cell homeostasis cannot be sufficiently restored to address the excess abnormal proteins, UPR can induce apoptosis (programmed cell death) *(16)*. This state of ER stress, manifested by increased lactate, CHOP production, and activation of UPR, also may result in systemic release of free fatty acids and inflammatory mediators *(16)*, as described below.

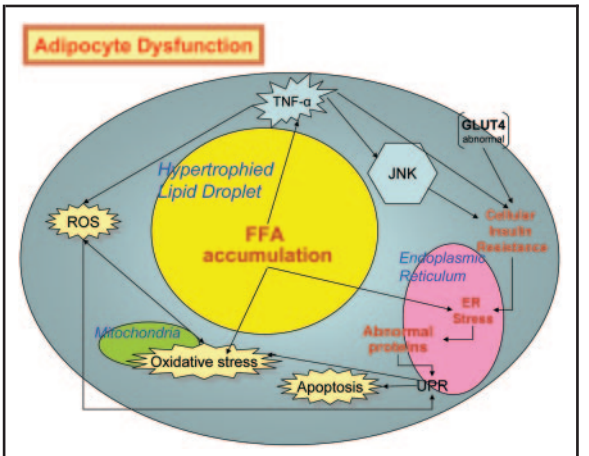

Fig. 2. Aspects of adipocyte dysfunction due to nutrient excess.

Excess lipid accumulation leads to increased ER activity, which ultimately can overwhelm the capacity of the ER to properly fold nascent proteins. The UPR can compensate for this situation to some extent. However, if the process proceeds unchecked, apoptosis may result. ER stress can lead to oxidative stress in the mitochondrion, as does the presence of excess free fatty acids (FFA). Oxidative stress produces reactive oxygen species (ROS). TNF-α production is stimulated by FFAs, which in turn act on JNK to contribute to cellular insulin resistance.

ER STRESS AND ADIPOCYTE INSULIN RESISTANCE

States of energy imbalance, excess adipose tissue, and increased circulating (and intracellular) lipid and glucose concentrations, as seen in obesity and type 2 diabetes, appear to cause ER stress (Figs. 1 and 2). ER stress, in turn, results in the UPR that can further induce cellular insulin resistance, thereby contributing to further increases in lipid and glucose concentrations and ER stress, generating a vicious cycle of worsening insulin resistance *(16)*. One major intracellular mediator of this effect appears to be the c-Jun N-terminal kinases (JNK). In wild-type mice, excess free fatty acids and inflammatory cytokines released in states of ER stress activate JNK in muscle, liver, and adipose cells *(19)*. Within the adipocyte, activated JNK re-

duces insulin sensitivity by decreasing the action of insulin receptor substrates (IRS) that are important in insulin signaling. For example, activation of JNK1 by TNF-α results in IRS-1 phosphorylation, which reduces cellular responses to circulating insulin and produces characteristic manifestations of insulin resistance *(20)*. Interestingly, some JNK mutations are protective against insulin resistance and obesity in mice *(19)*, and are associated with lower concentrations of inflammatory cytokines such as TNF-α, interleukin (IL)-6, and MCP-1. Additionally, a mutation in the mitogen-activated protein kinase 8 interacting protein 1 (*MAPK8IP1*)[4] gene leading to alterations in a JNK-binding protein results in increased JNK activity, and *MAPK8IP1* was proposed as a candidate gene associated with characteristics typical of type 2 diabetes in humans *(21)*.

The role of ER stress in inducing insulin resistance likely extends beyond the adipocyte. JNK in muscle and liver cells are activated in obesity and appear to promote insulin resistance in these tissues *(16, 19)*. Obese mice with JNK deletions did not demonstrate insulin resistance in hepatocytes despite stimulation with TNF-α *(19)*. Experimental evidence from animal studies suggests that lipotoxicity and glucotoxicity reduce pancreatic β-cell mass *(22)*, a process mediated at least in part by uncompensated ER stress in β-cells leading to apoptosis, further contributing to abnormal glucose-insulin homeostasis. In pancreatic islet cells, ER stress and associated JNK activation decrease insulin production and diminish β-cell insulin sensitivity *(23)*.

