NON-ALCOHOLIC FATTY LIVER DISEASE (NAFLD) IN CHILDREN

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1. ABSTRACT

Childhood NAFLD has become an important childhood liver disease, and it is probably highly prevalent. The full of spectrum of NAFLD has been identified in children. It is not currently known whether or not simple hepatic steatosis in children is benign or whether it evolves to NASH over time. In contrast, childhood NASH certainly can have serious consequences. Cirrhosis is apparently rare in children with NAFLD, but it definitely occurs.

Childhood NAFLD may occur in very young children, and there is no female predominance in the pediatric age bracket. Children present with vague abdominal pain, if they have any symptoms at all, but frequently hepatic steatosis is found incidentally on abdominal imaging. Laboratory studies show that serum aminotransferase abnormalities are rather moderate, with serum alanine aminotransferase (ALT) more elevated than serum aspartate aminotransferase (AST). Hypertriglyceridemia is the typical blood lipid abnormality, although hypercholesterolemia may occur. NASH may be more severe in children from certain ethnic groups, including Hispanics and Asians, or in association with certain metabolic disorders characterized by abnormalities in insulin receptor structure or signaling, such as lipodystrophy syndromes. Weight loss through dietary redesign and a regimen of regular exercise remains the mainstay for treatment for childhood NAFLD. A dietary strategy to minimize postprandial hyperinsulinemia and overall fat intake, such as a low glycemic index diet, may be the best dietary strategy. The real efficacy of drug treatments in children requires further investigation.

The overriding message is that childhood obesity poses important health problems, including but not limited to potentially severe chronic liver disease (1, 2). Early diagnosis of children who are only overweight is a worthy goal so that strategies to limit obesity can be instituted as early as possible. Identification of genetic risks is important, but management will invariably require changes in environmental factors. In addition to individual

treatment, a multifaceted, societal initiative is required for solving the childhood obesity epidemic.

2. INTRODUCTION

Non-alcoholic fatty liver disease (NAFLD) denotes a spectrum of liver disease, all of whose component entities involve some degree of accumulation of large droplets of fat in hepatocytes. Although the histological findings may resemble those of alcoholic liver disease, ethanol is not an etiological factor. The spectrum of disorders comprising NAFLD ranges from simple steatosis, through non-alcoholic steatohepatitis (NASH) where there is steatosis with inflammation and fibrosis, to cirrhosis in which fat accumulation may be subtle. The advantage of the term "NAFLD" is that it is less restrictive than "NASH," and in particular inflammation is not the *sine qua non* of the diagnosis. Importantly, NAFLD can be due to metabolic disorders or to drug hepatotoxicity (by definition, excluding ethanol). The major cause of NAFLD is a metabolic disorder affecting the liver which is a consequence of abnormal insulin action, namely hyperinsulinemia associated with insulin resistance. This condition most commonly arises in obese or overweight individuals, who may also have hyperlipidemia and/or type 2 diabetes mellitus. It has become apparent that NAFLD can occur in children.

3. CHILDHOOD OBESITY

Obesity is a worldwide health problem which affects people in wealthy industrialized countries, countries with emerging economies, and the developing world. It may be a health consequence of urbanization, labor-saving devices, and changing patterns of food availability. Childhood obesity is being identified as a major public health problem (2-5). Current estimates put the number of obese children at approximately 22 million worldwide. These estimates of the prevalence of overweight and obesity in children vary depending how "overweight" and "obesity" are defined for children. . Possible definitions include percent of ideal weight-for-height (where weight greater than 20% above ideal weight-for-height is defined as obese) or age- and gender-normative data for body-mass-index (BMI) (6, 7). Definitions of "overweight" and "obese" based on BMI are not as simple as those for adults (where overweight is BMI>25 and obesity BMI>30). Changes in normal BMI are not linear in childhood, and therefore the boundaries for these definitions shift throughout childhood for both sexes. Statistical analysis of childhood population weight and height data from six large and diverse national cross-sectional growth studies (from Brazil, Great Britain, Hong Kong, the Netherlands, Singapore and the United States) has permitted development of an international definition for "overweight" and "obesity" in children utilizing the BMI (8). This simple, internationalized scheme is easier to apply than percentile-based definitions. The normal variability of BMI through childhood can be standardized through the use of z-score. Waist circumference may also be informative, although this has not been validated for children as extensively as it has been for adults (9, 10).

