abstract

Despite a lack of consistent diagnostic criteria, the metabolic syndrome (MetS) is increasingly evident in children and adolescents, portending a tsunami of chronic disease and mortality as this generation ages. The diagnostic criteria for MetS apply absolute cutoffs to continuous variables and fail to take into account aging, pubertal changes, and race/ethnicity. We attempt to define MetS mechanistically to determine its specific etiologies and to identify targets for therapy. Whereas the majority of studies document a relationship of visceral fat to insulin resistance, ectopic liver fat correlates better with dysfunctional insulin dynamics from which the rest of MetS derives. In contrast to the systemic metabolism of glucose, the liver is the primary metabolic clearinghouse for 4 specific foodstuffs that have been associated with the development of MetS: trans-fats, branched-chain amino acids, ethanol, and fructose. These 4 substrates (1) are not insulin regulated and (2) deliver metabolic intermediates to hepatic mitochondria without an appropriate “pop-off” mechanism for excess substrate, enhancing lipogenesis and ectopic adipose storage. Excessive fatty acid derivatives interfere with hepatic insulin signal transduction. Reactive oxygen species accumulate, which cannot be quenched by adjacent peroxisomes; these reactive oxygen species reach the endoplasmic reticulum, leading to a compensatory process termed the “unfolded protein response,” driving further insulin resistance and eventually insulin deficiency. No obvious drug target exists in this pathway; thus, the only rational therapeutic approaches remain (1) altering hepatic substrate availability (dietary modification), (2) reducing hepatic substrate flux (high fiber), or (3) increasing mitochondrial efficiency (exercise). Pediatrics 2012;129:557–570
A recent documentary entitled *Fat, Sick, and Nearly Dead* (a Joe Cross film; Reboot Media, 2011) resembles *Super-size Me* (a Morgan Spurlock film; Roadside Attractions, 2004), in reverse. But “fat” does not automatically mean “sick” or “nearly dead.” Absolute mass of adiposity and ensuing metabolic complications overlap but are distinct phenomena. For instance, ~30% of obese adults are metabolically normal,1,2 whereas 5% to 45% of normal-weight people exhibit the same metabolic perturbations as seen in the obese.3,4 BMI, a calculation (kg/m²) based on weight and height used to define “overweight” and “obesity,” thus clearly does not account for all the variance in cardiometabolic risk.5

The metabolic syndrome (MetS) is a clinical condition composed of anthropometric, physiologic, and biochemical abnormalities predisposing affected individuals to the development of type 2 diabetes (T2DM) and cardiovascular disease (CVD). Rather than total adiposity, the core clinical component of the syndrome is visceral6–10 and/or ectopic fat (ie, fat in organs not designed for fat storage).11,12 whereas the principal metabolic abnormality is insulin resistance (IR).13–15

The concept that cardiovascular risk factors “cluster” in certain individuals has been known for nearly a century.16 However, it was not until the early 1980s that the relationship between obesity, dyslipidemia (particularly hypertriglyceridemia), and hypertension was recognized17 and not until the late 1980s that the central roles of IR and abdominal adiposity in the pathogenesis of T2DM became apparent.13,18 In 1988, Reaven19 described the central role of IR in human disease and its interrelationship in adults with obesity, hypertension, T2DM, dyslipidemia, and CVD. Although modulation of these MetS components by race, ethnicity, and age complicates the assignment of clear thresholds in the pediatric population, the same “clustering” of risk factors is increasingly apparent in obese children.20,21 Thus, MetS knows no age limit.

With heritability estimates for BMI ranging between 40% and 70%,22 extensive searches for the causative gene(s) have been undertaken. Both candidate gene analyses23 and genome-wide association scans24 have hinted at complex gene networks with pleiotropic effects but modest effect sizes. In fact, only ~10% of the variance of MetS appears to be explained by genetic susceptibility.25,26 leaving ~90% to changes in the environment and/or to epigenetic interactions with potential for hereditary imprinting.27 Yet, despite this, certain components of MetS, including nonalcoholic fatty liver disease (NAFLD), have preferential racial/ethnic variations in prevalence.28 One potential explanation for this phenomenon is the preferential presence of the rs738409 allele of the patatin-like phospholipase domain-containing protein 3 (PNPLA3), which encodes a protein under nutritional regulation.29 Although the exact role of PNPLA3 in lipid processing has yet to be elucidated, the rs738409 allele has been associated with the accumulation of intrahepatic lipid and is particularly frequent in Hispanic individuals, who have the highest prevalence of NAFLD in the United States.28 Moreover, another allele in the same gene is associated with low hepatic fat content in African Americans, who have the lowest risk of developing NAFLD. PNPLA3 may also affect other ectopic lipid depots because visceral adipose tissue is related to intrahepatic lipid accumulation, irrespective of race or ethnicity.30