MITOCHONDRIA AND OXIDATIVE STRESS IN THE ADIPOCYTE

In addition to effects on the ER, obesity is associated with oxidative stress at the level of the mitochondrion (Fig. 2). Oxidative stress can be defined as imbalance in levels of reactive oxygen species (ROS) vs the reducing substances that protect against damaging free radicals and peroxides. In states of adiposity, processing of excess free fatty acids by the mitochondrion causes mitochondrial uncoupling and release of ROS *(24)*, although exact mechanisms of this process are still debated *(25)*. Increased ROS, including malondialdehyde (MDA) and conjugated diene, are evident in adipose tissue of obese individuals *(26)*. ROS also appears to adversely affect insulin production by the pancreas and may lead to β-cell apoptosis *(27)*, further exacerbating glucose-insulin homeostasis. Additionally, once peripheral glucose homeostasis is compromised by skeletal muscle insulin resistance, hyperglycemia can lead to a further mitochondrial ROS production in both pancreatic β-cells and vascular endothelial cells *(28)*. Chronic excess ROS production may result in mitochondrial dysfunction in liver and also skeletal muscle, which may cause lipid accumulation in these tissues and further contribute to the vicious cycle of insulin resistance *(29, 30)*. Interestingly, infusion of free fatty acids leads to peripheral insulin resistance and oxidative stress, whereas giving glutathione blocks this effect, presumably by altering the activity of free radicals *(31)*. Inflammatory cytokines can also induce and worsen oxidative stress. For instance, TNF-α stimulates ROS production in human hepatic cells *(32)*. Reversing these processes can improve some of these abnormalities. For example, uncoupling oxidative phosphorylation decreases ROS production in animals and favorably modifies insulin sensitivity *(33)*.

ADIPOKINES

The adverse intracellular consequences of nutrient toxicity in the adipocyte, including ER and mitochondrial (oxidative) stress, also have systemic consequences. Systemic mediators of adipocyte dysfunction include adipokines, free fatty acids, and inflammatory mediators (Fig. 1). Adipokines, including adiponectin, leptin, resistin, and ghrelin, are circulating molecules produced by adipocytes that affect energy use and production and appear central to the pathophysiology of obesity and its systemic health effects, including nonalcoholic fatty liver disease, insulin resistance, atherosclerosis, and type 2 diabetes *(34–36)*. In addition to their effects on energy use, adipokines influence production of inflammatory mediators. For instance, adiponectin inhibits synthesis and actions of TNF-α, and in turn, TNF-α negatively affects adiponectin transcription *(34)*. Leptin increases synthesis of IL-6 and TNF-α by macrophages and also activates macrophages *(34)*. Resistin increases TNF-α and IL-6 synthesis, and resistin expression is in turn increased by those cytokines *(34)*.

CIRCULATING FATTY ACIDS

Obesity is associated with increased release of nonesterified fatty acids and triglycerides into the circulation *(37)*. Most nonesterified fatty acids, commonly termed "free" fatty acids, circulate in the bloodstream bound to albumin. Higher concentrations of circulating free fatty acids and triglycerides are associated with lipid accumulation in multiple tissues, including liver, skeletal muscle, heart, and pancreatic β-cells. Animal-experiments suggest that these nonadipose tissues, when exposed to excess free fatty acids and hypertriglyceridemia, are less capable of storing lipids than adipocytes and more adversely affected. For example,

[4] Human genes: *MAPK8IP1*, mitogen-activated protein kinase 8 interacting protein 1.