Childhood obesity is at epidemic proportions. Since 1986 the prevalence of overweight children in the United States has increased especially dramatically in certain ethnic groups there: to 22% for Afro-American children, 22% for Hispanic children, and 12% for non-Hispanic Caucasian children, according to 1998 data . Similar data are available from Canada (11) and Europe (12-14). Recent data from Canada indicate that nearly 40% of Canadian children aged 2-13 years are either overweight or obese. Particularly worrying is the rise in obesity and overweight among preschool children, aged 3-5 years. A recent study from Canada showed that in the 1997 birth cohort in the province of Newfoundland and Labrador, of which 4161 children (75%) were tested, 8% were obese and 17% were overweight: thus one-quarter of this cohort was either overweight or obese (15). Similar observations have been reported from England, Australia, and the United States (16, 17). Among the various circumstances responsible for this epidemic of childhood obesity, today's dietary patterns including fatty foods and snacks and beverages containing mainly highly refined carbohydrates play an important role. Recent studies have drawn attention to the greatly increased intake of sugary beverages, such as soda pop, in the past decade, notably among children to the exclusion of milk (18-20). Likewise highly prevalent sedentary behaviors, especially increased time spent watching television or working (or playing) at a computer, have been shown to be important contributors (21-24).

3.1. Consequences of childhood obesity

Childhood obesity has traditionally been regarded as mainly an important psychosocial problem, associated with lowered self-esteem and diminished social interactions (25, 26). Bullying can be an important problem at school (27). Important biological consequences of childhood obesity have become evident. Even though the relationship between childhood obesity and type 2 diabetes mellitus may be uncertain, the incidence of type 2 diabetes mellitus in children has risen dramatically in the past few years (6, 28). The incidence of primary hypertension in children has also risen in recent years (29). Hypertension is noteworthy for being within the spectrum of the metabolic syndrome and related to NASH in adults. Finally, numerous reports now exist describing the full spectrum of NAFLD in children.

3.2. Liver abnormalities in childhood obesity

Most reported series of childhood NAFLD involve some degree of selection, usually through referral bias. It is important to determine the prevalence of childhood NAFLD generally. From the practical point of view this means identifying children in an unselected manner who appear to have hepatic steatosis by non-invasive diagnostic methods. Studies in adults show that imaging can identify patients with hepatic steatosis accurately, especially if the steatosis is at least moderately severe (occupying >33% of hepatocytes on live biopsy), but imaging cannot distinguish simple steatosis from NASH or reliably detect fibrosis (30, 31). Thus children with mild degrees of hepatic steatosis will be missed. A sonographic study of 810 school children from northern

Japan demonstrated an overall prevalence of fatty liver by sonography in 2.6% of this unselected group, and there was a strong correlation to indices of obesity such as BMI (32). In studies where obese children were screened for abnormal serum aminotransferases the prevalence appears to be 10-25% (33). In one small study sonographic features of fatty liver were also found in 5 of 27 (19%) children who had normal ALT (34). Elevated ALT and fatty liver by sonography were more common in older children with more severe obesity, but no statistically significant differences were found in different age groups or with longer duration of obesity. In a study from Italy of 375 obese children aged 9-16 years-old and spanning all pubertal stages, 42% had hepatic steatosis by sonography (35). These children were all obese or very obese, and although presence of hepatic steatosis correlated significantly with BMI, mean BMI in all patients with or without steatosis was high (35.9 and 33.8, respectively). In a recent Chinese study of obese children hepatic steatosis was identified songraphically in 77% (36). Magnetic resonance imaging may be a more sensitive non-invasive method for detecting hepatic steatosis (37). A different approach is to examine the coincidence of obesity and abnormal liver biochemistries as a way to diagnose "presumed NASH." In the National Health and Examination Survey, cycle III, in the United States serum ALT and gamma-glutamyl transpeptidase (GGT) were measured in 2450 children 12-18 years-old who were classified as "obese" if the BMI was >95th percentile for age and gender or "overweight" if the BMI was between the 85th and 95th percentiles. In this relatively unselected study, 6% of overweight, and 10% of obese, adolescents had an elevated ALT (38). Alcohol use could not be excluded as a explanation for some of these findings. In a recent study of 182 overweight or obese but otherwise healthy German children, the serum ALT was at or above the normal limit for age in 48% whereas mean AST and GGT were normal (39).

4. LIVER BIOPSY FINDINGS IN NAFLD

Rigorous definition of NAFLD require liver histology (40, 41). In adults there is a broad spectrum of findings: macrovesicular steatosis without or with active inflammation; mild to moderate fibrosis; cirrhosis. Some fat is expected to be present (involving at least 5% of the hepatocytes) except perhaps with advanced cirrhosis, which may otherwise be regarded as cryptogenic (42, 43). When only fat is present, the lesion is simple steatosis. NASH involves inflammation or fibrosis or both in addition to steatosis. Signs of inflammation include ballooning degeneration of hepatocytes or focal hepatocyte drop-out. Mallory hyaline may be found. There may be some microvesicular fat in addition to macrovesicular fat. In adults inflammation and fibrosis are typically most severe in the perivenular zone. In adults, these histological findings can be associated with other disease processes, notably chronic hepatitis C, Wilson disease and medications or herbs causing a "pseudo-alcoholic hepatitis" lesion. No consensus exists as to how much alcohol can be consumed by an individual before the hepatic steatosis should be attributed to alcohol excess rather than to

NAFLD, but current recommendations consider 10-20 g of ethanol daily as the limit beyond which ethanol must be regarded as a causative factor for liver damage.