Rather than debate the role of genetics versus environment, or the veracity, validity, or utility of MetS screening criteria in children, our aim is to elucidate the most current understanding of the syndrome’s etiology and pathogenesis to provide a construct for future clinical and research endeavors.

**IR**

Conceptually, individuals can be classified as being “insulin sensitive” or “insulin resistant” on the basis of their response to an oral glucose challenge; specifically, how well a glucose load stimulates insulin release from the pancreas and the uptake of glucose by peripheral tissues.31 Whereas “insulin-sensitive” individuals have normal insulin secretion and rapid glucose clearance, “insulin-resistant” individuals manifest some degree of compensatory hyperinsulinemia to force glucose into peripheral tissues. Diabetes ensues when β-cells cannot compensate further to maintain euglycemia. However, it is important to not simply dichotomize an individual as being completely insulin sensitive or completely insulin resistant; rather, insulin sensitivity exists along a continuum and is modified by a host of factors.33,34 Furthermore, various cells and tissues have differential sensitivities to insulin,35–37 contributing to the variability of MetS expression and its phenotype. In fact, although multiple tissues are affected, IR in the liver is emerging as the likely primary lesion in the syndrome’s pathogenesis.

**Hepatic IR**

The liver plays a major role in substrate metabolism and is a primary target of insulin action. Thus, it is at the crossroads of metabolism and disease. After insulin’s release from the β-cell following a glucose load, it travels directly to the liver via the portal vein, where it binds to the insulin receptor and elicits 2 key actions at the level of gene transcription. First, insulin stimulates the phosphorylation of forkhead box protein 01 (FoxO1), which prevents it from entering the nucleus38 and thus diminishes the expression of genes required for gluconeogenesis, mainly
phosphoenolpyruvate carboxykinase and glucose-6-phosphatase. The net effect is diminished hepatic glucose output. Second, insulin activates the transcription factor sterol regulatory element-binding protein (SREBP)-1c, which in turn increases the transcription of genes required for fatty acid and triglyceride (TG) biosynthesis, most notably adenosine triphosphate-citrate lyase, acetyl-coenzyme A carboxylase, and fatty acid synthase. These 3 enzymes constitute the process of de novo lipogenesis (DNL). TGs synthesized by DNL are then packaged with apolipoprotein B (apoB) into very low-density lipoproteins (VLDL) for export to the periphery for storage or utilization by reciprocal activation of lipoprotein lipase on the surfaces of endothelial cells in adipose or muscle tissues. For reasons that remain unclear, insulin-resistant subjects typically have “selective” or “dissociated” hepatic IR; that is, they have impaired glucose homeostasis (mediated by the FoxO1 pathway) but enhanced insulin-mediated hepatic DNL (mediated by the SREBP-1c pathway) (Fig 1). The increase in free fatty acid (FFA) flux within the liver, either by DNL or FFA delivery via the portal vein, impairs hepatic insulin action, leading to increases in hepatic glucose output, the synthesis of proinflammatory cytokines, and major changes in lipoprotein metabolism. Specifically, hepatic DNL limits fatty acid β-oxidation via production of the intermediary malonyl CoA, which inhibits carnitine palmitoyltransferase-1 (CPT-1) and thus reduces the regeneration of carnitine, which normally shuttles fatty acids into the mitochondria. Thus, in the liver of insulin-resistant individuals, FFA flux is high, TG synthesis and intrahepatic lipid storage are increased, and excess TG is released with apoB as VLDL. This excess VLDL-TG secretion by the liver is considered the primary cause for MetS-associated dyslipidemia, characterized by elevated TG, low HDL cholesterol levels, and an elevated number of relatively cholesterol-depleted LDL particles. IR and MetS are also associated with intrahepatic lipid accumulation, oxidative stress, lipid peroxidation, and proinflammatory cytokine production. The intrahepatic accumulation of FFA and lipid are also detrimental to liver insulin sensitivity because they lead to the generation of toxic lipid-derived metabolites, such as diacylglycerol, fatty acyl CoA, and ceramides. These in turn trigger activation of protein kinase Cε (PKCe), and serine/threonine phosphorylation of insulin receptor substrate-1 (IRS-1), which attenuates hepatic insulin signal transduction.