intracellular lipid accumulation is associated with steatohepatitis, skeletal muscle insulin resistance, and β-cell dysfunction *(38)*. In rodent cardiomyocytes, lipid accumulation produces cellular damage and ventricular dysfunction *(38)*. In older adults, peripheral (skeletal muscle) insulin resistance correlates with intracellular accumulation of lipids and decreased mitochondrial function as assessed by nuclear magnetic resonance spectroscopy *(30)*. Free fatty acids may also directly induce peripheral insulin resistance in skeletal muscle and possibly the liver; it is unclear in humans whether this effect is secondary to, concomitant with, or a cause of the associated intracellular accumulation of fat stores in skeletal muscle and liver *(39)*. In skeletal muscle, accumulation of intracellular free fatty acids reduces insulin receptor substrates, with resulting skeletal muscle insulin resistance *(38)*. These processes lead to a cycle of increasing dysfunction: higher circulating free fatty acids induce intracellular lipid accumulation and peripheral insulin resistance, prompting increased insulin secretion *(37)*, whereas peripheral insulin resistance results in attenuated lipolysis of circulating chylomicrons and triglycerides, increasing concentrations of circulating free fatty acids *(40)*.

In addition to increasing peripheral insulin resistance and consequent pancreatic insulin secretion, excess circulating free fatty acids can ultimately lead to diminished function, and even apoptosis, of pancreatic β-cells in both in vivo rat models and in vitro human islet cells *(41)*, a process termed pancreatic lipotoxicity. Thus, excess circulating free fatty acids can act both to decrease responsiveness of peripheral tissues to insulin and, in the long term, decrease insulin supply.

In addition to systemic effects, adipose tissue likely induces local actions on neighboring tissues. For example, venous return from visceral fat drains directly into the liver via the portal system, and thus free fatty acids and inflammatory mediators secreted by hypertrophied adipocytes may have stronger effects on the liver. Furthermore, periarteriole fatty depots may modulate vascular reactivity to insulin in the arteriole and downstream, a process termed a vasocrine effect *(42)*.

INFLAMMATORY MEDIATORS

Alterations of inflammatory pathways are important local and systemic mediators of the health effects of adiposity (Fig. 1). Investigation of circulating and tissue-related inflammatory molecules involved in obesity and its health manifestations has led to greater suggested by the presence of macrophages in atherosclerotic plaques. Important inflammatory markers in obesity include TNF-α, serum retinol binding protein (sRBP)-4, and C-reactive protein (CRP).

TUMOR NECROSIS FACTOR α

TNF-α is an inflammatory marker associated with adiposity and cardiovascular risk factors. Produced by macrophages within adipose tissue and by adipocytes themselves *(43)* and stimulated by ER stress and UPR *(16)*, TNF-α inhibits lipoprotein lipase activity and increases lipolysis *(44)*. Among humans losing weight, macrophage expression of TNF-α decreases and is inversely proportional to lipoprotein lipase activity *(45)*. Because a major activity of lipoprotein lipase is breakdown of circulating triglycerides and VLDL, decreased lipoprotein lipase activity due to increased adipose tissue TNF-α concentrations may partly account for the hypertriglyceridemia of obesity *(45)*. TNF-α concentrations are higher in patients with type 2 diabetes and correlate with fasting glucose and insulin in obese individuals *(46)*. TNF-α inhibits insulin action in adipocytes, possibly through inhibition of IRS-1 by JNK *(29)*, leading to adipocyte insulin resistance *(47)*. TNF-α also appears involved in peripheral (skeletal muscle) insulin resistance; in obese rodents, blocking TNF-α increases skeletal muscle glucose uptake *(48)*. TNF-α is also likely involved in adiposity-related vascular dysfunction. In animal models of metabolic syndrome, endothelial cell expression of TNF-α and associated endothelial dysfunction were increased; blockade of TNF-α restored endothelial-mediated vasodilatation *(49)*. This effect of TNF-α on vascular function may relate to its importance as a stimulant of ROS and inhibitor of nitric oxide release *(50)*.