Hepatic histology in children with NASH is usually not identical to that found in adults with NASH. The inflammatory infiltrate often consists of mononuclear inflammatory cells instead of polymorphonuclear leukocytes. Inflammation is often periportal instead of perivenular. Mallory hyaline is exceptional. Fibrosis also tends to be periportal and is less frequently pericellular. The basis for these differences between children and adults is not yet determined. One possibility is that it represents a different response to injury. A predominantly portal lesion in adult NASH has recently been described (44).

Structural abnormalities of mitochondria have been described in adults with NASH. These findings consist of giant size (megamitochondria) with paracrystalline inclusions (45, 46). The pathogenesis of these changes is obscure, and it is not evident that the findings are specific for NAFLD. Similar changes have been reported in one study in children (47).

5. NAFLD IN CHILDREN

Childhood NAFLD was first reported in the English language literature in the 1980s. Moran and colleagues described three American children with fatty liver and steatohepatitis (48). These children, two boys and a girl, were 10-13 years-old and had body mass indices ranging from 28.5 to 33.2 at presentation: since the BMI boundary values for obesity in this age range are 24-28, these children were definitely obese. Liver biopsy revealed macrovesicular steatosis with variable degrees of inflammation and fibrosis, and no other specific liver disease was found. These patients had biochemical improvement when they lost weight. Since then there have been numerous reports from all over the world (49-54). Cirrhosis was first reported from Japan in an 11 year-old child who had been obese since 3 years-old and also had "maturity-onset" diabetes mellitus (49). Familial clustering of NAFLD was apparent in a large American study (55), in which one patient was a 14 year-old male with cirrhosis whose mother also had NAFLD with less severe fibrosis.

The first large pediatric clinical series included 36 patients from a variety of ethnic backgrounds collected prospectively and consecutively between 1985 and 1995 at the Hospital for Sick Children in Toronto; liver biopsy was obtained in 24 patients (56). The male-to-female ratio was approximately 3:2, and patients were 4 to 16 years-old at time of diagnosis. Notably, 13 of the 36 children in this series were less than 11 years-old at time of diagnosis. Most, but not all, patients were obese, where obesity was defined very conservatively as weight greater than 20% above ideal weight-for-height. The range for all patients was 114-192% of ideal weight for height. Approximately 30% of the cohort had acanthosis nigricans, known to be associated with insulin resistance. On liver biopsy 71% had some fibrosis. A 9 year-old child already had cirrhosis. Two brothers had Bardet-Biedl syndrome. One adolescent

Table 1. Inherited metabolic disorders associated with childhood NAFLD

Name of syndrome		Features
Bardet-Biedel syndrome	AR	Retinal dystrophy causing visual impairment, central obesity, renal dysgenesis leading to progressive renal insufficiency, male hypogonadism, insulin receptor defect
Alström syndrome	AR	Pigmentary retinopathy (infantile cone-rod dystrophy), obesity, sensorineural deafness, dilated cardiomyopathy, diabetes mellitus, insulin receptor defect
Polycystic ovary syndrome	Females	Structurally abnormal ovaries, chronic oligo-ovulation, hyperandrogenism (classic causes excluded), hirsutism, acne, obesity, abnormal insulin disposition
Lipodystrophy syndromes	AR (various genes including: *AGPAT2*, *BSCL2*, *LMNA*, *PPARG*)	Disordered insulin action, lipoatrophy, acanthosis nigricans, diabetes mellitus
Turner syndrome	Females: XO	Obesity, short stature, infertility
Cohen syndrome	AR	Neurodevelopmental delay, hypotonia, distinctive facies, myopia and retinal abnormalities, obesity, granulocytopenia
Prader-Willi syndrome	Paternal imprinting disorder	Obesity, hypogonadism, mental retardation, hypothalamic dysfunction

Note: AR = autosomal recessive

female appeared to have polycystic ovary syndrome, one child had severe hypothyroidism, and another had a partial deficiency of alpha-1-antitrypsin (phenotype PI MZ). Non-specific autoantibodies, most often low-titer anti-smooth muscle antibodies, were present in a few patients. Four children developed diabetes mellitus after the diagnosis of NAFLD was established. Weight loss was associated with improvement in serum aminotransferases. On the basis of these observations the authors postulated that childhood NASH involved a disorder of insulin action.