Adipose Tissue IR

Although alterations in hepatic insulin signaling may be a cardinal feature of MetS, adipose tissue IR also appears to be important. The expanded adipose tissue mass that accompanies obesity often leads to increased lipolysis and FFA turnover. Normally, insulin inhibits adipose tissue lipolysis; however, in the insulin-resistant state, the process is accelerated, leading to increased FFA release into the circulation. Moreover, visceral adipocytes and macrophage foam cells are more sensitive to catecholamine-stimulated lipolysis than subcutaneous adipocytes, further increasing FFA flux. Because FFAs released from the visceral adipose tissue drain directly into the liver via the portal system, 1 potential mechanism for increased hepatic FFA accumulation and subsequent hepatic IR is increased delivery from the visceral fat depot (the “portal theory” of hepatic IR). Increasing evidence suggests that...
macrophages also infiltrate into adipose tissue and contribute substantively to both adipocyte hypertrophy and cytokine release, with larger fat cells producing larger amounts. These circulating cytokines not only affect insulin action in other tissues, such as liver and muscle, but may also have paracrine effects locally in fat.

**Skeletal Muscle IR**

Downstream of an insulin-resistant liver, increased plasma FFA levels disrupt the glucose–fatty acid or “Randle” cycle and insulin-mediated glucose transport in skeletal muscle, facilitating the development of hyperglycemia. The ectopic deposition in skeletal muscle of fat as intramyocellular lipid may also play a direct role in the pathogenesis of IR and MetS via lipid metabolite-induced activation of protein PKCε with subsequent impairment of insulin signaling.

**DIETARY FACTORS**

The incidence of obesity, diabetes, and MetS has increased over the past few decades in conjunction with the rise in daily caloric intake. The continuous provision of energy via dietary carbohydrate, lipid, and protein fuels, unmatched by physical activity/energy demand, arguably creates a backlog of the products of mitochondrial oxidation, a process associated with progressive mitochondrial dysfunction and IR.

In the context of overconsumption, and in a glycogen-replete and nonanabolic/nonmuscle building state (which pertains to the vast majority of the population), specific macronutrients of the “Western diet” may differentially contribute to the pathogenesis of IR and MetS. Historically, 1 of the most common food constituents associated with MetS has been dietary fat. However, the absolute consumption of total dietary fat has not changed over the past 30 years; in fact, the percentage of calories ingested from saturated fats has decreased from 40% to 30%. The finding that high-fat, low-carbohydrate diets are protective against MetS further confirms the conventional wisdom that simple fat restriction is beneficial. In experimental models, saturated fats are robustly proinflammatory, omega-6 polyunsaturated fatty acids are weakly reactive, monounsaturated fatty acids are neutral, and omega-3 fatty acids have antiinflammatory properties. In humans, the story is much more complex because most fatty foods are composed of many types of fats, and cardiometabolic risk relates more to the balance of saturated versus unsaturated fats than to the total amount ingested. It may thus be the underrepresentation of unsaturated fatty acids in the typical American diet that gives saturated fat such a bad reputation. Studies suggest that monounsaturated fats such as oleic acid (found in olive oil) and polyunsaturated linoleic acid or omega-3 fatty acids all decrease inflammation and intrahepatic lipid deposition and improve postprandial TG levels, possibly by increasing peroxisomal activity and thereby limiting damage by reactive oxygen species (ROS).

**Trans-unsaturated Fatty Acids (Trans-fats)**

Trans-unsaturated fats in processed foods have been a staple in the Western diet since the early 20th century. This is because the trans-isomerization of the double bond prevents fatty acid breakdown by bacteria, prolonging the shelf life of foods. Like their bacterial predecessors, human mitochondria cannot subject trans-fats to β-oxidation in the liver, contributing to ectopic intrahepatic lipid accumulation. Fortunately, because of the recognized association between trans-fat consumption and cardiovascular disease in the mid-1980s and more stringent labeling requirements since 2006, the percent of calories from trans-fats consumed in the “Western diet” has been gradually declining. Trans-fats have no health benefit and cause hepatic steatosis and IR; however, their current consumption trends are temporally disparate with the current increasing prevalence of MetS, suggesting that other factors are involved.

