Nonalcoholic Fatty Liver Disease and Insulin Resistance in Youth

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This is the second in a series of four articles on presentations at the World Congress on the insulin resistance syndrome (IRS), reviewing the relationship between insulin resistance and nonalcoholic fatty liver disease, and aspects of insulin resistance in children and adolescents.

Nonalcoholic fatty liver disease

Arun Sanyal (Richmond, VA) reviewed the spectrum of disorders constituting nonalcoholic fatty liver disease (NAFLD), from fatty liver to nonalcoholic steatohepatitis (NASH). Analysis of liver fat content in steatosis, mainly di- and triglycerides, shows fatty acids derived from circulation as well as fatty acid synthesized de novo from Acetyl-CoA, derived from glucose, acetate, and oxidation of circulating fatty acids, ~60% are from plasma free fatty acids (FFAs), 15% from dietary sources, and 25% from de novo lipogenesis. The latter process is controlled by sterol regulatory element–binding protein (SREBP)1c, which is induced by overfeeding, an effect of insulin and the nuclear liver X receptor, to facilitate conversion of glucose to fatty acids and triglycerides for the storage. SREBP-1c acts at the levels of transcription and of cleavage from the endoplasmic reticulum. NAFLD is associated with depletion of polyunsaturated fatty acids, particularly γ-linoleic acid, but with increased overall availability of fatty acids and reduced fatty acid oxidation, although there is no specific abnormality in fatty acid β-oxidative capacity. Thus, the condition appears to be a function of increased fat accumulation from importation and synthesis, with reduced export. Transcriptional regulators include liver X receptor and SREBP, as well as the farnesyl X receptor and peroxisome proliferator–activated receptors, which in turn are modulated by endocrine, paracrine, and autocrine, factors such as insulin, tumor necrosis factor-α, adiponectin, and resistin, all of which are affected by diet, physical activity, genetics, and adipocyte mass.

There is a stepwise and significant increase in hepatic free cholesterol in NAFLD, and, to an even greater extent, in NASH, which Sanyal suggested may mediate cellular injury, while hepatic esterified cholesterol levels are similar to those in individuals without NAFLD. Free cholesterol can be converted to cholesterol esters by acyl-CoA: cholesterol acyltransferase, can be secreted directly into bile, or can be converted into bile acids. No genomic abnormality has been demonstrated in cholesterol and bile acid synthetic pathways in NAFLD, but hepatic cholesterol esters are enriched in polyunsaturated rather than saturated fatty acids, suggesting the presence of an abnormality in cholesterol metabolism in NAFLD, leading to increased biliary cholesterol, which may underlie the association between the IRS and gallstones.

There is increased lipid peroxidation in NAFLD (1) reflecting hepatic oxidative stress, which may be caused by mitochondrial dysfunction, by activation of the cytochrome P 450 system, or, less likely, by abnormality of peroxisomes or by iron overload. NASH is associated with paracrystalline mitochondrial inclusions similar to those in mitochondrial gene abnormalities, with decreased mitochondrial respiratory chain activity (2). Oxidative stress in turn causes liver injury, directly by damaging cellular organelles and DNA, by depleting mitochondrial DNA, and by inducing of redox-sensitive genes such as those of the nuclear factor-κB pathway, while increasing the degradation of inhibitors of κB, potentially explaining the increased apoptosis found in NASH (3). Another area receiving attention is the unfolded protein response, a signal transduction network activated by inhibition of protein folding in the endoplasmic reticulum, triggered by low cellular levels of ATP and calcium depletion, with free cholesterol potentially playing a role. This endoplasmic reticulum stress phenomenon leads to release of mediators such as activating transcription factor 6 from the endoplasmic reticulum, leading to expression of "adaptation" and "alarm" genes, with subsequent inflammation as well as apoptosis, worsened by cellular necrosis, cytokines, and further oxidative stress, then further activating nuclear factor-κB and mitogenic pathways. All of this occurs in the setting of insulin resistance, which itself worsens inflammation, causing macrophages to migrate into adipose tissue with proinflammatory cytokine release, increasing systemic inflammation and fibrosis, particularly in the liver. Hepatic fibrosis is mediated by stellate cells, which produce matrix. These cells are activated by oxidative stress, by cytokines, and by activation of the cannabinoid 1 receptor. As a subsequent step, insulin and leptin are needed to increase stellate cell matrix production, while endocannabinoids are among the factors producing stellate cell apoptosis. Another potential abnormality in NASH may be deficiency of stem cell–based reparative processes, which have the potential to restore normal hepatic architecture and physiology.