More recent reports have focused attention on some special features of children with NAFLD. Two boys, aged 10 and 14 years, were described with NAFLD which progressed rapidly to cirrhosis (57). The younger patient was Hispanic and massively obese for age (BMI 32) and the older patient had had a craniopharyngioma resected. A large series from San Diego included 43 children (30 male, 13 female), all of whom underwent liver biopsy. The ethnic distribution was 53% Hispanic, 25% Caucasian, 5% Afro-American, and ethnicity was not specified in 17%. Six of these children had type 2 diabetes mellitus. Nearly half (49%) had acanthosis nigricans, and 75% had fasting hyperinsulinemia. Insulin resistance was also highly prevalent as measured by indirect tests including the homeostasis model of insulin resistance (HOMA-IR). In a cross-sectional study of 84 obese Chinese children sonographic evidence of hepatic steatosis and elevated serum ALT was found in 24%: these children had higher waist-hip ratios than those with simple steatosis and affected males also had a greater degree of insulin resistance (36). Liver biopsy was not performed in this study.

Disorders resulting in hyperphagia have now been shown to be associated with childhood NAFLD. A series of patients was reported from the Mayo Clinic who had NAFLD associated with hypothalamic or pituitary dysfunction usually due to brain tumors such as craniopharyngioma or pituitary adenoma, but also including patients with idiopathic hypopituiarism. Seven were within the pediatric age-bracket (<18 years-old) at time of diagnosis of the liver disease (58), and two had cirrhosis. At least one of these pediatric patients had had a craniopharyngioma resected. Several other similar patients have been reported (57, 59, 60), and the data suggest a

comparatively rapid development of cirrhosis in this type of patient.

Some important pediatric clinical series are currently reported only in abstracts. In an early report from Texas, 39 children (28 males and 21 females) with NAFLD were described: 20 were Hispanic, 14 Caucasian, 4 Asian, and 1 Afro-American (61). Additionally, five children had recovered from childhood cancer. Fasting serum insulin was measured in 17 patients and was elevated on average three times the upper limit of normal. Acanthosis nigricans was present in 49% of these children. Thirty-one of these children underwent liver biopsy which revealed steatohepatitis in all 31, fibrosis in 21 and cirrhosis in two children. Children referred to King's College Hospital in London, UK, over a five-year period because of fatty liver without identifiable infectious or metabolic/genetic liver disease numbered seventeen, of whom 12 were male, and all but one obese. Anti-nuclear or anti-smooth muscle antibodies were found in 7 patients (41%). Liver biopsy performed in 13 patients disclosed steatosis in all, inflammation with fibrosis (5) or without fibrosis (3), and fibrosis without inflammation in 3 (62). A series from the Birmingham Children's Hospital in the UK included 38 children of whom 21 were male and 79% were obese. Insulin resistance was present in 17 children as measured by HOMA-IR, and in 11 had elevated serum concentrations of C-peptide, consistent with insulin overproduction. Non-specific autoantibodies were found in approximately one-third of patients. Liver biopsy was performed in the 30 children who had elevated serum ALT and showed cirrhosis in 3 children (63).

6. METABOLIC DISEASES WITH NAFLD USUALLY PRESENTING IN CHILDHOOD

Some inherited metabolic disorders of childhood, summarized in table 1, have a close etiological connection to childhood NAFLD because of known abnormalities in insulin receptor function (64).

6.1. Multisystem disorders with ocular abnormalities
6.1.1. Bardet-Biedl syndrome
Bardet-Biedl syndrome involves progressive loss of vision because of retinal dystrophy, central obesity, renal dysgenesis leading to progressive renal insufficiency, and

male hypogonadism. Polydactyly or other abnormalities of the extremities are variable features, and mental retardation appears to occur only sporadically (65). Type 2 diabetes mellitus may develop in these patients because of defective insulin receptor function (66). Bardet-Biedl syndrome is genetically heterogeneous (67, 68). Cirrhosis has been reported in one patient previously (69).

6.1.2. Alström syndrome

Alström syndrome is a rare autosomal recessive disorder whose features include pigmentary retinopathy with infantile cone-rod dystrophy, obesity, sensorineural deafness, dilated cardiomyopathy, and diabetes mellitus with insulin resistance, and normal intelligence (70, 71). Some features are thus similar to those of Bardet-Biedl syndrome. The genetic basis of Alström syndrome is being determined (72, 73). Modifier genes may also play an important role. There are numerous reports of associated hepatic dysfunction with Alström syndrome. One patient had mild steatosis, portal inflammation and moderate fibrosis (74), and others have had hepatic steatosis with cirrhosis (75). Still others have been described only as having hepatic dysfunction. An 8 year-old girl with Alström syndrome was obese and had hyperinsulinemia with type 2 diabetes mellitus and developed progressive liver disease with periportal and sinusoidal fibrosis as well as ballooned hepatocytes on liver biopsy at 5 years-old and decompensated cirrhosis at 8 years-old (76). Development of type 2 diabetes mellitus and hepatic dysfunction with steatosis has also been observed in adults with Alström syndrome (77). Recent reports indicate features of the metabolic syndrome in patients with Alström syndrome, who may have cardiac or hepatic disorders (78-80).

