Toward a Unifying Hypothesis of Metabolic Syndrome

AUTHORS: Andrew A. Bremer, MD, PhD,^a Michele Mietus-Snyder, MD,^b and Robert H. Lustig, MD^c

^aDepartment of Pediatrics, Vanderbilt University School of Medicine, Nashville, Tennessee; ^bDepartment of Pediatrics and Children's National Obesity Institute, Children's National Medical Center, Washington, District of Columbia; and ^cDepartment of Pediatrics, University of California, San Francisco, San Francisco, California

KEY WORDS

metabolic syndrome, insulin resistance, dyslipidemia, hypertension, diabetes, pediatrics

ABBREVIATIONS

apoB—apolipoprotein B
BCAA—branched-chain amino acid
CoA—coenzyme A
CPT-1—carnitine palmitoyltransferase-1
CVD—cardiovascular disease
DNL—de novo lipogenesis
ER—endoplasmic reticulum
F1P—fructose-1-phosphate
FFA—free fatty acid
FoxO1—forkhead box protein O1
HFCS—high-fructose corn syrup
IR—insulin resistance
IRS-1—insulin receptor substrate-1
JNK-1—*c-jun* N-terminal kinase 1
MetS—metabolic syndrome
mTOR—mammalian target of rapamycin
NAFLD—nonalcoholic fatty liver disease
PKCε—protein kinase C-ε
PNPLA3—patatin-like phospholipase domain-containing protein 3
PPAR—peroxisome proliferator-activated receptor
ROS—reactive oxygen species
SREBP—sterol regulatory element-binding protein
T2DM—type 2 diabetes
TG—triglyceride
UPR—unfolded protein response
VLDL—very low-density lipoprotein

www.pediatrics.org/cgi/doi/10.1542/peds.2011-2912

doi:10.1542/peds.2011-2912

Accepted for publication Nov 28, 2011

Address correspondence to Robert H. Lustig, MD, Department of Pediatrics, University of California, San Francisco, Suite S679, Box 0434, 513 Parnassus Ave, San Francisco, CA 94143-0434. E-mail: rlustig@peds.ucsf.edu

PEDIATRICS (ISSN Numbers: Print, 0031-4005; Online, 1098-4275).

Copyright © 2012 by the American Academy of Pediatrics

FINANCIAL DISCLOSURE: The authors have indicated they have no financial relationships relevant to this article to disclose.

FUNDING: Funded by the National Institutes of Health (NIH).

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/m^2) 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 visceral^{6–10} 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 challenge³¹; specifically, how well a glucose load stimulates insulin release from the pancreas and the uptake of glucose by peripheral tissues.³² 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 O1 (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.^{39,40} 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.^{41,42}

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)³⁷ (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,⁴³ 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 coenzyme A (CoA), which inhibits carnitine palmitoyltransferase-1 (CPT-1) and thus reduces the regeneration of carnitine, which normally shuttles fatty acids into the mitochondria.^{44–46} 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

FIGURE 1

Selective IR in the liver. Under normal conditions, dietary glucose stimulates insulin secretion from the pancreatic β -cells. Insulin then 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 FoxO1, which prevents it from entering the nucleus and thus diminishes the expression of genes required for gluconeogenesis; the net effect is diminished hepatic glucose output. Second, insulin activates the transcription factor SREBP-1c, which in turn increases the transcription of genes required for fatty acid and TG biosynthesis; the net effect is DNL. In the liver of individuals with MetS, insulin fails to decrease gluconeogenesis because of decreased signaling through the FoxO1 pathway but continues to stimulate the production of fatty acids and TGs through the SREBP-1c pathway. Resistance to the FoxO1 pathway leads to dysglycemia and subsequent compensatory hyperinsulinemia, whereas sensitivity to the SREBP-1c pathway leads to DNL, intrahepatic lipid deposition, and dyslipidemia/hypertriglyceridemia, enhancing ectopic lipid deposition. (Adapted from Brown and Goldstein⁵⁷.)

by elevated TG, low HDL cholesterol levels, and an elevated number of relatively cholesterol-depleted LDL particles.⁴⁷

IR and MetS are also associated with intrahepatic lipid accumulation, oxidative stress, lipid peroxidation, and proinflammatory cytokine production.³⁴ 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 C- ϵ (PKC ϵ), and serine/threonine phosphorylation of insulin receptor substrate-1 (IRS-1), which attenuates hepatic insulin signal transduction.^{48–50}

Adipose Tissue IR

Although alterations in hepatic insulin signaling may be a cardinal feature of

MetS, adipose tissue IR also appears to be important.^{19,36,51,52} The expanded adipose tissue mass that accompanies obesity often leads to increased lipolysis and FFA turnover.^{53,54} Normally, insulin inhibits adipose tissue lipolysis; however, in the insulin-resistant state, the process is accelerated,^{51,55} leading to increased FFA release into the circulation.^{34,52} Moreover, visceral adipocytes and macrophage foam cells are more sensitive to catecholamine-stimulated lipolysis than subcutaneous adipocytes,⁵⁶ 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).⁴³ Increasing evidence suggests that

macrophages also infiltrate into adipose tissue and contribute substantively to both adipocyte hypertrophy and cytokine release,^{57–59} with larger fat cells producing larger amounts.⁶⁰ 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.^{61,62}

Skeletal Muscle IR

Downstream of an insulin-resistant liver, increased plasma FFA levels disrupt the glucose–fatty acid or “Randle” cycle^{53,63} and insulin-mediated glucose transport in skeletal muscle,^{64–66} 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 PKC ϵ with subsequent impairment of insulin signaling.^{36,50,67,68}

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.⁶⁹ 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,⁷⁰ a process associated with progressive mitochondrial dysfunction and IR.⁷¹

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.^{72,73} 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%.⁷⁴ The finding that high-fat, low-carbohydrate diets are protective against MetS⁷⁵ further confounds 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.⁷⁶ 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.⁷⁷ It may thus be the underrepresentation of unsaturated fatty acids in the typical American diet⁷⁸ that gives saturated fat such a bad reputation. Studies suggest that monounsaturated fats such as oleic acid (found in olive oil)⁷⁹ and polyunsaturated linoleic acid⁸⁰ or omega-3 fatty acids⁸¹ 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).⁸²