SERUM RETINOL BINDING PROTEIN 4

sRBP-4, first identified in its role of binding and transporting retinol in the serum, is a circulating protein from the lipocalin family that is associated with visceral adiposity and insulin resistance. sRBP-4 is secreted by both the liver and adipocytes *(47)*, although primarily by the liver, where it is bound to transthyretin; transthyretin concentrations decrease with acute infection and stress, and sRBP-4 concentrations are higher in states of chronic low-grade inflammation. In mouse models, sRBP-4 released from adipocytes induces insulin resistance in liver and skeletal muscle *(51)*. In mice gene-knockout models, lower sRBP-4 concentrations are associated with improved responses to oral glucose challenge *(47)*. In humans, some studies have shown associations between sRBP-4 and obesity *(52)*, specifically visceral adiposity *(53)*, as well as insulin resistance and type 2 diabetes *(54)*. Conversely, in other studies comparing lean, overweight, and obese women, differences in sRBP-4 concentrations were not seen; and 5% weight loss did not induce significant changes in serum sRBP-4, although adipose-tissue expression of sRBP-4 did decline *(55)*. In Japanese adults

with abnormal glucose tolerance, sRBP-4 concentrations did not correlate with body mass index but were related to lower HDL and higher triglyceride concentrations *(56)*, prompting some to suggest that sRBP-4 concentrations are more closely related to abnormal lipid concentrations present in obesity and related conditions, rather than to adiposity per se *(57)*. On the other hand, pediatric studies have found associations between sRBP-4 concentrations and prevalence of obesity and the metabolic syndrome *(58, 59)*, independent of serum retinol concentrations *(59)*.

C-REACTIVE PROTEIN

CRP is a circulating marker of inflammation produced predominantly by the liver in response to IL-6. Very high concentrations of CRP are seen in acute infectious and systemic inflammatory states, but more modest elevations of CRP, measured as high-sensitivity CRP, can occur chronically, providing a relatively stable indicator of low-grade inflammation over months to years. Excess adiposity is associated with increased serum IL-6 and CRP *(60)* and higher concentrations correlate with adipocyte hypertrophy *(7)*. In addition to liver production, one-third of circulating IL-6 is released by adipose tissue *(44)*, and IL-6 production is more strongly associated with visceral adiposity than with subcutaneous fat *(61)*. Circulating CRP concentrations are also higher in adults with metabolic syndrome, and increased CRP is an independent risk factor for type 2 diabetes and CVD *(62)*. An association between adiposity and CRP is also seen in children at the age of 10 to 11 years *(63)*, suggesting that this relationship is one of the earliest steps in the path to chronic disease. The process of inflammation in obesity is complex, and there are likely multiple pathways of interactions between inflammatory markers. For example, although correlations are seen between IL-6 and CRP concentrations and between IL-6 and TNF-α concentrations, CRP concentrations do not necessarily correlate with TNF-α concentrations *(64)*.

LABORATORY IMPLICATIONS

Although elucidation of the intracellular consequences of adiposity have furthered our understanding of the pathophysiology of obesity-related health effects, these findings have yet to be translated into definitive advances for clinical evaluation or treatment of overweight patients. However, several assays currently available as research tests are likely to enter the clinical arena in the near future.

Measurement of CRP concentrations is the laboratory test that is perhaps nearest to influencing clinical care of obesity, because CRP measurement is generally standardized and available, and concentrations appear to correlate closely with adipocyte dysfunction and its systemic consequences. Indeed, in the absence of infection or other systemic inflammation, CRP concentrations may be interpreted as a circulating biomarker of adipocyte dysfunction. However, the potential effects of CRP concentration information on clinical practice and, more importantly, health outcomes, once other markers of adiposity such as body mass index, waist circumference, and triglyceride and HDL concentrations are taken into account, are not yet established. There are recommendations for use of CRP measurement in cardiovascular disease evaluation *(62)*; the use of CRP measurement for evaluation of the metabolic consequences of adiposity may prove important and requires further investigation.