Mary Rinella (Chicago, IL) discussed the evaluation and management of NAFLD. The distinction between simple steatosis and NASH is important. For in-

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Abbreviations: ALT, alanine transaminase; AST, aspartate transaminase; CVD, cardiovascular disease; FFA, free fatty acid; IGT, impaired glucose tolerance; IMCL, intramyocellular lipid; IRS, insulin resistance syndrome; IUGR, intrauterine growth retardation; NAFLD, nonalcoholic fatty liver disease; NASH, nonalcoholic steatohepatitis; NHANES, National Health and Nutrition Examination Survey; SREBP, sterol regulatory element–binding protein.

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dividuals with NASH, over a 5-year period 25% will develop fibrosis and 15% cirrhosis (4). Histological signs such ballooning degeneration indicate active disease, with a proposed NASH activity score allowing discrimination between mild and more severe disease, although there is disagreement at the intermediate stages (5). Typically, levels of alanine transaminase (ALT) exceed those of aspartate transaminase (AST), the opposite finding to that of alcoholic fatty liver; the presence of an increased alkaline phosphatase suggests that another disease process may be present. Six of 10 individuals with NASH are asymptomatic, 30% have fatigue, 30% have right upper abdominal pain, and 25% have enlargement of the liver on physical examination. A normal ALT is seen in 30% of individuals with NASH and in 60% of those with cirrhosis, and when the ALT is normal with NASH, Rinella commented that diabetes is particularly likely to be present. NASH should then be suspected in high-risk individuals, particularly with metabolic syndrome and obesity, even with normal liver enzymes, as well as in individuals with persistently abnormal liver enzymes, recognizing that other causes must be excluded, such as alcohol, chronic viral hepatitis B (particularly in individuals of Asian origin), hepatitis C (particularly with a history of drug use or with a tattoo), autoimmune hepatitis (which may be associated with positive antinuclear or antismooth muscle antibodies), or with abnormality on serum protein electrophoresis, iron overload, and drugs including tamoxifen, prednisone, diltiazem, and amiodarone. Using such an approach, in a study of 354 individuals with persistently abnormal liver enzymes, 34 and 32% had NAFL and NASH, with more than one-third having other etiologies (6). Imaging may be useful in excluding other liver diseases. Ultrasound features include a "bright" liver with echo texture increased compared with that of the kidney and with vascular blurring, although ultrasound suffers from low sensitivity, as it may be normal with fat comprising <30% of the liver, as well as low specificity as it may be difficult to distinguish fat from fibrosis. Computerized tomography detects the presence of steatosis by decreased attenuation compared with spleen, a finding exaggerated with contrast, and magnetic resonance imaging is the most sensitive test in detecting steatosis; however, Rinella noted that no imaging modality distinguishes NAFLD from NASH. It is, she stated, important to exclude hepatic decompensation and to assess the stage of disease in an individual with NASH, with "liver biopsy . . . the only way at this time to stage disease accurately." There is a 66% prevalence of bridging fibrosis, the stage just before cirrhosis, in individuals aged >50 years with diabetes or with obesity (7,8). She suggested that specialist referral is appropriate in accurately staging disease and before initiating therapy that might lead to adverse outcome, as well as for clinical trials.

Management approaches include avoidance of alcohol and drugs causing steatohepatitis, correction of underlying risk factors with lipid-lowering agents and insulin sensitizers, and weight loss, with diet, exercise, and pharmacologic approaches including orlisat, sibutramine, GLP-1 receptor agonists, and antagonists of endogenous cannabinoids having potentially beneficial effects, with consideration potentially given to bariatric surgery in morbidly obese individuals. There may, however, be harm from rapid weight loss, as seen with jejeuno-ileal bypass, and as has been found in diabetes prevention trials relatively small degrees of weight loss may be effective, recognizing the many barriers that exist to achieving this goal. Other pharmacologic approaches being explored include antioxidants such as vitamin E, pentoxifylline, agents blocking tumor necrosis factor-α, betaine, *S*-adenosyl-methionine, and ursodeoxycholic acid.