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,⁸³ contributing to ectopic intrahepatic lipid accumulation.⁸⁴ 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.⁷⁴ Trans-fats have no health benefit and cause hepatic steatosis and IR⁸⁵; however, their current consumption trends are temporally disparate with the current increasing prevalence of MetS,³⁴ 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.”⁸⁶ Although normally used for protein biosynthesis and cell growth, when provided in excess, they are diverted away from protein synthesis and toward energy utilization.⁸⁷

In the liver, BCAAs increase transcription of carbohydrate regulatory element-binding protein and SREBP-1c,⁸⁸ facilitating DNL. Furthermore, BCAAs limit insulin-induced phosphoinositide 3-kinase (PI3-K) signaling^{89,90} and stimulate the activation of the mammalian target of rapamycin (mTOR),^{91,92} promoting the serine phosphorylation of IRS-1 and impairment of insulin signaling.^{87,89} 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.⁸⁷ Mechanistically, this appears to be accounted for by a high rate of flux through the BCAA catabolic pathway (Fig 2A), 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.⁸⁷ Furthermore, the increased α -ketoacids generated by increased flux of the

FIGURE 2

A, Hepatic BCAA metabolism. BCAAs induce: excess formation of alanine, which serves as a substrate for gluconeogenesis, contributing to hyperglycemia; excess formation of α -ketoacids, leading to increased levels of C5 and C3 acylcarnitine levels and subsequently excess formation of malonyl-CoA, which inhibits β -oxidation; hepatic lipid droplet formation and steatosis; activation of mTOR and its

BCAAs through their catabolic pathways also potentially suppress mitochondrial β -oxidation.⁹³

Because of the liver's relatively low activity of the branched-chain aminotransferase enzyme, dietary BCAAs also reach the systemic circulation in levels proportional to dietary intake,^{86,94} increasing their exposure to peripheral tissues. This increased peripheral delivery of BCAAs 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, BCAAs cause overaccumulation of C3 and C5 acylcarnitines, which may saturate capacity for mitochondrial β -oxidation, leading to the accumulation of incompletely oxidized lipid-derived metabolites.⁸⁷ Alterations in adipose tissue BCAA metabolism also appear to influence circulating BCAA levels,^{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 BCAAs,^{87,99} potentially facilitating subsequent β -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, BCAAs 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 sensitivity¹⁰² and wine consumption with reduced cardiovascular risk,¹⁰³ other cross-sectional^{104,105} and prospective studies¹⁰⁶ 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).¹⁰⁷

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.¹⁰⁸ Acetaldehyde is then metabolized by the enzyme aldehyde dehydrogenase-2 to acetic acid, which in turn is metabolized by the enzyme acyl-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.¹⁰⁹ 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.¹¹⁰ The ethanol-induced suppression of PPAR- α also suppresses microsomal triglyceride transfer protein,¹¹¹ thereby altering the liver's lipid export machinery.^{112–114} Buildup of intrahepatic lipid metabolites leads to subsequent activation of the enzyme *c-jun* N-terminal kinase 1 (JNK-1)¹¹⁵ and serine-phosphorylation of the IRS-1, driving further hepatic IR. Thus, ethanol metabolism results in intrahepatic lipid accumulation and liver injury,^{116–118} driving hepatic IR^{119,120} and promoting MetS.

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 $\geq 25\%$ of energy from added sugars.¹²¹ Adolescents are by far the highest fructose consumers, consuming >70 g per day (~12% of total calories); and more than 20% of adolescents consume $\geq 25\%$ of their total calories as fructose.^{121,122}

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.¹²³ 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.^{124–128} Importantly, increased fructose consumption, particularly in the form of sugar-sweetened beverages, has been implicated in promoting weight gain,^{129–131} visceral adiposity,^{132,133} dyslipidemia,^{133,134} and IR/glucose intolerance,^{133,135,136} as well as hepatic steatosis.^{137–140}

Fructose in the gut is transported into the enterocyte via the fructose transporter Glut5, independent of adenosine triphosphate hydrolysis and sodium absorption.¹⁴¹ Once inside in 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. (Reproduced from Lustig¹⁰⁷.) ACC, acetyl CoA carboxylase; ACL, adenosine triphosphate citrate lyase; FAS, fatty acid synthase; MTP, microsomal transfer protein. C, Hepatic fructose metabolism. Fructose induces substrate-dependent phosphate depletion, which increases uric acid and contributes to hypertension through inhibition of endothelial nitric oxide synthase and reduction of NO; DNL and dyslipidemia; hepatic lipid droplet formation and steatosis; muscle IR; JNK-1 activation, contributing to hepatic IR, which promotes hyperinsulinemia and influences substrate deposition into fat; and CNS hyperinsulinemia, which antagonizes central leptin signaling and promotes continued energy intake. (Reproduced from Lustig¹⁰⁷.) ACC, acetyl CoA carboxylase; ACL, adenosine triphosphate citrate lyase; ACS2, acyl-CoA synthetase short-chain family member 2; AMP, adenosine monophosphate; BP, blood pressure; CNS, central nervous system; FAS, fatty acid synthase; Glut5, glucose transporter 5; MTP, microsomal transfer protein; NO, nitric oxide; pSer-IRS-1, serine phosphorylated IRS-1.

load is converted to lactic acid and released in the portal circulation¹⁴²; another small portion may also be converted to glucose.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,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.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.^{44–46} F1P also stimulates SREBP-1c via peroxisome proliferator-activated receptor- γ coactivator-1 β ¹⁴³ independently of insulin, which activates the genes involved in DNL^{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 7,¹⁴⁷ which subsequently stimulates JNK-1,¹⁴⁸ a hepatic enzyme considered to act as a bridge between hepatic metabolism and inflammation.¹⁴⁹ In addition, the lipogenic intermediate diacylglycerol (formed during fructose metabolism in the liver) activates PKC ϵ .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.^{133,151}

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 β -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.¹⁵² Excessive intracellular ROS formation occurs via 3 pathways: (1) inflammatory cytokines derived from

visceral fat accumulation^{153,154}; (2) dysfunctional mitochondrial energetics¹⁵⁵; 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.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.^{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 ϵ -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.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

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 al¹⁴⁰.)