Other inflammatory markers such as IL-6 and TNF-α can be measured using commercial reagent sets but are not routinely available in clinical practice. IL-6 concentrations correlate closely with CRP but have diurnal variation and are less stable over the long term; thus there is little current clinical rationale for measuring IL-6 rather than CRP. Similarly, given the relatively short half-life of serum TNF-α, circulating TNF-α concentrations may not be reliable indicators of tissue activity *(42, 48)*; furthermore, TNF-α is not easily released from adipose tissue beds *(61)*, and thus circulating concentrations may have only weak associations with adipose tissue concentrations. The soluble TNF-α receptors 1 and 2 are more stable circulating measures of TNF-α system activation *(65)*; further understanding of their utility in clinical practice is needed.

Other candidates for laboratory evaluation of overweight patients include measurement of adiponectin, leptin, and free fatty acids. Several oligomers of adiponectin can be assayed, including low, medium, and high molecular weight oligomers, but controversy exists over the utility of measuring these subfractions *(34)*. Leptin, measured by specialized laboratories, can be used clinically in patients who have been obese since early infancy to exclude congenital leptin deficiency and to diagnose Prader-Willi syndrome (high leptin concentrations) *(66)*. Concentrations of nonesterified free fatty acids can be assayed in specialized laboratories using a commercially available and inexpensive assay *(67)*. Although free fatty acid concentrations are independently associated with insulin resistance *(39)* and predict incidence of total mortality, cardiovascular mortality, and sudden cardiac death *(68)*, recognition of their predictive utility in clinical practice is not yet widespread.

Oxidative stress is a multifaceted process and difficult to quantify. Urinary (or, less stable, serum) F2α-isoprostanes are often considered the gold standard for evaluating lipid peroxidation, but measurement is difficult and relatively expensive. Measurements of

blood MDA and oxygen radical absorbance capacity are simpler alternatives. A summary measure of cellular oxidative stress can be obtained by assessing 8-hydroxydeoxyguanosine, a biomarker of oxidative DNA damage that correlates with some measures of diabetes severity *(69)*, in blood and urine, but measurement is labor-intensive. The ratio of the antioxidant glutathione to the oxidized glutathione disulfide can be determined in plasma as a measure of the reduced/oxidized state (care must be taken to avoid hemolysis of the blood sample); in one study, this measure correlated weakly with carotid intima-media thickness *(70)*. These or other novel methods to measure oxidative stress may be useful in the future to assess mitochondrial stress before clinical manifestations of disease, but no measurement method is currently ready for widespread clinical use.

CLINICAL IMPLICATIONS

Given the complexity of the interactions involved in the health effects of obesity, it is not surprising that, at least to date, pharmacotherapy has proven inefficient for addressing the multiple abnormalities associated with excess adiposity. For instance, pharmacologic alteration of one pathway can prompt compensatory responses from other pathways, and effects on downstream targets (e.g., skeletal muscle insulin resistance) are unlikely to ameliorate much of the health consequences of the upstream causes (e.g., adipocyte dysfunction). For example, pharmacologic therapies that decrease circulating glucose concentrations by improving peripheral insulin sensitivity or skeletal muscle glucose uptake have had disappointing effects on incidence of coronary heart disease. Based on the pathophysiologic cascade of obesity, the most promising interventions would target cellular stress and dysfunction at the adipocyte level to mitigate or reverse the adverse affects of adiposity.