**Branched-Chain Amino Acids**

Branched-chain amino acids (BCAAs: valine, leucine, and isoleucine) are essential amino acids that account for >20% of the amino acids in the typical “Western diet.” Although normally used for protein biosynthesis and cell growth, when provided in excess, they are diverted away from protein synthesis and toward energy utilization.

In the liver, BCAAs increase transcription of carbohydrate regulatory element-binding protein and SREBP-1c, facilitating DNL. Furthermore, BCAAs limit insulin-induced phosphoinositide 3-kinase (PI3-K) signaling and stimulate the activation of the mammalian target of rapamycin (mTOR), promoting the serine phosphorylation of IRS-1 and impairment of insulin signaling. In addition, just as there are obesity-related changes in adipokines and cardiovascular risk markers, there also appears to be obesity-associated changes in BCAA metabolism and subsequent serum levels. In particular, valine and leucine/isoleucine levels have been reported to be 20% and 14% higher, respectively, in obese compared with lean subjects. Mechanistically, this appears to be accounted for by a high rate of flux through the BCAA catabolic pathway, resulting in the increased production of alanine. Because alanine is a highly gluconeogenic amino acid, increased BCAA catabolism may thus contribute to increased hepatic glucose output. Furthermore, the increased α-ketocacids generated by increased flux of the
BCAs through their catabolic pathways also potentially suppress mitochondrial \( \beta \)-oxidation.\(^{93}\)

Because of the liver’s relatively low activity of the branched-chain aminotransferase enzyme, dietary BCAs also reach the systemic circulation in levels proportional to dietary intake,\(^{86,94}\) increasing their exposure to peripheral tissues. This increased peripheral delivery of BCAs when combined with a high-fat diet may promote IR by causing the accumulation of lipid-derived metabolites such as diacylglycerols and ceramides and/or the activation of the mTOR pathway along with serine phosphorylation of IRS-1.\(^{87,89,95,96}\) In addition, in the setting of a high-fat diet, BCAs cause overaccumulation of C3 and C5 acylcarnitines, which may saturate capacity for mitochondrial \( \beta \)-oxidation, leading to the accumulation of incompletely oxidized lipid-derived metabolites.\(^{87}\) Alterations in adipose tissue BCAA metabolism also appear to influence circulating BCAs,\(^{97,98}\) and the exaggerated insulin secretion response to glucose that is often observed in obese individuals may be due to potentiation of glucose-induced insulin secretion by BCAs,\(^{87,99}\) potentially facilitating subsequent \( \beta \)-cell failure.

Furthermore, chronic BCAA elevation impairs the transport of aromatic amino acids into the brain; the reduced production of serotonin (derived from tryptophan) and catecholamines (derived from phenylalanine and tyrosine) may drive hunger.\(^{87}\) The “BCAA overload” hypothesis suggests that in the context of a dietary pattern that includes high fat consumption, BCAs may make an independent contribution to the development of IR,\(^{87}\) a hypothesis supported by metabolomics studies demonstrating high BCAA levels in normoglycemic individuals who subsequently develop IR and diabetes.\(^{100,101}\)
Ethanol
Although adult epidemiologic studies associate light to moderate ethanol consumption with improved insulin sensitivity and wine consumption with reduced cardiovascular risk, other cross-sectional and prospective studies implicate a dose-dependent effect of alcohol in MetS and suggest that chronic consumption of large amounts of ethanol worsens insulin sensitivity. Furthermore, its metabolism (Fig 2B) bears important similarities to fructose (see below).