mitochondria, and act as “support staff” for ROS processing.¹⁶⁰ Mouse models of peroxisomal disorders result in mitochondrial and ER dysfunction.¹⁶¹ Furthermore, cytokines such as tumor necrosis factor- α can reduce peroxisomal number and function, rendering cells even more vulnerable.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.¹⁶³ Furthermore, an *in vivo* study in antioxidant-deficient mice demonstrated that intrahepatic lipid toxicity and hepatocellular death occurred after sucrose administration.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.¹⁶⁵ Accumulation of ROS and misfolded proteins within the ER activates the UPR,¹⁶⁶ designed to decrease protein synthesis to allow for their clearance.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.¹⁶⁸ ER stress in the liver is a specific mechanism of hepatic injury in NAFLD,¹⁶⁹ and ER stress in the pancreas reduces β -cell number and promotes diabetes.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.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 glyce-mic load, which is most easily accomplished by increasing the fiber content of food.¹⁷² Furthermore, lipogenesis and hepatic lipid export can also be reduced with improved dietary fiber intake.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

FIGURE 4

Intracellular ROS formation and consequences of fructose metabolism. Dietary fructose, because of its metabolic processing in the mitochondria and the fructosylation of protein ϵ -amino groups via the Maillard reaction, and circulating inflammatory cytokines, because of their receptor-mediated activation of NADPH oxidase, increase intracellular levels of ROS. In the absence of sufficient peroxisomal quenching and degradation, the ROS moieties lead to the UPR response, causing either cell death (apoptosis) or cellular/metabolic dysfunction. The formation of acetyl-CoA also leads to lipid deposition and IR through the activation of inflammatory pathways as described in the text. (Adapted from Parola and Marra¹⁵³.) NADPH, nicotinamide adenine dinucleotide phosphate; pSer-IRS-1, serine phosphorylated IRS-1.

the transcription factor peroxisome proliferator-activated receptor γ coactivator-1 α , exercise causes mitochondrial biogenesis in liver and muscles.¹⁷⁴ The age of the mitochondria is relevant because new mitochondria are more efficient, generating fewer ROS.¹⁷⁵ Exercise

also burns acetyl-CoA and prevents the buildup of fatty acids, which improves insulin sensitivity in the liver and muscles.¹⁷⁶

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.

REFERENCES

1. Chan JM, Rimm EB, Colditz GA, Stampfer MJ, Willett WC. Obesity, fat distribution, and weight gain as risk factors for clinical diabetes in men. *Diabetes Care*. 1994; 17(9):961–969
2. Wildman RP. Healthy obesity. *Curr Opin Clin Nutr Metab Care*. 2009;12(4):438–443
3. Conus F, Rabasa-Lhoret R, Péronnet F. Characteristics of metabolically obese normal-weight (MONW) subjects. *Appl Physiol Nutr Metab*. 2007;32(1):4–12
4. Ruderman N, Chisholm D, Pi-Sunyer X, Schneider S. The metabolically obese, normal-weight individual revisited. *Diabetes*. 1998;47(5):699–713
5. Abbasi F, Brown BW, Jr, Lamendola C, McLaughlin T, Reaven GM. Relationship between obesity, insulin resistance, and coronary heart disease risk. *J Am Coll Cardiol*. 2002;40(5):937–943
6. Després JP, Lemieux S, Lamarche B, et al. The insulin resistance-dyslipidemic syndrome: contribution of visceral obesity and therapeutic implications. *Int J Obes Relat Metab Disord*. 1995;19(suppl 1):S76–S86
7. Després JP. Is visceral obesity the cause of the metabolic syndrome? *Ann Med*. 2006;38(1):52–63
8. Fox CS, Massaro JM, Hoffmann U, et al. Abdominal visceral and subcutaneous adipose tissue compartments: association with metabolic risk factors in the Framingham Heart Study. *Circulation*. 2007; 116(1):39–48