6.1.3. Alström/Bardet-Biedl combination

A brother and sister both had a multisystemic disease with some features of each syndrome as well as polycystic ovaries in the girl and both had decreased insulin receptor binding: one had hepatic steatosis (81).

6.2. Polycystic ovary syndrome (PCOS)

PCOS is a multi-system endocrine disorder of adolescent (82) and young adult women characterized mainly by disordered ovulation leading to menstrual disorders, features of androgen excess including hirsutism and acne, structurally abnormal ovaries. Obesity, usually central, occurs in only approximately half of patients. Acanthosis nigricans is frequently present (83). Insulin resistance appears to be due to insulin receptor abnormalities and to post-receptor mechanisms (84, 85). Hyperinsulinemia causes or intensifies the adverse effects of androgen excess. Hypertriglyceridemia is often present. To date no definite pediatric cases of PCOS with NAFLD have yet been reported, but this is probably because PCOS is often not diagnosed in this age-group. Modest weight loss (5-10% overall) or metformin has been found to improve ovarian function and diminish other features of androgen excess (86-88).

6.3. Lipodystrophy syndromes

Persons affected by lipodystrophy or lipoatrophy syndromes are strikingly thin and may look disproportionately muscular because they have complete or partial lack of adipose tissue. These syndromes are primary disorders of insulin action. Their key feature is hyperinsulinemia associated with relative insulin resistance. The most severely affected patients develop diabetes mellitus (89). NAFLD has been found in patients with congenital forms of lipodystrophy, including one who later required liver transplantation (90, 91). The severity of the hepatic steatosis is proportional to the extent of extrahepatic fat loss.

Lipodystrophy syndromes are genetically and clinically heterogeneous. In one type of congenital generalized lipodystrophy the gene *AGPAT2* is mutated (92). Its gene product, 1-acylglycerol-3-phosphate O-acyltransferase, isinvolved in production of triacylglycerol and glycerophospholipids. In a different form of congenital generalized lipodystrophy, mutations are found in BSCL2, which encodes a novel human protein, seipin, whose function is unknown (93). The gene abnormal in some autosomal familial partial lipodystrophies is *LMNA*, which encodes lamin A/C, a nuclear envelope protein (94). Lamin A has been shown to interact with sterol regulatory element binding proteins (SREBP) 1 and 2 (95). SREBP-1 may play a role in the pathogenesis of NAFLD. Other autosomal familial partial lipodystrophies are associated with mutations in *PPARG*, the gene for the peroxisome proliferator-activated receptor-gamma (96), also implicated in the pathogenesis of NAFLD. Clinical observations regarding severe fatty liver disease in lipodystrophy syndromes provide strong evidence that hyperinsulinemia associated with insulin resistance is critical to the pathogenesis of NAFLD. The diversity of genes and gene products abnormal in these syndromes may provide clues to the details of the disease mechanism of NAFLD.

6.4. Turner syndrome

Girls with Turner syndrome (XO) are often obese. In one report of liver abnormalities in Turner syndrome, two girls, 13 and 14 years-old, were shown to have steatosis and fibrosis on liver biopsy (97). In another report, a 4 year-old with Turner syndrome and severe obesity (body weight 159% of ideal weight for height) was reported as having elevated ALT years before starting hormone treatment, but NAFLD was not diagnosed definitively (98).

6.5. Cohen syndrome

Cohen syndrome is very rare and heterogeneous. The pattern of inheritance is autosomal recessive. Its main features are neurodevelopmental delay, hypotonia, distinctive facies, myopia and retinal abnormalities, granulocytopenia and obesity (99). NAFLD as such occurring with Cohen syndrome has not yet been reported in either adults or children, but a child with Cohen syndrome plus acanthosis nigricans and hyperinsulinemia/insulin resistance has been described (100).

6.6. Prader-Willi syndrome

This is a genetic disorder of imprinting which leads to a hypothalamic dysfunction syndrome whose

features include hyperphagia and obesity; other features include hypogonadism and mental retardation (101). Diabetes mellitus with insulin resistance may develop. A single patient has been reported who had NAFLD: the liver disease was diagnosed when the patient was 20 years-old, and insufficient detail was provided to determine actual age of onset (58). Acanthosis nigricans was present.

6.7. Kabuki (Niikawa-Kuroki) syndrome

This rare syndrome displays a characteristic facies as well as short stature, skeletal and palatal abnormalities, congenital heart disease, mental retardation, and various minor dysmorphisms (102). These children have poor growth in infancy but eventually become obese in mid-childhood or adolescence. Diabetes mellitus was reported in two patients but was characterized as being insulin-dependent and may indeed have been type 1 (103). Liver disease has been reported and in some cases appears to be a definable disorder of the biliary system such as extrahepatic biliary atresia. NAFLD as such has not yet been reported.