Ethanol neither elicits an insulin response nor requires a transporter to enter the liver. Once inside the hepatocyte, it bypasses glycolysis and is converted by alcohol dehydrogenase-1B to form acetaldehyde, which promotes ROS formation and toxic damage to the liver if not quenched by hepatic antioxidants such as glutathione or ascorbic acid. Acetaldehyde is then metabolized by the enzyme aldehyde dehydrogenase-2 to acetic acid, which in turn is metabolized by the enzyme acetyl-CoA synthetase short-chain family member 2 to form acetyl-CoA. The acetyl-CoA can then enter the mitochondrial tricarboxylic acid cycle (as per acetyl-CoA derived from glucose metabolism); however, in the presence of other caloric substrates, it is preferentially used for the synthesis of fatty acids through DNL (as per acetyl-CoA derived from fructose metabolism). The excess malonyl-CoA produced from ethanol metabolism inhibits CPT-1, thereby limiting mitochondrial fatty acid β-oxidation. Ethanol also blocks fatty acid β-oxidation by inhibiting both peroxisome proliferator-activated receptor (PPAR)-α and adenosine monophosphate-activated protein kinase, which lead to increased activity of acetyl-CoA carboxylase and increased levels of malonyl-CoA. PPAR-α is expressed mainly in the liver, kidney, and heart and stimulates the transcription of genes involved in fatty acid uptake and both mitochondrial and peroxisomal fatty acid β-oxidation. The ethanol-induced suppression of PPAR-α also suppresses microsomal triglyceride transfer protein, thereby altering the liver’s lipid export machinery. Buildup of intrahepatic lipid metabolites leads to subsequent activation of the enzyme c-Jun N-terminal kinase 1 (JNK-1) and serine-phosphorylation of the IRS-1, driving further hepatic IR. Thus, ethanol metabolism results in intrahepatic lipid accumulation and liver injury.

Fructose
Another dietary component that has been clearly implicated in the pathogenesis of MetS is the monosaccharide fructose. Fructose is typically consumed either as sucrose (50% fructose) or as high-fructose corn syrup (HFCS; 42% or 55% fructose). Unlike those of trans-fats or ethanol, the secular consumption trends for fructose have paralleled the rise of obesity and MetS, especially in children. Before World War II, Americans consumed ~24 g per day of fructose; by the mid-1970s, it had increased to ~37 g per day, and by the mid-1990s, to ~55 g per day (a progressive increase from ~5% to 7% to 10% of total calories). In fact, recent NHANES data suggest that ~15% of the US population consumes ≥25% of energy from added sugars. Adolescents are by far the highest fructose consumers, consuming >70 g per day (~12% of total calories); and more than 20% of adolescents consume ≥25% of their total calories as fructose.

Although considerable debate exists as to whether HFCS is different than sucrose, examination of the data suggests that HFCS and sucrose have similar endocrine and metabolic effects. Unlike the hepatic metabolism of glucose, which principally leads to glycogen synthesis, the hepatic metabolism of fructose (Fig 2C) results in sustained elevations in postprandial TG levels. Importantly, increased fructose consumption, particularly in the form of sugar-sweetened beverages, has been implicated in promoting weight gain, visceral adiposity, dyslipidemia, and IR/glucose intolerance as well as hepatic steatosis.

Fructose in the gut is transported into the enterocyte via the fructose transporter Glut5, independent of adenosine triphosphate hydrolysis and sodium absorption. Once inside the enterocyte, a small portion of the fructose downstream target S6K, which contributes to serine phosphorylation of IRS-1 and hepatic IR, which in turn promotes hyperinsulinemia and influences substrate deposition into fat, and export of free fatty acids, which leads to VLDL formation and muscle IR. α-KG, α-ketoglutarate; BCKA, branched-chain keto acid; pSer-IRS-1, serine phosphorylated IRS-1; S6K, S6 kinase. B, Hepatic ethanol metabolism. Ethanol induces: DNL and dyslipidemia; JNK-1 activation, which serine phosphorylates hepatic IRS-1, rendering it inactive, and contributing to hepatic IR, which promotes hyperinsulinemia and influences substrate deposition into fat; hepatic lipid droplet formation, leading to steatosis; and stimulation of the reward pathway, promoting continuous consumption. PPAR-α is expressed mainly in the liver, kidney, and heart and stimulates the transcription of genes involved in fatty acid uptake and both mitochondrial and peroxisomal fatty acid β-oxidation. The ethanol-induced suppression of PPAR-α also suppresses microsomal triglyceride transfer protein, thereby altering the liver’s lipid export machinery. Buildup of intrahepatic lipid metabolites leads to subsequent activation of the enzyme c-Jun N-terminal kinase 1 (JNK-1) and serine-phosphorylation of the IRS-1, driving further hepatic IR. Thus, ethanol metabolism results in intrahepatic lipid accumulation and liver injury.