9. Porter SA, Massaro JM, Hoffmann U, Vasan RS, O'Donnel CJ, Fox CS. Abdominal subcutaneous adipose tissue: a protective fat depot? *Diabetes Care*. 2009;32(6):1068–1075
10. Carr DB, Utzschneider KM, Hull RL, et al. Intra-abdominal fat is a major determinant of the National Cholesterol Education Program Adult Treatment Panel III criteria for the metabolic syndrome. *Diabetes*. 2004;53(8):2087–2094
11. Fabbrini E, Magkos F, Mohammed BS, et al. Intrahepatic fat, not visceral fat, is linked with metabolic complications of obesity. *Proc Natl Acad Sci USA*. 2009;106(36):15430–15435
12. Cali AM, De Oliveira AM, Kim H, et al. Glucose dysregulation and hepatic steatosis in obese adolescents: is there a link? *Hepatology*. 2009;49(6):1896–1903
13. DeFronzo RA, Ferrannini E. Insulin resistance. A multifaceted syndrome responsible for NIDDM, obesity, hypertension, dyslipidemia, and atherosclerotic cardiovascular disease. *Diabetes Care*. 1991;14(3):173–194
14. Haffner SM, Valdez RA, Hazuda HP, Mitchell BD, Morales PA, Stern MP. Prospective analysis of the insulin-resistance syndrome (syndrome X). *Diabetes*. 1992;41(6):715–722
15. Balkau B, Charles MA; European Group for the Study of Insulin Resistance (EGIR). Comment on the provisional report from the WHO consultation. *Diabet Med*. 1999;16(5):442–443
16. Kylin E. Studien über das Hypertonie-Hyperglykämie-Hyperurikämie-Syndrom. *Zentralbl Inn Med*. 1923;44:105–127
17. Albrink MJ, Krauss RM, Lindgren FT, von der Groeben J, Pan S, Wood PD. Inter-correlations among plasma high density lipoprotein, obesity and triglycerides in a normal population. *Lipids*. 1980;15(9):668–676
18. Després JP. Abdominal obesity as important component of insulin-resistance syndrome. *Nutrition*. 1993;9(5):452–459
19. Reaven GM. Banting lecture 1988. Role of insulin resistance in human disease. *Diabetes*. 1988;37(12):1595–1607
20. Luciano A, Zoppi G. Blood insulin values after the oral glucose tolerance test (OGTT) and the body composition in 30 obese children. *Pediatr Med Chir*. 1994;16(5):471–473
21. Biloft CA, Muir A. The metabolic syndrome in children and adolescents: a clinician's guide. *Adolesc Med State Art Rev*. 2009;20(1):109–120
22. Allison DB, Kaprio J, Korkeila M, Koskenvuo M, Neale MC, Hayakawa K. The heritability of body mass index among an international sample of monozygotic twins reared apart. *Int J Obes Relat Metab Disord*. 1996;20(6):501–506
23. Povel CM, Boer JM, Reiling E, Feskens EJ. Genetic variants and the metabolic syndrome: a systematic review. *Obes Rev*. 2011;12(11):952–967
24. Monda KL, North KE, Hunt SC, Rao DC, Province MA, Kraja AT. The genetics of obesity and the metabolic syndrome. *Endocr Metab Immune Disord Drug Targets*. 2010;10(2):86–108
25. Chen Y, Zhu J, Lum PY, et al. Variations in DNA elucidate molecular networks that cause disease. *Nature*. 2008;452(7186):429–435
26. Lusis AJ, Attie AD, Reue K. Metabolic syndrome: from epidemiology to systems biology. *Nat Rev Genet*. 2008;9(11):819–830
27. Sebert S, Sharkey D, Budge H, Symonds ME. The early programming of metabolic health: is epigenetic setting the missing link? *Am J Clin Nutr*. 2011;94(suppl 6):1953S–1958S
28. Romeo S, Kozlitina J, Xing C, et al. Genetic variation in PNPLA3 confers susceptibility to nonalcoholic fatty liver disease. *Nat Genet*. 2008;40(12):1461–1465
29. Huang Y, He S, Li JZ, et al. A feed-forward loop amplifies nutritional regulation of PNPLA3. *Proc Natl Acad Sci USA*. 2010;107(17):7892–7897
30. Kantartzis K, Peter A, Machicao F, et al. Dissociation between fatty liver and insulin resistance in humans carrying a variant of the patatin-like phospholipase 3 gene. *Diabetes*. 2009;58(11):2616–2623
31. Pacini G. The hyperbolic equilibrium between insulin sensitivity and secretion. *Nutr Metab Cardiovasc Dis*. 2006;16(suppl 1):S22–S27
32. Chiarelli F, Marcovecchio ML. Insulin resistance and obesity in childhood. *Eur J Endocrinol*. 2008;159(suppl 1):S67–S74
33. Bergman RN. Lilly lecture 1989. Toward physiological understanding of glucose tolerance. Minimal-model approach. *Diabetes*. 1989;38(12):1512–1527
34. Cornier MA, Dabelea D, Hernandez TL, et al. The metabolic syndrome. *Endocr Rev*. 2008;29(7):777–822
35. Poretsky L. On the paradox of insulin-induced hyperandrogenism in insulin-resistant states. *Endocr Rev*. 1991;12(1):3–13
36. McGarry JD. Banting lecture 2001: dysregulation of fatty acid metabolism in the etiology of type 2 diabetes. *Diabetes*. 2002;51(1):7–18
37. Brown MS, Goldstein JL. Selective versus total insulin resistance: a pathogenic paradox. *Cell Metab*. 2008;7(2):95–96
38. Matsumoto M, Han S, Kitamura T, Accili D. Dual role of transcription factor FoxO1 in controlling hepatic insulin sensitivity and lipid metabolism. *J Clin Invest*. 2006;116(9):2464–2472
39. Brown MS, Goldstein JL. The SREBP pathway: regulation of cholesterol metabolism by proteolysis of a membrane-bound transcription factor. *Cell*. 1997;89(3):331–340
40. Horton JD, Goldstein JL, Brown MS. SREBPs: activators of the complete program of cholesterol and fatty acid synthesis in the liver. *J Clin Invest*. 2002;109(9):1125–1131
41. Goldberg IJ. Lipoprotein lipase and lipolysis: central roles in lipoprotein metabolism and atherogenesis. *J Lipid Res*. 1996;37(4):693–707
42. Lewis GF, Uffelman KD, Szeto LW, Steiner G. Effects of acute hyperinsulinemia on VLDL triglyceride and VLDL apoB production in normal weight and obese individuals. *Diabetes*. 1993;42(6):833–842
43. Bergman RN, Kim SP, Hsu IR, et al. Abdominal obesity: role in the pathophysiology of metabolic disease and cardiovascular risk. *Am J Med*. 2007;120(2 suppl 1):S3–S8
44. McGarry JD. Malonyl-CoA and carnitine palmitoyltransferase I: an expanding partnership. *Biochem Soc Trans*. 1995;23(3):481–485
45. McGarry JD. The mitochondrial carnitine palmitoyltransferase system: its broadening role in fuel homeostasis and new insights into its molecular features. *Biochem Soc Trans*. 1995;23(2):321–324
46. McGarry JD, Brown NF. The mitochondrial carnitine palmitoyltransferase system. From concept to molecular analysis. *Eur J Biochem*. 1997;244(1):1–14
47. Ginsberg HN, Zhang YL, Hernandez-Ono A. Regulation of plasma triglycerides in insulin resistance and diabetes. *Arch Med Res*. 2005;36(3):232–240
48. Shulman GI. Cellular mechanisms of insulin resistance. *J Clin Invest*. 2000;106(2):171–176
49. Petersen KF, Shulman GI. Etiology of insulin resistance. *Am J Med*. 2006;119(5 suppl 1):S10–S16
50. Samuel VT, Petersen KF, Shulman GI. Lipid-induced insulin resistance: unravelling the mechanism. *Lancet*. 2010;375(9733):2267–2277

