

Chapter 10: Non-Alcoholic Steatosis

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Introduction

Steatosis, the accumulation of fat in hepatocytes, is a feature of many liver diseases. Steatosis is categorized as 'large-droplet' (macrovesicular) when one or a few fat droplets nearly fill the hepatocyte or a 'small-droplet' (microvesicular) when numerous tiny droplets are found, giving the hepatocyte a somewhat foamy appearance. Inflammation may also be present. In adults, alcoholic liver disease is a common cause of steatosis, but although alcoholic liver disease may occur in adolescents it is rare in younger children. In children the causes of steatosis include certain drugs, such as methotrexate, inherited metabolic disorders, and liver disease associated with obesity and/or disordered action of insulin, known as non-alcoholic fatty liver disease (NAFLD), which is the major focus of this chapter. Microvesicular steatosis, associated with mitochondrial dysfunction, is discussed in detail in Chapters 5 and 12.

NAFLD in adults

Childhood NAFLD needs to be considered in the context of the disease in adults. In the late 1970s chronic liver disease associated with obesity (with or without hyperlipidaemia and with or without non-insulin-dependent diabetes mellitus) was described as a new and separate disease entity (Adler & Schaffner 1979; Ludwig *et al.* 1980). This condition was originally called 'non-alcoholic steatohepatitis' (NASH) because it resembled alcoholic hepatitis histologically but was not due to ethanol abuse. Recently, the more inclusive term 'non-alcoholic fatty liver disease' (NAFLD) has been favoured because it includes the entire spectrum: simple hepatic steatosis without inflammation, NASH, and the resulting cirrhosis in which steatosis may no longer be prominent. NAFLD is highly prevalent in adult populations (Lee 1989; Wanless & Lentz 1990). NAFLD occurs in both men and women,

and although most adult patients with NAFLD are obese (body mass index >30), and many have type 2 diabetes mellitus, not all affected patients are obese (Bacon *et al.* 1994). They may be asymptomatic or have only nonspecific constitutional symptoms. The serum aminotransferases are modestly elevated, but serum bilirubin is normal or near-normal and biochemical features of cholestasis are not present. Findings suggestive of an autoimmune process may be present, such as increased serum IgG and detectable nonspecific tissue autoantibodies. Hyperlipidaemia is usually due to hypertriglyceridaemia.

Hepatic histology is required for defining the NAFLD lesion rigorously (Brunt 2001). In adults there is a broad spectrum of findings: macrovesicular or rarely microvesicular steatosis without or with active inflammation; mild to moderate fibrosis or cirrhosis. If there is no inflammation, then this is considered to be simple steatosis, as by definition NASH involves inflammation and/or fibrosis (see Plate 35, Atlas: p. 452). Signs of inflammation include ballooning degeneration of hepatocytes or focal hepatocyte drop-out. With mild NASH, macrophages may be the only inflammatory cell present, but in more active lesions neutrophils are usually found, typically adjacent to degenerating hepatocytes. Mallory's hyaline may be found in hepatocytes. If it is not present, histological staining for ubiquitin may reveal this feature (Banner *et al.* 2000). 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's disease and medications/herbs causing a 'pseudo-alcoholic hepatitis' lesion. Only a shaky consensus currently 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.

Until recently NAFLD in adults has been regarded as a trivial disorder. Its increasing prevalence and the accu-

mulating evidence for severe outcomes have reversed this assessment. Most adults with pure steatosis do not have progressive liver disease (Teli *et al.* 1995) and weight reduction generally leads to improvement in serum aminotransferases (Palmer & Schaffner 1990). However, at least 8–17% of adults with steatosis with inflammation and fibrosis ultimately develop cirrhosis (Lee 1989; Powell *et al.* 1990; Wanless & Lentz 1990; Willner *et al.* 2001). In one study of adults with an average follow-up of 6.5 years, Kaplan–Meier analysis showed that the 5-year probability of survival in NAFLD was 67% and the 10-year survival was 59% (Propst *et al.* 1995). Some adult patients with NAFLD eventually require liver transplantation, and fatty liver disease may reoccur after liver transplant (Molloy *et al.* 1997; Charlton *et al.* 2001; Contos *et al.* 2001; Ong *et al.* 2001). It appears that hepatocellular carcinoma can complicate NAFLD in adults (Zen *et al.* 2001; Ratziu *et al.* 2002; Shimada *et al.* 2002).