7. PATHOGENESIS

7.1. Hyperinsulinemia with insulin resistance

The pathogenesis of NAFLD is complex. Hyperinsulinemia, in association with insulin resistance, is now recognized as an essential component of the disease mechanism (45, 55, 104-106). In many adult patients, NAFLD represents the hepatic presentation of the "metabolic syndrome" (107, 108), sometimes denoted "dysmetabolic syndrome" or "Syndrome X." Increased plasma free fatty acids and/or increased free fatty acid concentrations in hepatocytes likely play an important role in the development of steatosis and inflammation (109, 110). Free fatty acids are highly destructive in tissues, free fatty acids damage intracellular membranes through lipid peroxidation and injure mitochondria resulting in decreased beta-oxidation of hepatic free fatty acids. Insulin inhibits oxidation of free fatty acids, and thus hyperinsulinemia may enhance free fatty acid hepatotoxicity. NAFLD is apparently polygenic, and candidate predisposing genes are being identified.

The disease mechanisms in childhood NAFLD are probably the same as in adults; however, few specific data regarding the pathogenesis of childhood NAFLD are currently available. Acanthosis nigricans, which has been reported in several clinical series of childhood NAFLD (56), provides evidence for hyperinsulinemia in the etiology of childhood NAFLD (111-114). Acanthosis nigricans is darkening of the skin at the nape of the neck, in the axillae, groin and sometimes in other flexural areas. It is occasionally mistaken for dirty skin due to poor personal hygiene. Acanthosis nigricans is due to hyperplasia of pigmented skin cells bearing receptors for insulin and insulin-like growth factors (115). In an unselected cohort of adolescents aged 12-15 years 128 (19%) had acanthosis nigricans; of these 39% had hyperinsulinemia and 49% were obese. Multiple linear regression revealed that only acanthosis nigricans, obesity and physical activity were statistically significant predictors of hyperinsulinemia.

Children with acanthosis nigricans and obesity early in life may be exhibiting the earliest clinical features of the metabolic syndrome (116). Acanthosis nigricans is a feature of several of the paediatric metabolic/genetic diseases where insulin receptors or their function is abnormal (81, 117, 118). Acanthosis nigricans was present in 92% of children with type 2 diabetes mellitus (119). Recent pediatric studies document biochemically both the presence of hyperinsulinemia and insulin resistance in children with NAFLD (63, 120). In a very recent study of 490 children, 10 to 13 years-old including Blacks and Hispanics as well as Caucasians, 39% of moderately obese and 50% of severely obese children met strict, childhood-appropriate criteria for the metabolic syndrome whereas no overweight child did (121). A strong correlation was found between BMI expressed as z score and insulin resistance. Prevalence of metabolic syndrome in these children increased significantly with increasing insulin resistance irrespective of race or ethnic background. In this study girls were at a lower risk for the metabolic syndrome than boys.

7.2. Adipocytokines

Adipose tissue is not simply a fat depot where mainly triglyceride is stored; it produces metabolically active proteins (hormones or cytokines), collectively known as "adipocytokines." Some of these modulate insulin action: tumor necrosis factor-alpha (TNF-alpha), adiponectin, resistin, adiponutrin, soluble preadipocyte factor-1. Of these, adiponectin may play a most important role in the pathogenesis of NAFLD. Adiponectin is a small protein in the collagen superfamily and has a tertiary similar to that of TNF-alpha. In addition to anti-inflammatory actions, adiponectin modulates insulin action, in part by affecting insulin receptor function and by influencing hepatocellular free fatty acid metabolism (122). Deficient adiponectin secretion is associated with insulin resistance (122, 123). Numerous studies indicate that obesity in children is typically associated with low serum adiponectin concentrations (124, 125). Moreover, severity of insulin resistance in obese children is inversely correlated with serum adiponectin concentration (121, 126). Understanding the regulation of the adiponectin gene and the physiological regulation of adiponectin itself may afford new options for treatment of childhood NAFLD.

Leptin is another adipocytokine regulating eating behaviours and nutrient utilization (127, 128). Leptin resistance is a feature of the metabolic syndrome (129). Since elevated serum concentrations of leptin, along with leptin resistance, may be found in NAFLD, simple supplementation along this regulatory pathway is not likely to be effective and thus is inappropriate. This is not, however, the case in generalized lipodystrophy syndromes characterized by defective expression of leptin where leptin supplementation has improved hepatic steatosis (130).