51. Ginsberg HN. Insulin resistance and cardiovascular disease. *J Clin Invest.* 2000;106(4):453–458
52. Eckel RH, Grundy SM, Zimmet PZ. The metabolic syndrome. *Lancet.* 2005;365(9468):1415–1428
53. Randle PJ, Garland PB, Hales CN, Newsholme EA. The glucose fatty-acid cycle. Its role in insulin sensitivity and the metabolic disturbances of diabetes mellitus. *Lancet.* 1963;1(7285):785–789
54. Randle PJ. Regulatory interactions between lipids and carbohydrates: the glucose fatty acid cycle after 35 years. *Diabetes Metab Rev.* 1998;14(4):263–283
55. Kahn BB, Flier JS. Obesity and insulin resistance. *J Clin Invest.* 2000;106(4):473–481
56. Large V, Arner P. Regulation of lipolysis in humans. Pathophysiological modulation in obesity, diabetes, and hyperlipidaemia. *Diabetes Metab.* 1998;24(5):409–418
57. Gustafson B, Hammarstedt A, Andersson CX, Smith U. Inflamed adipose tissue: a culprit underlying the metabolic syndrome and atherosclerosis. *Arterioscler Thromb Vasc Biol.* 2007;27(11):2276–2283
58. Heilbronn LK, Campbell LV. Adipose tissue macrophages, low grade inflammation and insulin resistance in human obesity. *Curr Pharm Des.* 2008;14(12):1225–1230
59. Bremer AA, Devaraj S, Afify A, Jialal I. Adipose tissue dysregulation in patients with metabolic syndrome. *J Clin Endocrinol Metab.* 2011;96(11):E1782–E1788
60. Skurk T, Alberti-Huber C, Herder C, Hauner H. Relationship between adipocyte size and adipokine expression and secretion. *J Clin Endocrinol Metab.* 2007;92(3):1023–1033
61. Sopasakis VR, Sandqvist M, Gustafson B, et al. High local concentrations and effects on differentiation implicate interleukin-6 as a paracrine regulator. *Obes Res.* 2004;12(3):454–460
62. Hube F, Hauner H. The role of TNF-alpha in human adipose tissue: prevention of weight gain at the expense of insulin resistance? *Horm Metab Res.* 1999;31(12):626–631
63. Schachl DS, Kipnis DM. Abnormalities in carbohydrate tolerance associated with elevated plasma nonesterified fatty acids. *J Clin Invest.* 1965;44(12):2010–2020
64. Boden G, Chen X, Ruiz J, White JV, Rossetti L. Mechanisms of fatty acid-induced inhibition of glucose uptake. *J Clin Invest.* 1994;93(6):2438–2446
65. Dresner A, Laurent D, Marcucci M, et al. Effects of free fatty acids on glucose transport and IRS-1-associated phosphatidylinositol 3-kinase activity. *J Clin Invest.* 1999;103(2):253–259
66. Roden M, Krssak M, Stingl H, et al. Rapid impairment of skeletal muscle glucose transport/phosphorylation by free fatty acids in humans. *Diabetes.* 1999;48(2):358–364
67. Krssak M, Falk Petersen K, Dresner A, et al. Intramyocellular lipid concentrations are correlated with insulin sensitivity in humans: a ¹H NMR spectroscopy study. *Diabetologia.* 1999;42(1):113–116
68. Petersen KF, Dufour S, Savage DB, et al. The role of skeletal muscle insulin resistance in the pathogenesis of the metabolic syndrome. *Proc Natl Acad Sci USA.* 2007;104(31):12587–12594
69. Centers for Disease Control. Trends in intake of energy and macronutrients - United States. *Morb Mortal Wkly Rep.* 2004;53(4):80–82
70. Muoio DM, Newgard CB. Obesity-related derangements in metabolic regulation. *Annu Rev Biochem.* 2006;75:367–401
71. Bonnard C, Durand A, Peyrol S, et al. Mitochondrial dysfunction results from oxidative stress in the skeletal muscle of diet-induced insulin-resistant mice. *J Clin Invest.* 2008;118(2):789–800
72. Zivkovic AM, German JB, Sanyal AJ. Comparative review of diets for the metabolic syndrome: implications for nonalcoholic fatty liver disease. *Am J Clin Nutr.* 2007;86(2):285–300
73. Cave M, Deaciuc I, Mendez C, et al. Non-alcoholic fatty liver disease: predisposing factors and the role of nutrition. *J Nutr Biochem.* 2007;18(3):184–195
74. Chanmugam P, Guthrie JF, Cecilio S, Morton JF, Basiotis PP, Anand R. Did fat intake in the United States really decline between 1989-1991 and 1994-1996? *J Am Diet Assoc.* 2003;103(7):867–872
75. York LW, Puthalapattu S, Wu GY. Non-alcoholic fatty liver disease and low-carbohydrate diets. *Annu Rev Nutr.* 2009;29:365–379
76. Lee JY, Sohn KH, Rhee SH, Hwang D. Saturated fatty acids, but not unsaturated fatty acids, induce the expression of cyclooxygenase-2 mediated through Toll-like receptor 4. *J Biol Chem.* 2001;276(20):16683–16689
77. Willett WC. The great fat debate: total fat and health. *J Am Diet Assoc.* 2011;111(5):660–662
78. Kris-Etherton PM, Harris WS, Appel LJ; American Heart Association. Nutrition Committee. Fish consumption, fish oil, omega-3 fatty acids, and cardiovascular disease. *Circulation.* 2002;106(21):2747–2757
79. Assy N, Nassar F, Nasser G, Grosovski M. Olive oil consumption and non-alcoholic fatty liver disease. *World J Gastroenterol.* 2009;15(15):1809–1815
80. Nagao K, Inoue N, Wang YM, Shirouchi B, Yanagita T. Dietary conjugated linoleic acid alleviates nonalcoholic fatty liver disease in Zucker (fa/fa) rats. *J Nutr.* 2005;135(1):9–13
81. Cussons AJ, Watts GF, Mori TA, Stuckey BG. Omega-3 fatty acid supplementation decreases liver fat content in polycystic ovary syndrome: a randomized controlled trial employing proton magnetic resonance spectroscopy. *J Clin Endocrinol Metab.* 2009;94(10):3842–3848
82. Zandbergen F, Plutzky J. PPARalpha in atherosclerosis and inflammation. *Biochim Biophys Acta.* 2007;1771(8):972–982
83. Rasooly R, Kelley DS, Greg J, Mackey BE. Dietary trans 10, cis 12-conjugated linoleic acid reduces the expression of fatty acid oxidation and drug detoxification enzymes in mouse liver. *Br J Nutr.* 2007;97(1):58–66
84. Tetri LH, Basaranoglu M, Brunt EM, Yeran LM, Neuschwander-Tetri BA. Severe NAFLD with hepatic necroinflammatory changes in mice fed trans fats and a high-fructose corn syrup equivalent. *Am J Physiol Gastrointest Liver Physiol.* 2008;295(5):G987–G995
85. Dorfman SE, Laurent D, Gounarides JS, et al. Metabolic implications of dietary trans-fatty acids. *Obesity (Silver Spring).* 2009;17(6):1200–1207
86. Layman DK, Walker DA. Potential importance of leucine in treatment of obesity and the metabolic syndrome. *J Nutr.* 2006;136(suppl 1):319S–323S
87. Newgard CB, An J, Bain JR, et al. A branched-chain amino acid-related metabolic signature that differentiates obese and lean humans and contributes to insulin resistance. *Cell Metab.* 2009;9(4):311–326
88. Higuchi N, Kato M, Miyazaki M, et al. Potential role of branched-chain amino acids in glucose metabolism through the accelerated induction of the glucose-sensing apparatus in the liver. *J Cell Biochem.* 2011;112(1):30–38
89. Patti ME, Brambilla E, Luzi L, Landaker EJ, Kahn CR. Bidirectional modulation of insulin action by amino acids. *J Clin Invest.* 1998;101(7):1519–1529
90. Baum JI, O'Connor JC, Seyler JE, Anthony TG, Freund GG, Layman DK. Leucine reduces the duration of insulin-induced PI 3-kinase activity in rat skeletal muscle.