References
load is converted to lactic acid and released in the portal circulation; another small portion may also be converted to glucose.\textsuperscript{142} However, the majority of ingested fructose is secreted into the portal circulation and delivered to the liver. There, fructose is rapidly metabolized to fructose-1-phosphate (F1P) via fructokinase,\textsuperscript{141} an insulin-independent process which also bypasses the negative feedback regulation of phosphofructokinase in the glycolytic pathway. Thus, fructose metabolism generates lipogenic substrates (eg, glyceraldehyde-3-phosphate and acetyl-CoA), which are delivered straight to the mitochondria, in an unregulated fashion. This excessive mitochondrial substrate then drives hepatic DNL, which can then overwhelm apoB and the lipid export machinery, leading to intrahepatic lipid deposition and steatosis.\textsuperscript{140} Hepatic DNL also limits further fatty acid oxidation in the liver via excess production of malonyl-CoA, which reduces entry of fatty acids into the mitochondria by inhibiting CPT-1.\textsuperscript{44–46} F1P also stimulates SREBP-1c via peroxisome proliferator-activated receptor-\(\gamma\) coactivator-1\(\beta\)\textsuperscript{145} independently of insulin, which activates the genes involved in DNL\textsuperscript{144–146}; moreover, fructose has been shown to induce activation of carbohydrate regulatory element-binding protein and increase the expression of all the enzymes of DNL. Furthermore, F1P activates dual-specificity mitogen-activated protein kinase 7,\textsuperscript{147} which subsequently stimulates JNK-1,\textsuperscript{148} a hepatic enzyme considered to act as a bridge between hepatic metabolism and inflammation.\textsuperscript{149} In addition, the lipogenic intermediate diacylglycerol (formed during fructose metabolism in the liver) activates PKC\(\varepsilon\).\textsuperscript{150} Both of these events stimulate serine phosphorylation of IRS-1, leading to hepatic IR. This impairs insulin-mediated phosphorylation of FoxO1, leading to increased expression of the genes required for gluconeogenesis and promoting increased hepatic glucose output, possibly contributing to hyperglycemia and the development of T2DM. The excess TGs secreted from the liver into the circulation as fat-laden VLDL particles after the ingestion of fructose may couple with a fructose-induced reduction in lipoprotein lipase activity to cause sustained postprandial dyslipidemia, thereby augmenting the risk for CVD.\textsuperscript{153,151}

How are these 4 dietary foodstuffs similar? Each share 3 biochemical properties: (1) they are metabolized for energy primarily within the liver; (2) they are not insulin regulated; and (3) they do not have a “pop-off” mechanism to form glycogen for storage. So, although the kinetics of their metabolism may differ, virtually all their intermediates are delivered directly to the mitochondria, which cannot process the volume of substrate, resulting in a backlog of metabolic intermediates, ROS generation, excessive DNL, and impaired \(\beta\)-oxidation, driving IR and the downstream comorbidities of MetS.

Although the data supporting a role of BCAAs in the development of MetS components are currently only associative in nature, the “BCAA overload” hypothesis is intriguing and relevant. Alternatively, data supporting the role of the other 3 dietary foodstuffs (ie, trans-fats, ethanol, and fructose) in the development of MetS components are more definitive and demonstrate causation.

**SUBCELLULAR ROS METABOLISM**

**Mitochondria and ROS Formation**

The “free radical theory” holds that imbalance between ROS generation and antioxidant defenses is a major factor in the determination of lipid peroxidation and protein misfolding, with resultant DNA and cellular damage.\textsuperscript{152} Excessive intracellular ROS formation occurs via 3 pathways: (1) inflammatory cytokines derived from visceral fat accumulation;\textsuperscript{153,154} (2) dysfunctional mitochondrial energetics;\textsuperscript{155} and (3) glycation (see below). Excessive nutrient processing by mitochondria can result in uncoupling of oxidative phosphorylation and increased generation of ROS; this, in turn, leads to altered mitochondrial function and further ROS generation.\textsuperscript{156} ROS accumulation can also impair endoplasmic reticulum (ER) function, causing ER stress and the compensatory unfolded protein response (UPR). The UPR can itself be overwhelmed by persistent excessive nutrient processing and ROS generation, leading to cellular shutdown, defective insulin secretion, and T2DM.\textsuperscript{157,158}