Rinella reviewed a number of studies of these approaches. In analysis of the effect of bariatric surgery in individuals with morbid obesity, the prevalence of hepatic steatosis decreased from 88% before to 8% after the procedure, with the prevalences of fibrosis decreasing from 31 to13% and that of inflammation from 23 to 2% (9). Another study used gastric banding, again showing improvement of steatosis and fibrosis (10). The majority of individuals with NASH, however, do not have morbid obesity, and there are no long-term follow-up studies showing continued benefit. In studies of thiazolidinediones, pioglitazone administration has been associated with decreased fat, ballooning, Mallory bodies, and pericellular fibrosis (11), although the potential for weight gain has been a concern. (A 6-month study of 55 individuals with NASH who had diabetes or glucose intolerance was reported subsequent to Rinella's talk; compared with hypocaloric diet alone, 45 mg pioglitazone daily was associated with improvement in glycemia and transaminase levels, with decreased histological evidence of steatosis, ballooning necrosis, and inflammation, although not with significant difference in fibrosis [12].) Metformin has been administered in a number of studies (13,14). In a comparison of metformin with rosiglitazone, only the latter agent was associated with improvement of hepatic fat content and reduction in transaminase levels (15). Given the evidence that administration of metformin with thiazolidinediones reduces the likelihood of weight gain among individuals with diabetes, Rinella suggested that consideration should be given to studying the combination in the treatment of NAFLD. Another potentially useful approach is intervention in the renin-angiotensin-aldosterone system. Mineralcorticoid blockade reduces cardiac fibrosis. Animal models show improvement in hepatic steatosis with losartan, and in the DREAM (Diabetes REduction Assessment with ramipril and rosiglitazone Medication) trial ALT levels decreased with ramipril (16). Given the evidence of toxicity of free cholesterol, statins might be of benefit, with promising preliminary results using atorvastatin. Consideration should always be given, Rinella noted, to excluding the presence of hepatitis C, which is associated with NAFLD, with the presence of NAFLD affecting the response to antiviral treatment.

Insulin resistance in childhood and adolescence

Alan Sinaiko (Minneapolis, MN) introduced a symposium on insulin resistance in pediatrics preceding the Annual World Congress on the IRS with the observation that "the roots of the metabolic syndrome go back into childhood." Proper appreciation of its complexities, then, must include the study of children with insulin resistance. Stephen Cook (Rochester, NY) examined the characterization of metabolic syndrome in children and adolescents. Certainly, cardiovascular disease (CVD) risk factors cluster in families, and in individual children, and tracking these factors over time in children provides insight into their development. Over several decades, progressive increase has been seen in overweight among children and adolescents (17). Mean waist circumference increased by 1.6 and 2.4 cm for male and female children, respectively, from the time of the National Health and Nu-

trition Examination Survey (NHANES) III survey in 1988–1994 to the next survey in 1999–2000, with the prevalence of elevated waist circumference increasing by >70% (18,19). Similar studies in British children have shown increased central adiposity (20).

Analysis of the NHANES III data shows that 23.7% of adults have metabolic syndrome, including 6–7% of those aged 20–29 years (21). Using 110 mg/dl for both triglyceride and glucose cutoff levels, 40 and 50 mg/dl HDL, respectively, in male and female adolescents, and the 90th percentiles of abdominal circumference and blood pressure, 4% of adolescents had metabolic syndrome (22). In studies of younger children using 90 mg/dl as triglyceride cutoff, 40 mg/dl for HDL cholesterol, and A1C >6% (rather than fasting glucose) as criteria, 4.3% of 5- to 11-year-old children had metabolic syndrome, including 20% of those who were overweight.

There is evidence that risk factor clustering tracks over time (23). The Young Finns study followed high-risk children with increased cholesterol, low HDL, and increased diastolic blood pressure, finding that one-quarter still had high-risk abnormality in these parameters after 6 years of follow-up (24). A 12-year Canadian study similarly showed the IRS to continue in young individuals over a 12-year period. In the Bogalusa study, abnormalities of insulin sensitivity index, BMI, blood pressure, triglyceride, and HDL cholesterol clustered after 8 years. Similar studies show that IRS tracks into adulthood (25). Furthermore, offspring studies in Minnesota and Bogalusa show that parents with IRS have children with higher BMI, waist circumference, insulin, and blood pressure levels, presumably reflecting both genetic and environmental influences. Interesting, not only those children with high levels of CVD risk factors, but also those in the lowest quartile levels of risk factors track together, suggesting that with appropriate intervention there is the potential to offer long-term protection.