- Am J Physiol Endocrinol Metab.* 2005;288(1):E86–E91
91. Chotechuang N, Azzout-Marniche D, Bos C, et al. mTOR, AMPK, and GCN2 coordinate the adaptation of hepatic energy metabolic pathways in response to protein intake in the rat. *Am J Physiol Endocrinol Metab.* 2009;297(6):E1313–E1323
 92. Um SH, D'Alessio D, Thomas G. Nutrient overload, insulin resistance, and ribosomal protein S6 kinase 1, S6K1. *Cell Metab.* 2006;3(6):393–402
 93. Eaton S. Control of mitochondrial beta-oxidation flux. *Prog Lipid Res.* 2002;41(3):197–239
 94. Harper AE, Miller RH, Block KP. Branched-chain amino acid metabolism. *Annu Rev Nutr.* 1984;4:409–454
 95. Krebs M, Krssak M, Bernroider E, et al. Mechanism of amino acid-induced skeletal muscle insulin resistance in humans. *Diabetes.* 2002;51(3):599–605
 96. Krebs M, Brehm A, Krssak M, et al. Direct and indirect effects of amino acids on hepatic glucose metabolism in humans. *Diabetologia.* 2003;46(7):917–925
 97. She P, Van Horn C, Reid T, Hutson SM, Cooney RN, Lynch CJ. Obesity-related elevations in plasma leucine are associated with alterations in enzymes involved in branched-chain amino acid metabolism. *Am J Physiol Endocrinol Metab.* 2007;293(6):E1552–E1563
 98. Herman MA, She P, Peroni OD, Lynch CJ, Kahn BB. Adipose tissue branched chain amino acid (BCAA) metabolism modulates circulating BCAA levels. *J Biol Chem.* 2010;285(15):11348–11356
 99. Floyd JC, Jr, Fajans SS, Conn JW, Knopf RF, Rull J. Stimulation of insulin secretion by amino acids. *J Clin Invest.* 1966;45(9):1487–1502
 100. Wang TJ, Larson MG, Vasan RS, et al. Metabolite profiles and the risk of developing diabetes. *Nat Med.* 2011;17(4):448–453
 101. Tai ES, Tan ML, Stevens RD, et al. Insulin resistance is associated with a metabolic profile of altered protein metabolism in Chinese and Asian-Indian men. *Diabetologia.* 2010;53(4):757–767
 102. Facchini F, Chen YD, Reaven GM. Light-to-moderate alcohol intake is associated with enhanced insulin sensitivity. *Diabetes Care.* 1994;17(2):115–119
 103. Di Castelnuovo A, Costanzo S, di Giuseppe R, de Gaetano G, Iacoviello L. Alcohol consumption and cardiovascular risk: mechanisms of action and epidemiologic perspectives. *Future Cardiol.* 2009;5(5):467–477
 104. Athyros VG, Liberopoulos EN, Mikhailidis DP, et al. Association of drinking pattern and alcohol beverage type with the prevalence of metabolic syndrome, diabetes, coronary heart disease, stroke, and peripheral arterial disease in a Mediterranean cohort. *Angiology.* 2007;58(6):689–697
 105. Sakurai Y, Umeda T, Shinchi K, et al. Relation of total and beverage-specific alcohol intake to body mass index and waist-to-hip ratio: a study of self-defense officials in Japan. *Eur J Epidemiol.* 1997;13(8):893–898
 106. Baik I, Shin C. Prospective study of alcohol consumption and metabolic syndrome. *Am J Clin Nutr.* 2008;87(5):1455–1463
 107. Lustig RH. Fructose: metabolic, hedonic, and societal parallels with ethanol. *J Am Diet Assoc.* 2010;110(9):1307–1321
 108. Dey A, Cederbaum AI. Alcohol and oxidative liver injury. *Hepatology.* 2006;43(2 suppl 1):S63–S74
 109. García-Villafranca J, Guillén A, Castro J. Ethanol consumption impairs regulation of fatty acid metabolism by decreasing the activity of AMP-activated protein kinase in rat liver. *Biochimie.* 2008;90(3):460–466
 110. Dreyer C, Keller H, Mahfoudi A, Laudet V, Krey G, Wahli W. Positive regulation of the peroxisomal beta-oxidation pathway by fatty acids through activation of peroxisome proliferator-activated receptors (PPAR). *Biol Cell.* 1993;77(1):67–76
 111. Sozio M, Crabb DW. Alcohol and lipid metabolism. *Am J Physiol Endocrinol Metab.* 2008;295(1):E10–E16
 112. Steinberg D, Pearson TA, Kuller LH. Alcohol and atherosclerosis. *Ann Intern Med.* 1991;114(11):967–976
 113. Suter PM, Schutz Y. The effect of exercise, alcohol or both combined on health and physical performance. *Int J Obes (Lond).* 2008;32(suppl 6):S48–S52
 114. Schneider J, Tesdorpf M, Kaffarnik H, Hausmann L, Zöfel P, Zilliken F. Alteration of plasma lipids and intermediates of lipid metabolism in healthy fasting volunteers by ethanol and fructose. *Res Exp Med (Berl).* 1976;167(2):159–170
 115. Lee YJ, Aroor AR, Shukla SD. Temporal activation of p42/44 mitogen-activated protein kinase and c-Jun N-terminal kinase by acetaldehyde in rat hepatocytes and its loss after chronic ethanol exposure. *J Pharmacol Exp Ther.* 2002;301(3):908–914
 116. You M, Crabb DW. Molecular mechanisms of alcoholic fatty liver: role of sterol regulatory element-binding proteins. *Alcohol.* 2004;34(1):39–43
 117. Crabb DW, Galli A, Fischer M, You M. Molecular mechanisms of alcoholic fatty liver: role of peroxisome proliferator-activated receptor alpha. *Alcohol.* 2004;34(1):35–38
 118. Guzmán M, Castro J. Alterations in the regulatory properties of hepatic fatty acid oxidation and carnitine palmitoyl-transferase I activity after ethanol feeding and withdrawal. *Alcohol Clin Exp Res.* 1990;14(3):472–477
 119. Yokoyama H, Hiroshi H, Ohgo H, Hibi T, Saito I. Effects of excessive ethanol consumption on the diagnosis of the metabolic syndrome using its clinical diagnostic criteria. *Intern Med.* 2007;46(17):1345–1352
 120. Onishi Y, Honda M, Ogihara T, et al. Ethanol feeding induces insulin resistance with enhanced PI 3-kinase activation. *Biochem Biophys Res Commun.* 2003;303(3):788–794
 121. Marriott BP, Olsho L, Hadden L, Connor P. Intake of added sugars and selected nutrients in the United States, National Health and Nutrition Examination Survey (NHANES) 2003–2006. *Crit Rev Food Sci Nutr.* 2010;50(3):228–258
 122. Vos MB, Kimmons JE, Gillespie C, Welsh J, Blanck HM. Dietary fructose consumption among US children and adults: the Third National Health and Nutrition Examination Survey. *Medscape J Med.* 2008;10(7):160
 123. Stanhope KL, Havel PJ. Endocrine and metabolic effects of consuming beverages sweetened with fructose, glucose, sucrose, or high-fructose corn syrup. *Am J Clin Nutr.* 2008;88(6):1733S–1737S
 124. Abraha A, Humphreys SM, Clark ML, Matthews DR, Frayn KN. Acute effect of fructose on postprandial lipaemia in diabetic and non-diabetic subjects. *Br J Nutr.* 1998;80(2):169–175
 125. Cohen JC, Schall R. Reassessing the effects of simple carbohydrates on the serum triglyceride responses to fat meals. *Am J Clin Nutr.* 1988;48(4):1031–1034
 126. Teff KL, Elliott SS, Tschöp M, et al. Dietary fructose reduces circulating insulin and leptin, attenuates postprandial suppression of ghrelin, and increases triglycerides in women. *J Clin Endocrinol Metab.* 2004;89(6):2963–2972
 127. Stanhope KL, Griffen SC, Bair BR, Swarbrick MM, Keim NL, Havel PJ. Twenty-four-hour endocrine and metabolic profiles following consumption of high-fructose corn syrup, sucrose, fructose, and glucose-sweetened