PHARMACOLOGICAL INTERVENTIONS

Affecting adipocyte metabolism directly, for example by decreasing ER stress, could be a powerful mechanism for altering health because such interventions may be less subject to systemic counterregulatory changes *(16)*. ER stress might be mitigated by multiple mechanisms. For example, augmenting activity of protein chaperones that facilitate protein folding could decrease production of abnormal proteins that induce UPR and adversely affect ER function. Exogenous administration of 2 such candidate chaperones decreased UPR in adipose tissues and showed promise in reducing insulin resistance *(16)*. Another approach would be attenuation of ER protein synthesis: an eIF2alpha dephosphorylation inhibitor (salubrinal) decreased ER protein translation and reduced apoptosis due to ER stress in a rat model *(16)*. Medications currently in use have been shown to affect ER protein synthesis. Salicylates are antiinflammatory agents that improve eIF2alpha phosphorylation by activating PERK [RNA-activated protein kinase (PKR)-like ER kinase], the native mechanism for phosphorylating eIF2alpha and decreasing protein production *(16)*. Thiazolidinediones, which activate PPAR-γ, decrease adipocyte size *(11)*, and improve skeletal muscle insulin sensitivity *(71)*, have been shown to decrease ER protein synthesis by increasing eIF2alpha phosphorylation *(16)*.

Efforts to develop pharmaceutical means to decrease mitochondrial stress have focused on vitamin-based antioxidant therapy, and although the results of some smaller trials have been favorable, larger studies such as the Heart Outcomes Prevention Evaluation trial, the Primary Prevention Project trial have, to date, been largely unsuccessful in showing benefit *(72)*. Some researchers have suggested systemic delivery of antioxidant therapy may be ineffective due to the intracellular nature of the process of mitochondrial stress. The American Heart Association advises that evidence is not sufficient to support the use of antioxidant therapy in clinical care at present *(73)*.

In contrast, there is data to suggest that other pharmaceutical therapies with established cardiovascular prevention indications may have favorable effects on inflammation, circulating free fatty acids, and adipokines. Statins are known to decrease CRP, the most commonly measured marker of inflammation. Thiazolidinediones (PPARγ agonists), statins, and angiotensin-converting enzyme inhibitors have shown some antioxidant effects in animal models *(72)*. In a study of patients with type 2 diabetes, rosiglitazone therapy decreased sRBP-4 concentrations and improved insulin sensitivity *(53)*. Agonists of PPARα include fibrates, which lower triglyceride concentrations and increase HDL. PPARγ agonists (thiazolidinediones) increase the uptake of free fatty acids into the fat cell as they promote fat cell differentiation *(71)*, as well as decreasing some markers of inflammation (CRP, TNFα, IL-6) and increasing adiponectin expression *(71)*. Metformin, an insulin-sensitizing agent, reduced production of ROS in insulin resistant adults *(74)* and lowered CRP concentrations *(75)*.

LIFESTYLE MODIFICATION

Given that the fundamental cause of adiposity in most individuals is energy imbalance, the optimal approach to restoring caloric balance is lifestyle modification. Indeed, relatively modest (favorable and unfavorable) changes in lifestyle habits powerfully affect multiple pathways related to obesity.

NUTRITIONAL INTAKE

Although full review of effects of nutritional factors is beyond the scope of this report, it is clear that dietary habits are central to induction and maintenance of adipocyte stress. Reduction in caloric intake, if properly maintained, is effective for both prevention and reversal of adiposity and its associated health consequences *(76)*. Different diets can be effective in reducing total caloric consumption, including diets focused on particular macronutrient intakes (e.g., from very low fat diets to very high fat/low carbohydrate diets) and diets based on increased or decreased consumption of specific foods *(76)*. In randomized trials, specific dietary factors impact numerous established and novel cardiovascular and obesity-related risk factors; some of these dietary factors may be involved in mitigating or reversing adipocyte changes. In rodent models of obesity, a diet high in marine ω-3 fatty acids reduced adipocyte hypertrophy *(77)*; conversely, consumption of trans fatty acids may increase visceral adipocyte size or number *(4)*. Other fatty acids may stimulate adipocyte hyperplasia by altering gene expression to increase preadipocyte proliferation *(77)*. Consumption of high glycemic index carbohydrates *(78)* and trans fats *(79)* may be particularly detrimental for both weight gain and adipocyte stress. Dietary habits may also interact directly with adipokines. Animals genetically deficient in adiponectin placed on high-sugar, high-fat diets develop insulin resistance and are less responsive to PPAR-γ agonists, whereas intravenous injection of adiponectin in these animals improved insulin sensitivity and lowered glucose concentrations *(80)*, suggesting another possible opportunity for intervention. In cross-sectional analyses, adults consuming low glycemic-index diets had higher adiponectin concentrations *(81)*. Changes in dietary habits also influence inflammatory mediators: a diet extremely low in carbohydrates (13% of total calories) and high in fat (60%) induced weight loss and reduction in TNF-α and CRP *(82)*.