Cook reviewed other studies suggesting further factors reflecting metabolic risk. Children who survive malignancy have increased risk of developing IRS, with greater likelihood of obesity, high triglyceride levels, and low HDL cholesterol levels. Analysis of C-reactive protein showed levels of 3.8 vs. 1.4 mg/l in individuals with versus without IRS, respectively (26). Cigarette use is associated with overweight and with the metabolic syndrome, suggesting another contributory factor.

Studies of preadolescent girls, using modified Adult Treatment Panel III and World Health Organization criteria, found IRS prevalence ranging from <0.5 to 25% (27). Applying a common set of criteria, 3% of Korean, 4% of North American, 6% of Brazilian, and 10% of Iranian children had metabolic syndrome. Interestingly, analyses of 1,826 adolescents in the NHANES 1999–2002 dataset using three different definitions showed IRS prevalence varying from 2 to 9.4%, with 12–44% of the overweight group meeting the criteria for the syndrome, suggesting the need to design definitions having greater agreement with one another.

Sherin Devaskar (Los Angeles, CA) described the fetal origins of the IRS, with multiple complex contributory factors, including intrauterine growth retardation (IUGR), which may reflect a gene-environment interaction leading to obesity and type 2 diabetes, so that increased energy intake and inactivity may "propagate what was already set in utero." A number of maternal factors influence postnatal outcome, including maternal prepregnancy weight/body composition, weight gain during pregnancy, ethnicity, pregestational and gestational diabetes, whether diet- or insulin-controlled, pregestational and gestational hypertension, and dyslipidemia. Maternal obesity is associated with still birth and both with macrosomia and with IUGR. Presentations at birth may include injuries, such as shoulder dystocia and nerve palsies, and prematurity leading to respiratory distress syndrome. Long-term outcome has been studied in Pima Indian and Chicago observations of adolescents born to diabetic or obese mothers, showing increased likelihood of hyperinsulinemia, elevated leptin levels, and childhood obesity. IUGR is also common in the developing world in the setting of low maternal caloric intake, and here too it is associated with long-term complications including insulin resistance, central obesity, hypertension, and CVD (28,29). One possibility is that IUGR leads to exaggerated "catch-up growth," with excessive consequent energy balance causing the insulin-resistant state (30). Conversely, an important postnatal influence on insulin sensitivity is breast-feeding, which is associated with lower BMI at age 6 through 72 months (31), suggesting that they "self-regulate better." A therapeutic implication is the need to focus on measures to improve self-regulation and on nutrients that improve regulation of energy balance, rather than on efforts to increase body weight in infants with IUGR, as this may promote the development of obesity. Devaskar reviewed cross-sectional computed tomography scan studies showing that offspring with IUGR exhibiting catch-up growth have increases both in subcutaneous and visceral fat, while those with lower childhood weight have lesser increase in visceral fat, without increased subcutaneous fat. The infants with IUGR exhibiting catch-up have the greatest degree of postload hyperinsulinemia when tested in adulthood. This leads to the concept of transgenerational propagation, which has been explored in animal studies, with either over- or undernutrition predisposing the infant to insulin resistance. With intervention in the grandmother, mothers show IUGR, and their infants, even with optimal maternal nutrition during pregnancy, have normal growth pattern but postload hyperinsulinemia, lower hepatic glucose production, and altered insulin signaling. A potential mechanism may be epigenetic change, perhaps with DNA methylation of genes leading to abnormality of nutrient metabolism.