- beverages with meals. *Am J Clin Nutr*. 2008; 87(5):1194–1203
128. Swarbrick MM, Stanhope KL, Elliott SS, et al. Consumption of fructose-sweetened beverages for 10 weeks increases postprandial triacylglycerol and apolipoprotein-B concentrations in overweight and obese women. *Br J Nutr*. 2008;100(5):947–952
 129. Havel PJ. Dietary fructose: implications for dysregulation of energy homeostasis and lipid/carbohydrate metabolism. *Nutr Rev*. 2005;63(5):133–157
 130. Bray GA, Nielsen SJ, Popkin BM. Consumption of high-fructose corn syrup in beverages may play a role in the epidemic of obesity. *Am J Clin Nutr*. 2004;79(4):537–543
 131. Malik VS, Schulze MB, Hu FB. Intake of sugar-sweetened beverages and weight gain: a systematic review. *Am J Clin Nutr*. 2006;84(2):274–288
 132. Stanhope KL, Havel PJ. Fructose consumption: potential mechanisms for its effects to increase visceral adiposity and induce dyslipidemia and insulin resistance. *Curr Opin Lipidol*. 2008;19(1):16–24
 133. Stanhope KL, Schwarz JM, Keim NL, et al. Consuming fructose-sweetened, not glucose-sweetened, beverages increases visceral adiposity and lipids and decreases insulin sensitivity in overweight/obese humans. *J Clin Invest*. 2009;119(5):1322–1334
 134. Welsh JA, Sharma A, Abramson JL, Vaccarino V, Gillespie C, Vos MB. Caloric sweetener consumption and dyslipidemia among US adults. *JAMA*. 2010;303(15):1490–1497
 135. Malik VS, Popkin BM, Bray GA, Després JP, Hu FB. Sugar-sweetened beverages, obesity, type 2 diabetes mellitus, and cardiovascular disease risk. *Circulation*. 2010; 121(11):1356–1364
 136. Bremer AA, Auinger P, Byrd RS. Relationship between insulin resistance-associated metabolic parameters and anthropometric measurements with sugar-sweetened beverage intake and physical activity levels in US adolescents: findings from the 1999–2004 National Health and Nutrition Examination Survey. *Arch Pediatr Adolesc Med*. 2009;163(4):328–335
 137. Thuy S, Ladurner R, Volynets V, et al. Nonalcoholic fatty liver disease in humans is associated with increased plasma endotoxin and plasminogen activator inhibitor 1 concentrations and with fructose intake. *J Nutr*. 2008;138(8):1452–1455
 138. Collison KS, Saleh SM, Bakheet RH, et al. Diabetes of the liver: the link between nonalcoholic fatty liver disease and HFCS-55. *Obesity (Silver Spring)*. 2009;17(11):2003–2013
 139. Lê KA, Ith M, Kreis R, et al. Fructose overconsumption causes dyslipidemia and ectopic lipid deposition in healthy subjects with and without a family history of type 2 diabetes. *Am J Clin Nutr*. 2009;89(6):1760–1765
 140. Lim JS, Mietus-Snyder M, Valente A, Schwarz JM, Lustig RH. The role of fructose in the pathogenesis of NAFLD and the metabolic syndrome. *Nat Rev Gastroenterol Hepatol*. 2010;7(5):251–264
 141. Tappy L, Lê KA. Metabolic effects of fructose and the worldwide increase in obesity. *Physiol Rev*. 2010;90(1):23–46
 142. Bjorkman O, Crump M, Phillips RW. Intestinal metabolism of orally administered glucose and fructose in Yucatan miniature swine. *J Nutr*. 1984;114(8):1413–1420
 143. Nagai Y, Yonemitsu S, Erion DM, et al. The role of peroxisome proliferator-activated receptor gamma coactivator-1 beta in the pathogenesis of fructose-induced insulin resistance. *Cell Metab*. 2009;9(3):252–264
 144. Faeh D, Minehira K, Schwarz JM, Periasamy R, Park S, Tappy L. Effect of fructose overfeeding and fish oil administration on hepatic de novo lipogenesis and insulin sensitivity in healthy men. *Diabetes*. 2005; 54(7):1907–1913
 145. Matsuzaka T, Shimano H, Yahagi N, et al. Insulin-independent induction of sterol regulatory element-binding protein-1c expression in the livers of streptozotocin-treated mice. *Diabetes*. 2004;53(3):560–569
 146. Nagai Y, Nishio Y, Nakamura T, Maegawa H, Kikkawa R, Kashiwagi A. Amelioration of high fructose-induced metabolic derangements by activation of PPARalpha. *Am J Physiol Endocrinol Metab*. 2002;282(5): E1180–E1190
 147. Wei Y, Wang D, Pagliassotti MJ. Fructose selectively modulates c-jun N-terminal kinase activity and insulin signaling in rat primary hepatocytes. *J Nutr*. 2005;135(7): 1642–1646
 148. Wei Y, Wang D, Topczewski F, Pagliassotti MJ. Fructose-mediated stress signaling in the liver: implications for hepatic insulin resistance. *J Nutr Biochem*. 2007; 18(1):1–9
 149. Hirosumi J, Tuncman G, Chang L, et al. A central role for JNK in obesity and insulin resistance. *Nature*. 2002;420(6913):333–336
 150. Morino K, Petersen KF, Shulman GI. Molecular mechanisms of insulin resistance in humans and their potential links with mitochondrial dysfunction. *Diabetes*. 2006; 55(suppl 2):S9–S15
 151. Teff KL, Grudziak J, Townsend RR, et al. Endocrine and metabolic effects of consuming fructose- and glucose-sweetened beverages with meals in obese men and women: influence of insulin resistance on plasma triglyceride responses. *J Clin Endocrinol Metab*. 2009;94(5):1562–1569
 152. Civitarese AE, Ravussin E. Mitochondrial energetics and insulin resistance. *Endocrinology*. 2008;149(3):950–954
 153. Parola M, Marra F. Adipokines and redox signaling: impact on fatty liver disease. *Antioxid Redox Signal*. 2011;15(2):461–483
 154. Subasinghe W, Syed I, Kowluru A. Phagocyte-like NADPH oxidase promotes cytokine-induced mitochondrial dysfunction in pancreatic β -cells: evidence for regulation by Rac1. *Am J Physiol Regul Integr Comp Physiol*. 2011;300(1):R12–R20
 155. Turrens JF. Mitochondrial formation of reactive oxygen species. *J Physiol*. 2003; 552(pt 2):335–344
 156. Fan X, Hussien R, Brooks GA. H2O2-induced mitochondrial fragmentation in C2C12 myocytes. *Free Radic Biol Med*. 2010;49(11):1646–1654
 157. Malhotra JD, Kaufman RJ. Endoplasmic reticulum stress and oxidative stress: a vicious cycle or a double-edged sword? *Antioxid Redox Signal*. 2007;9(12):2277–2293
 158. de Ferranti S, Mozaffarian D. The perfect storm: obesity, adipocyte dysfunction, and metabolic consequences. *Clin Chem*. 2008; 54(6):945–955
 159. Bunn HF, Higgins PJ. Reaction of monosaccharides with proteins: possible evolutionary significance. *Science*. 1981;213(4504):222–224
 160. Thoms S, Grønborg S, Gärtner J. Organelle interplay in peroxisomal disorders. *Trends Mol Med*. 2009;15(7):293–302
 161. Dirix R, Vanhorebeek I, Martens K, et al. Absence of peroxisomes in mouse hepatocytes causes mitochondrial and ER abnormalities. *Hepatology*. 2005;41(4): 868–878
 162. Schrader M, Fahimi HD. Peroxisomes and oxidative stress. *Biochim Biophys Acta*. 2006;1763(12):1755–1766
 163. Lee O, Bruce WR, Dong Q, Bruce J, Mehta R, O'Brien PJ. Fructose and carbonyl metabolites as endogenous toxins. *Chem Biol Interact*. 2009;178(1-3):332–339
 164. Pickens MK, Yan JS, Ng RK, et al. Dietary sucrose is essential to the development of liver injury in the methionine-choline-deficient model of steatohepatitis. *J Lipid Res*. 2009;50(10):2072–2082