8. DIAGNOSIS OF CHILDHOOD NAFLD

At the present time the typical patient with childhood NAFLD is at least of kindergarten age (4-5 years-old) and usually is 10-14 years old. With respect to gender distribution, childhood NAFLD is somewhat more

Table 2. Causes in children of fatty liver with mainly large-droplet fat accumulation (macrovesicular steatosis)

- ❑ **General**
  - Acute systemic disease: dehydration, severe infection
  - Protein-calorie malnutrition (kwashiorkor)
  - Acute starvation
  - Schwachman-Diamond syndrome (pancreatic insufficiency)
  - Celiac disease
  - Inflammatory bowel disease (ulcerative colitis, Crohn's disease)
  - Diabetes mellitus (including Mauriac syndrome)
  - Nephrotic syndrome
  - Total parenteral nutrition
  - Status post surgical treatment of obesity: jejuno-ileal bypass; gastric reduction
  - Non-alcoholic fatty liver disease (and related inherited diseases—*see table 1*)
- ❑ **Infections**
  - Hepatitis C
- ❑ **Metabolic**
  - Cystic fibrosis
  - Wilson disease
  - Galactosemia
  - Hereditary fructose intolerance
  - Glycogen storage diseases (mainly types I, VI)
  - Sialidosis
  - Mannosidosis
  - Fucosidosis
  - Hereditary tyrosinemia, type 1 (fumaryl acetoacetate hydrolase mutations)
  - α_1-antitrypsin deficiency
  - Homocystinuria
  - Refsum disease
  - Abeta- or hypobetalipoproteinemia
  - Neutral lipid storage disease
  - Wolman disease
  - Cholesterol ester storage disease
  - Tangier disease
  - Familial hyperlipoproteinemias
  - Citrullinemia
  - Argininemia
  - Arginosuccinic aciduria
  - Acute intermittent porphyria
  - Weber-Christian disease
  - Chronic granulomatous disease
  - Porphyria cutanea tarda
  - Dorfman-Chanarin syndrome
- ❑ **Drug toxicity**
  - Amiodarone
  - Methotrexate
  - Prednisone/glucocorticoids
  - L-asparaginase
  - Vitamin A
  - Ethanol

frequent in males. The child is usually overweight or obese by age- and gender-normative reference data. Some affected children are tall for age and proportionally heavy, typical of generalized overnutrition. Most children are asymptomatic, but some present with non-specific abdominal pain. Serum aminotransferases may be normal, but if they are elevated, the overall abnormality is only moderate (2-3 times the upper limit of normal). Serum ALT may be higher than AST. Hyperlipidemia may be present. It is usually due to elevated serum triglycerides, although hypercholesterolemia may occur. Children may have both NAFLD and some type of chronic viral hepatitis. The clinical management of chronic hepatitis B or C coincident with NAFLD is an unresolved problem.

Many inherited metabolic diseases involve the liver, and in some disorders, as shown in table 2, large-droplet steatosis may be present (131). In general the mechanisms by which steatosis develops with these diseases differ from the pathogenesis of NAFLD, and therefore hepatic steatosis associated with specific metabolic diseases must be differentiated from childhood NAFLD. Since NAFLD has not yet been identified in infants or toddlers, the differential diagnosis really focuses on inherited metabolic disorder with hepatic steatosis in early to mid childhood. Many of these disorders can be identified by typical findings on medical history or with specific laboratory testing. Wilson disease requires specific consideration in the pediatric age bracket. Wilson disease may present with prominent hepatic steatosis, and it can be notoriously difficult to diagnose in younger patients. Chronic hepatitis C, which may also have some degree of steatosis, can be identified by virological testing. The relative contribution of chronic hepatitis C and the metabolic syndrome to childhood NAFLD must be judged on an individual basis for each patient. Drug hepatotoxicity, especially with methotrexate, should be excluded. The propensity of corticosteroids to cause fatty liver is highly variable. If regularly feeding wine or other alcoholic beverages to children is a local custom, this cause of hepatic steatosis and potentially progressive liver disease should be identified and stopped. Fatty liver is found in 30-40% of cystic fibrosis: cystic fibrosis should be excluded by sweat testing. Rare genetic/metabolic disorders with hepatic steatosis are usually easy to diagnose by their extrahepatic features.

Microvesicular steatosis is sometimes identified by liver sonography. Associated childhood liver diseases (such as inherited mitochondrial disorders, urea cycle disorders, and valproic acid hepatotoxicity) should be investigated if any clinical features of these disorders are present or if the child is receiving treatment with any medication which can cause mitochondrial injury. With respect to urea cycle disorders, although citrullinemia, argininemia and argininosuccinic acidura can present with macrovesicular steatosis, ornithine transcarbamylase deficiency usually presents with microvesicular fat. Likewise, systemic carnitine deficiency almost always presents with microvesicular steatosis.