Donna M. Dabelea (Denver, CO) discussed ethnic differences in the IRS in youth, emphasizing that the syndrome represents clustering of CVD risk factors including central obesity, abnormal glucose tolerance, dyslipidemia with high triglyceride and low HDL cholesterol, and hypertension and rapid heart rate, all reflecting insulin resistance. The association of pulse pressure and heart rate was shown in the Bogalusa study (32), with autopsy of children who died accidentally in this study showing an association of the number of IRS components with the likelihood of aortic and coronary artery fatty streaks and fibrous plaques (33). A number of studies in adults have shown greater prevalence of insulin resistance and of CVD risk factors in ethnic minorities, in part related to obesity. There is evidence of elevated fasting insulin in African-American versus non-Hispanic white children ages 7–11 (34), with similar findings in American Indian and in Hispanic children, to some extent independent of obesity. Dabelea suggested that obesity may influence the development of IRS to greater extent in white and Hispanic than African-American chil-

dren. African-American and Hispanic youth have lower insulin sensitivity than non-Hispanic white youth, with higher acute insulin response in African-American than in Hispanic or in non-Hispanic white youth and total insulin secretion higher in Hispanic children (35). There may be both genetic and social class components to the reduced insulin sensitivity and greater acute insulin response to glucose in African American than non-Hispanic white (36), with the greater insulin response associated with reduced hepatic insulin extraction, while Hispanic youth have greater levels of insulin secretion. There is a stronger relationship of total body fat to insulin secretion (37) and to triglyceride levels (38) in non-Hispanic white than in African-American youth. This data suggests, Dablea commented, that minority children might be more likely than non-Hispanic white children to develop type 2 diabetes due to obesity-independent increase in insulin resistance but may be less likely to develop obesity-dependent insulin resistance, although this conclusion appears somewhat at variance with clinical experience. In terms of IRS, one must recognize that it is difficult to compare studies, as different definitions are used. There appears to be a higher prevalence of the syndrome in African-American than in non-Hispanic white or Hispanic children (22). Comparing non-Hispanic white with African-American and Hispanic children in the NHANES III study, the prevalence of central obesity appeared to increase over time in the latter group, blood pressure levels increased in all, and HDL cholesterol levels were lower in the minority groups (39).

Type 2 diabetes in youth may be considered "the extreme manifestation of the IRS," and, although increasing in incidence, it remains sufficiently rare on a population basis that it has not been possible to directly ascertain whether it begins in children with insulin resistance. The Search for Diabetes in Youth data has given some information on the topic. For children up to 9 years of age developing diabetes, the prevalence is 0.79 cases per 1,000, somewhat higher among non-Hispanic white, with >80% having type 1 diabetes, while among those aged 10–19 years the prevalence is 2.80 cases per 1,000, with type 2 diabetes in 6% of non-Hispanic white but in 76% of American Indian youth (40). Among youth with diabetes, the prevalence of insulin resistance and of IRS components is highest in American Indians, lower in those of Asian-Pacific island ethnicity, with somewhat lower prevalence among Hispanics, and then among African American, and lowest among non-Hispanic white (41). Although evidence of insulin resistance is common among type 2 diabetic youth, it also is seen in 30% of youth with type 1 diabetes. The prevalence of microalbuminuria is also higher in type 2 than type 1 diabetic youth, varying with race in youth with type 2 diabetes but not in those with type 1 diabetes.

Sonia Caprio (New Haven, CT) reviewed the spectrum of insulin resistance among obese children. Given the prediction that diabetes is growing in epidemic proportions in Asia, South America, and Africa, she speculated that the "hidden epidemic" of impaired glucose tolerance (IGT) may be of even greater consequence, as there are currently some 200 million individuals with diabetes and >300 million with IGT worldwide. An important consideration is the mechanism of the effect of obesity in causing insulin resistance. Obesity is associated with excess tissue lipid deposition, causing increased hepatic glucose production and impaired muscle insulin-mediated glucose disposal, both contributing to insulin resistance and eventually to hyperglycemia. Skeletal muscle specimens obtained from adults with type 2 diabetes contain droplets of ectopic lipid (42), and Caprio reviewed studies in children and adolescents using ^{1}H nuclear magnetic resonance spectroscopy to quantitate extra- and intramyocellular lipid (IMCL), the latter associated with insulin resistance. In her study of adolescents with IGT, euglycemic and hyperglycemic clamps were performed to assess insulin sensitivity and secretion, and body composition measurements were made in 14 adolescents with IGT and 14 control subjects with normal glucose tolerance, matched for obesity. Fasting glucose levels were identical, but the 2-h glucose level was 111 vs. 162 mg/dl, fasting and 2-h insulin levels were ~1.5- and 3-fold higher, and adiponectin 25% lower in the IGT group. Insulin sensitivity was reduced in the nonoxidative pathway in a fashion similar to that seen in individuals with type 2 diabetes, and IMCL was 30% higher in the IGT children (43). Total body fat was similar in the two groups, but those with IGT had more visceral and less subcutaneous fat. IMCL may directly reduce insulin signaling by increasing levels of ceramide, diacyl glycerol, fatty acyl-CoA, and protein kinase C-θ, leading to decreased insulin receptor substrate 1 activity (44).