Fructose is a well-known driver of excessive ROS formation. Molecularly, glucose is found in 2 stereoisomeric forms (Fig 3): the majority in the glucopyranose (6-membered ring) form and the minority in the linear aldehyde form, which is highly reactive with e-amino groups of lysine, generating a ROS with each reaction, known as the Maillard or “browning” reaction. Fructose is also found in 2 stereoisomeric forms: the majority in the linear ketone form (also highly reactive) and the minority in the fructofuranose (5-membered ring) form. The latter has 2 axial (abutting) hydroxymethyl groups, which exert allosteric and ionic forces to the unstable furanose ring and drive it toward the linear form. This difference explains why nonenzymatic fructosylation is 7 times more rapid than protein glycation and why fructose generates 100 times more ROS than glucose.\textsuperscript{159}

**Peroxisomes and ROS Quenching**

Because ROS are inherent by-products of cellular metabolism, endogenous cellular antioxidants (eg, catalase and glutathione) quench the ROS before they have a chance to promote peroxidation. These antioxidants are found primarily in peroxisomes, which abut the
mitochondria, and act as “support staff” for ROS processing.\textsuperscript{160} Mouse models of peroxisomal disorders result in mitochondrial and ER dysfunction.\textsuperscript{161} Furthermore, cytokines such as tumor necrosis factor-\textalpha\textsuperscript{a} can reduce peroxisomal number and function, rendering cells even more vulnerable.\textsuperscript{162} In the absence of antioxidant quenching, fructose can cause cellular damage. In an in vitro study, incubation of hepatocytes with fructose yielded no direct damage; however, when these hepatocytes were preincubated with sublethal doses of hydrogen peroxide to reduce their peroxisomal ROS-quenching ability, fructose was as hepatotoxic as other organic aldehydes.\textsuperscript{163} In the absence of antioxidant quenching, fructose can cause cellular damage. In an in vitro study, incubation of hepatocytes with fructose yielded no direct damage; however, when these hepatocytes were preincubated with sublethal doses of hydrogen peroxide to reduce their peroxisomal ROS-quenching ability, fructose was as hepatotoxic as other organic aldehydes.\textsuperscript{163} Furthermore, an in vivo study in antioxidant-deficient mice demonstrated that intrahepatic lipid toxicity and hepatocellular death occurred after sucrose administration.\textsuperscript{164} These data thus suggest that excessive ROS, in combination with micronutrient insufficiencies which impair antioxidant reserves, can lead to cellular damage (Fig 4).

**ER, ROS, and UPR**

Excessive ROS that are not quenched by peroxisomes find their way to the adjacent ER, where they alter the redox environment crucial for proper protein folding.\textsuperscript{165} Accumulation of ROS and misfolded proteins within the ER activates the UPR,\textsuperscript{166} designed to decrease protein synthesis to allow for their clearance.\textsuperscript{167} However, excessive ROS impairs the ability to clear misfolded proteins and activates the enzyme caspase-3, leading to even further ROS generation, apoptosis, and cellular demise.\textsuperscript{168} ER stress in the liver is a specific mechanism of hepatic injury in NASH,\textsuperscript{169} and ER stress in the pancreas reduces \( \beta \)-cell number and promotes diabetes.\textsuperscript{170} Thus, dietary foodstuff-induced increases in intracellular ROS formation in conjunction with hepatic DNL and ectopic fat deposition (see above) lead to IR and metabolic dysfunction, increasing an individual’s risk for MetS and its associated comorbidities.

**PREVENTION OF MetS**

Unfortunately, there is no actionable drug target in either the lipogenesis or ROS pathways. Currently, the only options to prevent MetS are to reduce mitochondrial substrate metabolism to prevent these 2 phenomena. This can be accomplished in 3 ways, as follows.