EXERCISE

Physical activity is critically important for both prevention and treatment of obesity. Favorable effects of physical activity include raising of HDL-C, lowering of triglycerides, lowering of blood pressure, improvement in fasting and postprandial glucose-insulin homeostasis, induction and maintenance of weight loss, and likely lowering of inflammation and improvement in endothelial function, even with moderate activity such as 30 min of brisk walking on most days *(83)*. Preliminary evidence suggests that at least some of the benefits of exercise may be related to reductions in intracellular ER stress and oxidative stress. For example, exercise training lowered sRBP-4 concentrations in adults and overweight children, correlating with improved insulin sensitivity and lower CRP and IL-6 *(58)*. Lifestyle modification in obese children reduced CRP and MDA concentrations, correlating with improvements in endothelial function *(84)*. Among older adults, 6 months of resistance training improved oxidative stress, assessed by measurement of exercised-induced lipid hydroperoxides *(85)*.

WEIGHT LOSS

Weight gain and loss are fundamental to the pathoetiology of adiposity and its health effects, although independent effects of weight change can be challenging to quantify given their necessary association with changes in caloric intake and/or physical activity. In the Diabetes Prevention Program, a modest lifestyle intervention decreased median CRP concentrations by approximately 30% over 12 months, largely correlating with weight loss *(75)*. In a metaanalysis of weight loss studies, CRP decreased by 0.13 mg/L for each 1 kg of weight loss *(86)*. In a 1-month dietary trial, adiponectin concentrations increased with weight reduction *(87)*. One study showed that measurements indicative of oxidative stress declined with weight loss *(88)*.

SMOKING

Smoking affects numerous pathways related to adipocyte stress, including worsening of inflammation and oxidative stress, endothelial dysfunction *(89)*, and insulin resistance and glucose intolerance *(90)*. Smoking also directly impairs pancreatic β-cell function *(91)*. Although smoking modestly reduces body weight, this effect is associated with increased central (visceral) adiposity *(92)* and therefore probably increases diabetes risk. In a metaanalysis of observational studies, smoking was associated with 44% higher multivariable-adjusted relative risk of new-onset diabetes (95% CI, 31%–58%), with evidence of dose response *(93)*.

Conclusions

Obesity is a well-established metabolic and cardiovascular risk factor. Recent advances have increased our understanding of the cellular mechanisms whereby adiposity induces adverse local and systemic effects. These include adipocyte intracellular lipid accumulation, ER and mitochondrial stress, and insulin resistance, with associated changes in circulating adipokines, free fatty acids, and inflammatory mediators. Although the human body possesses numerous mechanisms to protect itself from perturbations, the evolutionary drive to preserve and store excess calories (which was essential for previously rare periods of abundant food supply), together with the modern en-

ergy imbalance resulting from ubiquitous excess caloric intake and inadequate physical activity, has created the perfect storm for the present obesity epidemic and its related health consequences. Over time, relatively small alterations in energy balance produce significant changes in weight, and thus modest lifestyle modifications to address energy imbalance represent highly effective interventions to reverse adiposity and its adverse health effects *(94, 95)*. Unfortunately, the optimal methods to implement effective lifestyle changes to reduce obesity on a population level are not well understood. Additional research is needed to clarify the intracellular mechanisms of adiposity and the related health consequences and, perhaps more importantly, the most effective and generalizable means to implement healthier lifestyle habits in both children and adults.

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