NAFLD is the major cause of unexplained increased ALT in adults and children, termed by Caprio "the hepatic component of the metabolic syndrome," present in 2–3% of nonobese and 30–50% of obese children and in 70% of children with IRS. Hepatic fat accumulation is seen in the setting of increased circulating FFAs, leading to increased hepatic VLDL production and glucose production, perhaps mediated to a large part by an associated decrease in levels of adiponectin, which has metabolic and anti-inflammatory effects including actions on adenosine monophosphate–activated protein kinase and on peroxisome proliferator–activated receptor-γ (45). This phenomenon led Caprio's group to study 392 obese adolescents, none receiving medications, comparing ALT tertiles. The highest ALT level was seen in 14% of African-American, but in 43% of non-Hispanic white and 37% of Hispanic, adolescents in the group, with stepwise worsening of glucose tolerance and of hyperinsulinemia as ALT increased, in association with decreasing insulin sensitivity and increasing triglyceride and FFA levels (46). Magnetic resonance imaging studies in 72 of these children allowed quantitation of liver, visceral, and subcutaneous fat. The African-American children had less and the Hispanic children more hepatic fat, and different subcutaneous fat patterns were seen in the two groups, with African Americans having more superficial abdominal and less deep subcutaneous fat, while the Hispanic children had more deep subcutaneous fat. Children with increased hepatic fat had lower adiponectin, higher visceral fat, and a greater ratio of deep-to-superficial subcutaneous fat.

Jeffrey Schwimmer (San Diego, CA) further reviewed the association of liver disease with obesity and metabolic syndrome in children. The normal liver is ~1% fat. NAFLD is associated with large fat droplets in liver, with steatosis (≥5% fat content) the defining feature. The mechanism of NASH may involve excess delivery of fat to the liver, with increased FFA uptake, or potentially caused by increased de novo lipogenesis, although an alternative concept is that increased liver fat reflects decreased oxidation or decreased VLDL secretion. Inflammation is present in some individuals with NAFLD and may reach the level of NASH. Other individuals with NAFLD develop fibrosis,

some with cirrhosis, and 3–10% of children with NAFLD have cirrhosis at the time of a diagnostic liver biopsy. Schwimmer commented that one should never consider NAFLD "just fatty liver," stating that NASH progresses to cirrhosis as frequently as hepatitis C.

In Schwimmer's studies of pediatric NASH, the disease typically presents around 12 years of age (47). On autopsy studies the prevalence of NASH increases with age, with twice as many boys than girls (48). ALT levels are typically higher than levels of AST, but Schwimmer pointed out that a normal level does not exclude NASH. Most pediatric NASH is associated with obesity, and abnormal liver function is seen in 10–20% of obese children, while half of obese children have ultrasound findings suggesting NASH, with some of these probably false-positive given the 38% prevalence of NASH on liver biopsy of obese children. Morbid obesity is an important risk factor for progression; interestingly, hypopituitarism also increases the likelihood of progression of NASH. Of children with NAFLD, 8–10% have diabetes, 75% have hyperinsulinemia, and 95% have insulin resistance using homeostasis model assessment. Conversely, half of type 2 diabetic children have NAFLD. Half of children with NAFLD have acanthosis, and dyslipidemia is common. Approximately one-quarter of children have pain from NASH, with Schwimmer characterizing these as "the lucky ones [who] get picked up."

Two types of NASH are seen in children: the first type with fat particularly in the central vein area, similar to the typical appearance in adults, affects 20%, while in the second, more common type lipid is mainly present in hepatocytes, with portal inflammation and fibrosis (47). Non-Hispanic white children typically have the first type, while the second form of NASH in children is typically seen in those of Asian, particularly Filipino and Japanese, and Hispanic ethnicity, with obese African-American children having NASH relatively infrequently.