1. **Reduction in substrate availability:** caloric restriction, particularly of lipogenic substrates, improves insulin sensitivity and reduces liver fat accumulation in humans, thus reducing risk for MetS.\textsuperscript{171}

2. **Reduction in hepatic substrate flux:** by reducing the rate of substrate absorption and resultant liver metabolic capacity, the serum glucose rise and subsequent insulin response can be attenuated. This can be accomplished by reducing glycemic load, which is most easily accomplished by increasing the fiber content of food.\textsuperscript{172} Furthermore, lipogenesis and hepatic lipid export can also be reduced with improved dietary fiber intake.\textsuperscript{173}

3. **Increase in substrate clearance:** by increasing hepatic mitochondrial substrate metabolism, the availability of substrate for lipogenesis will be reduced, and hepatic IR can be mitigated. This can be directly accomplished by exercise. By stimulating the sympathetic nervous system and inducing

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**FIGURE 3**

Molecular renditions of (A) glucose and (B) fructose, in the linear, chair, and space-occupying projections. In the linear form, both glucose and fructose possess a reactive aldehyde or one ketone moiety, which can bind nonenzymatically to freely available amino groups of proteins. At normal body temperature and pH, the chair form of glucose predominates. This conformation is a glucopyranose (6-membered ring), with equatorial hydroxyl groups and is molecularly stable, which limits its protein reactivity. However, the chair form of fructose is a fructofuranose (5-membered ring) with 2 axial hydroxymethyl groups that exert allosteric and ionic forces on the unstable furanose ring, which favors the linear form. Thus, at normal body temperature and pH, the majority of fructose exists in the linear form and is more reactive with proteins than is glucose. (Reproduced from Lim et al\textsuperscript{162}.)

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**TABLE 3**

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<thead>
<tr>
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**DIAGRAM 3**

**A** Glucose (linear form)

**B** Fructose (linear form)
the transcription factor peroxisome proliferator-activated receptor γ coactivator-1α, exercise causes mitochondrial biogenesis in liver and muscles. The age of the mitochondria is relevant because new mitochondria are more efficient, generating fewer ROS. Exercise also burns acetyl-CoA and prevents the buildup of fatty acids, which improves insulin sensitivity in the liver and muscles.

CONCLUSIONS

The pathways delineated in this article make it clear how our current food environment is mismatched to our biochemistry. Although we have drugs that can treat individual cardiometabolic complications such as dyslipidemia, hypertension, and IR, there is no clear drug target that can mitigate the underlying cellular damage wrought by dysfunctional mitochondrial ROS and protein misfolding. The only rational approaches to reduce mitochondrial ROS formation and toxicity are preventive, by limiting specific substrate availability (dietary modification), reducing hepatic substrate flux (high fiber), and/or increasing hepatic mitochondrial biogenesis to improve mitochondrial capacity and efficiency (exercise). Dietary recommendations to restrict trans-fats, refined carbohydrates (including fructose), and excessive protein loads can be bolstered by policy changes. The food industry has been increasingly successful in reintroducing whole grains and adding fiber back to processed foods, but dietary intake of fiber continues to fall short, and policies for removal of added sugar are currently nonexistent. Improved access to healthful unprocessed foods, and to regular physical activity in schools and communities, has been a policy priority for the current administration. Although such fundamental goals of lifestyle modification are laudable, their implementation is easier said than done.

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MUSIC TO MY EARS: We routinely perform blind taste comparisons of wine, beer, and chocolate with guests at our home. Sometimes I’m “surprised” when a favorite wine performs poorly, compared head-to-head with another wine. Guests are always surprised that a chocolate made by a famous luxurious chocolate maker is the least favorite of the group. We know that part of a wine’s allure includes the setting, company, and significance of the event. Does the same apply to classic stringed instruments such as those made by the Guarneri and Stradivari families? Several studies have shown that concert attendees cannot determine the difference between the sound of high-end modern and classic violins when the violinist plays behind a screen. As reported in The New York Times (Science: January 2, 2012), a researcher conducted a study to determine if violinists themselves could tell the difference. Violinists at an international competition in Indianapolis were instructed to wear goggles and asked to play three well-made modern instruments they could not see, a Guarneri and two Stradivari. Not surprisingly, the violinists could not tell them apart. According to the article, 8 of the 21 violinists chose an old violin as the one they would bring home. In head-to-head competitions, a Stradivarius was the least preferred while a new violin the most. Violin makers welcomed the news. However, some violinists are skeptical as the experiment was conducted in a hotel room, not a concert hall. The violinists were not able to develop a relationship with the violin so as to coax the best sound from their play. Finally, some sniff that on the first attempt even experienced musicians cannot discern quality in an instrument. While I greatly enjoy music, I am not an audiophile. Still, the study seems very credible and the results consistent with blinded comparisons of other objects. At a certain level, all violins are going to sound great. How I view the experience will be dependent on the musician, the setting, and my partner rather than just the instrument.

Noted by WVR, MD