165. Kapoor A, Sanyal AJ. Endoplasmic reticulum stress and the unfolded protein response. *Clin Liver Dis.* 2009;13(4):581–590
166. Merksamer PI, Trusina A, Papa FR. Real-time redox measurements during endoplasmic reticulum stress reveal interlinked protein folding functions. *Cell.* 2008;135(5):933–947
167. Haynes CM, Titus EA, Cooper AA. Degradation of misfolded proteins prevents ER-derived oxidative stress and cell death. *Mol Cell.* 2004;15(5):767–776
168. Santos CX, Tanaka LY, Wosniak J, Laurindo FR. Mechanisms and implications of reactive oxygen species generation during the unfolded protein response: roles of endoplasmic reticulum oxidoreductases, mitochondrial electron transport, and NADPH oxidase. *Antioxid Redox Signal.* 2009;11(10):2409–2427
169. Mollica MP, Lionetti L, Putti R, Cavaliere G, Gaita M, Barletta A. From chronic over-feeding to hepatic injury: role of endoplasmic reticulum stress and inflammation. *Nutr Metab Cardiovasc Dis.* 2011;21(3):222–230
170. Hummasti S, Hotamisligil GS. Endoplasmic reticulum stress and inflammation in obesity and diabetes. *Circ Res.* 2010;107(5):579–591
171. Yki-Järvinen H. Nutritional modulation of nonalcoholic fatty liver disease and insulin resistance: human data. *Curr Opin Clin Nutr Metab Care.* 2010;13(6):709–714
172. Chandalia M, Garg A, Lutjohann D, von Bergmann K, Grundy SM, Brinkley LJ. Beneficial effects of high dietary fiber intake in patients with type 2 diabetes mellitus. *N Engl J Med.* 2000;342(19):1392–1398
173. Brynes AE, Mark Edwards C, Ghatei MA, et al. A randomised four-intervention crossover study investigating the effect of carbohydrates on daytime profiles of insulin, glucose, non-esterified fatty acids and triacylglycerols in middle-aged men. *Br J Nutr.* 2003;89(2):207–218
174. Lira VA, Benton CR, Yan Z, Bonen A. PGC-1alpha regulation by exercise training and its influences on muscle function and insulin sensitivity. *Am J Physiol Endocrinol Metab.* 2010;299(2):E145–E161
175. Starkov AA. The role of mitochondria in reactive oxygen species metabolism and signaling. *Ann N Y Acad Sci.* 2008;1147:37–52
176. Hannukainen JC, Borra R, Linderborg K, et al. Liver and pancreatic fat content and metabolism in healthy monozygotic twins with discordant physical activity. *J Hepatol.* 2011;54(3):545–552

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