In general, in children the first diagnostic steps involve identification of hepatic steatosis by a non-invasive technique, excluding other causes of fatty liver, and documenting overweight or obesity as well as evidence for the metabolic syndrome. Obese/overweight children with no other cause for hepatic steatosis and normal liver biochemistries are considered as having simple steatosis, and those with elevated serum ALT may be regarded as having "presumed NASH." Cirrhosis due to NASH in children may be under-diagnosed. Liver biopsy is important for making the definitive diagnosis of NAFLD and should be included in the assessment of children presenting with hepatic steatosis and abnormal serum aminotransferases. It may elucidate other potential diagnoses besides NASH. The essential findings of macrovesicular steatosis, inflammation or its residua, and fibrosis are present in the

majority of cases (56). Whether liver biopsy should be part of the initial evaluation is controversial, and liver biopsy is not uncommonly deferred until after a 3-6 month trial of weight loss treatment.

9. TREATMENT OF CHILDHOOD NAFLD

The only treatment thus far shown to be really effective in childhood NAFLD is weight loss; however, very few pharmacological treatments have been investigated in children. The usual reported endpoints for judging efficacy are normalization of serum aminotransferases or loss of steatosis by sonography. Histological improvement on liver biopsy has rarely been evaluated in children.

9.1. Weight loss

Reduction in body weight has been effective in leading to normalization of serum aminotransferases in several clinical series (50, 56, 132). Substantial improvement in liver histology was reported in one pediatric patient (50). Whereas in adults a 5-10% loss of initial weight is thought to be adequate, it is not known how much weight must be lost to achieve improvement in childhood NAFLD. The usual strategy is to reduce caloric intake and increase moderately intense aerobic exercise. Adequate nutrition for general growth must be maintained. Well-designed studies examining treatment of childhood obesity have shown that a diet specifically designed to minimize postprandial hyperinsulinemia may be more effective than the conventional low-calorie diet (133). This "low glycemic index diet" may be easier to maintain long-term than a calorie-restricted diet and consists of comparatively straightforward food inclusions/restrictions. Fruits, vegetables, legumes, whole-grain or high fiber or traditionally-processed grains and pasta, and dairy products are included. Consumption of white potatoes, sugar (sucrose), and highly refined white flour is greatly limited (134). Complementing the child's weight reduction regimen with family-based behavioral intervention may also enhance success (135). Instituting a regimen of regular physical exercise is important because exercise also reduces hyperinsulinemia. Exercise may also have strategic benefits by giving the child a sense of well-being along with some control and input into design of the weight reduction program.

A perceived drawback of the low glycemic index diet is that it is somewhat complicated and complex for regular use, especially by children and teen-agers. Additionally, excessive dietary intake of saturated fats impairs insulin sensitivity (136). A possible alternate dietary approach, based mainly on practicality, to a great extent mimics the low glycemic index diet: excessively fatty foods are avoided, and specifically table sugar (sucrose) is eliminated from the child's diet. This has the effect of removing sugary drinks including soda pop and sugary snack foods which are also high in fat from the child's regular diet. Most children can figure out whether or not a food has white (that is, table) sugar in it. The normal pattern of nutrients required for normal growth is not disturbed. No systematic data are yet available as to the efficacy of this dietary strategy.

9.2. Drug treatment

Data about treating childhood NAFLD pharmacologically are scarce. A small randomized controlled trial involving 31 children with NAFLD diagnosed by sonography showed that ursodeoxycholic acid (10-12.5 mg/kg/day) provided no additional benefit over weight reduction diet, and was ineffective on its own, in improving the liver biochemistries and the sonographic appearance (132). Although this trial was well-designed it was quite small. Treatment with vitamin E (400-1200 IU/day by mouth) in an open-label pilot study in 11 children with NASH was associated with improvements in serum aminotransferases, despite no major change in body mass index or in sonographic appearance of the liver. Biochemical relapse occurred in two children who discontinued vitamin E (137).

Although metformin has been used for treatment of NASH in adults (138), it has not been used in children with NAFLD. Some pediatric experience with metformin is available in relevant childhood diseases. Metformin has also been used in children with acanthosis nigricans with improvement (114) and in obese adolescents not necessarily having NASH (139). In these adolescents the combination of metformin plus low-calorie diet worked better than the low-calorie diet alone. It has also been used effectively for treatment of children with type 2 diabetes mellitus (119, 140), polycystic ovary syndrome (86, 141), and Prader-Willi syndrome (142). Betaine has been used in a small number of adults with NASH, but it has not been examined in childhood NASH. Although betaine is commonly used in children for various disorders of homocysteine metabolism, its overall safety remains undetermined. Thiazolidinediones are being investigated as treatment for NASH in adults but have not been studied in childhood NAFLD.

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Abbreviations: ALT: alanine aminotransferase; AST: aspartate aminotransferase; BMI: body mass index; GGT: gamma-glutamyl transpeptidase; HOMA-IR: homeostasis model assessment of insulin resistance; NAFLD: non-alcoholic fatty liver disease; NASH: non-alcoholic steatohepatitis; PCOS: polycystic ovary syndrome; SREBP: sterol response element binding protein; TNF-alpha: tumor necrosis factor-alpha

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