Schwimmer described a trial of 10 nondiabetic children aged 8–17 years with NASH treated for 24 weeks with 500 mg metformin twice daily, with ALT and AST decreased to normal levels in half. Liver fat by magnetic resonance spectroscopy decreased in all cases, with a direct dose-response effect between metformin (in milligrams per kilograms per day) and the decrease in liver fat.

Alan Sinaiko presented his studies of the natural history of insulin resistance and its relationship to the IRS in children and adolescents. The prevalence of "overweight" (the term used to designate obesity among children) increased from 4 to10% from 1971 to 1994. Pediatric overweight is associated with abnormal carotid intima-media thickness and flow-mediated vasodilation, the findings of the IRS in adults. Sinaiko reviewed his three cohort studies, started in 1978, in 1986, and in 1997. The first study showed an extremely high correlation of BMI at age 7 years with that at age 24 years (49), so that the notion that when children "go through their growth spurt" they lose weight is incorrect. HDL cholesterol, triglyceride, and blood pressure track similarly from age 13 to 23 years, but insulin levels do not track as well, leading Sinaiko to conclude that the "fasting insulin falls far short of other measures of insulin sensitivity." Insulin sensitivity measured by euglycemic clamp shows somewhat better correlation at the two time points than fasting insulin, with an insulin resistance score based on "*Z*-scores" of triglyceride, HDL, systolic blood pressure, and fasting insulin showing the greatest correlation. Sinaiko reviewed other evidence that the fasting insulin correlates poorly with clamp insulin sensitivity (50) and noted that given the low variability in fasting glucose, homeostasis model assessment (calculated from the ratio of insulin to glucose) is no better than the fasting insulin.

Insulin sensitivity decreases at the beginning of of puberty, particularly for boys, returning to baseline at end of puberty (51). During the period from age 8 to 19 years, blood pressure and triglyceride levels increase, particularly in boys, and HDL cholesterol levels decrease. Insulin sensitivity, however, decreases in boys and increases in girls, and the proportion of body fat decreases in boys and increases in girls, suggesting that although insulin sensitivity is related to obesity, there must be determinants other than fatness. The degree of obesity does, however, correlate with levels of insulin, triglyceride, HDL, blood pressure, and inflammatory markers.

Grouping children into four categories, based on BMI and on insulin sensitivity above versus below the median gives another approach to dissecting the relative contributions of obesity versus insulin resistance to CVD risk factors. The heavier half of children had higher blood pressure, triglyceride, and fasting insulin and lower HDL but similar insulin sensitivity to that seen in the thinner half, while, comparing those with low versus high insulin sensitivity, triglyceride levels were higher and HDL cholesterol lower, but blood pressure was similar. Those who were both in the heavier and less insulin-sensitive halves of the population had higher blood pressure, higher triglyceride, lower HDL, and higher fasting insulin, suggesting this to be the key group displaying all the metabolic abnormalities, an observation that became more apparent using the *Z*-score measure (52).

Sinaiko pointed out that the goal of an analysis of the sort he described is to derive approaches to the prediction of future risk. In addition to baseline insulin resistance, an important predictive factor is the degree of change in insulin resistance, and although the baseline BMI is not a significant predictor of CVD risk in multivariate analysis, the change in BMI from age 13 to 19 years was strongly correlated with blood pressure and triglyceride (53), suggesting that initiatives to change obesity are important, although we do not know how to reproducibly achieve this goal.

Sinakio described the relationship between the metabolic syndrome and insulin resistance in his studies. The syndrome was present in 2–6%, 4–9%, and 8% of children in his survey at ages 13, 15, and 19 years, depending on the approach used, with waist, triglyceride, and HDL cholesterol more important than elevated blood pressure and glucose levels as contributors to the diagnosis. Almost all of the children with metabolic syndrome had BMI above and insulin sensitivity below the median, and this group tended not to show improvement during the period of follow-up, further suggesting the importance of developing measures to achieve weight loss or to improve insulin sensitivity. Comparing insulin-sensitive with insulin-resistant children whose BMI was below median, visceral fat measured at age 23 years was lower among the former group, suggesting that fatness may be important even among those in the thinner group. Differences between these groups in triglyceride and in the insulin sensitivity *Z*-score continued to be present over time. Sinaiko concluded that the metabolic syndrome does begin in childhood, that it is related to increased risk, and that its treatment will require measures both to address obesity and to improve insulin sensitivity.

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