Pathogenesis of NAFLD

Hyperinsulinaemia

The pathogenesis of NAFLD remains unknown (Fig. 10.1). Hyperinsulinaemia, in association with insulin resistance, is accepted as an essential component of the disease mechanism (Marchesini *et al.* 1999; Tankurt *et al.* 1999; Paradis *et al.* 2001; Sanyal *et al.* 2001; Willner *et al.* 2001; Chitturi *et al.* 2002; Pagano *et al.* 2002). In many patients, NAFLD appears to be a part of the metabolic syndrome currently known as 'syndrome X' (Cortez-Pinto *et al.* 1999; Luyckx *et al.* 2000). Differences between insulin resistance in the liver and peripheral tissues may account for some of the features of NAFLD. The greater insulin resistance in muscles and adipose tissue compared with liver leads to mobilization of free fatty acids, and hepatocellular deposition of free fatty acids. Increased plasma free fatty acids and/or increased free fatty acid concentrations in hepatocytes probably also play an important role in the development of steatosis and inflammation (Wanless & Lentz 1990; de Almeida *et al.* 2002). Free fatty acids are highly destructive in tissues and cause damage to intracellular membranes through lipid peroxidation and injury to mitochondria resulting in decreased β -oxidation of hepatic free fatty acids. Insulin inhibits oxidation of free fatty acids, and thus hyperinsulinaemia may enhance free fatty acid hepatotoxicity. Recent findings implicate SREBP-1c (sterol response element binding protein-1c), which regulates hepatocellular glucose, triglyceride synthesis and fat metabolism, as a key player in the intracellular mechanisms leading to hepatocyte damage (Foufelle & Ferre 2002; Horton *et al.* 2002). Up-regulation of SREBP-1c due to hyperinsulinaemia probably accounts for increased hepatocellular production of triglycerides and very low-density lipoproteins, and thus

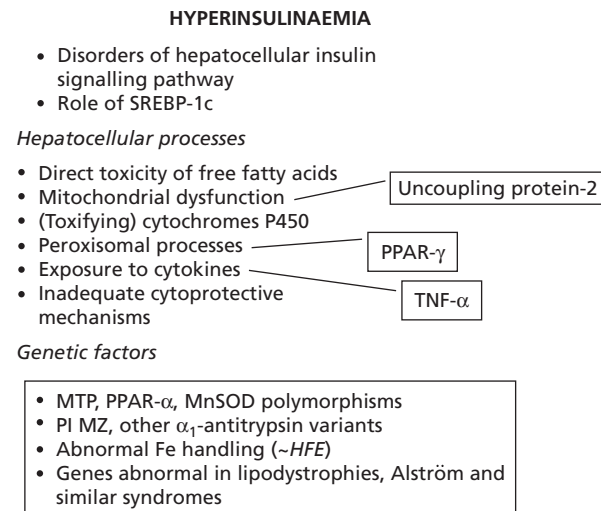


Fig. 10.1 Elements in the pathogenesis. Non-alcoholic fatty liver disease (NAFLD) is typical of a polygenic disorder with a complex interaction between environment (increased carbohydrate and fat intake, increased sedentary activity) and host, including genetic factors. Contributors to the pathogenesis of NAFLD may differ from patient to patient. Details of this disease mechanism remain obscure at the current time.

the hypertriglyceridaemia, which is characteristic of NAFLD.

Current concepts about the pathogenesis of NASH suggest that a second factor is needed to account for the inflammation and fibrosis (Day & James 1998; Chitturi & Farrell 2001). Candidate factors include mitochondrial dysfunction, functional changes due to cytokines such as tumour necrosis factor (TNF)- α , increased toxification (for example, through hepatocellular cytochromes P450) or decreased cytoprotection in hepatocytes. Ongoing low-grade inflammation has been documented in obese adults, mainly with C-reactive protein and serum fibrinogen as sensitive markers for systemic inflammation (Visser *et al.* 1999; Festa *et al.* 2001; Pannacciulli *et al.* 2001); similar findings have been reported in obese children (Visser *et al.* 2001). Mitochondrial dysfunction may be important in the more severe forms of NAFLD since mitochondria play an important role in fat metabolism and are also an important source of reactive oxygen species in hepatocytes (Pessayre *et al.* 2001, 2002). Low serum levels of antioxidants such as vitamin E have been found in obese children (Decsi *et al.* 1997; Strauss 1999).

It is likely that similar mechanisms operate in children with NAFLD, but there are few studies documenting this. As in adults, hyperinsulinaemia has an important role in the aetiology (Cruz & Hud 1992; Brockow *et al.* 1995; Stuart *et al.* 1998; Hermanns-Le *et al.* 2002). Acanthosis nigricans (darkening of the skin) has been reported in several clinical series of childhood NAFLD (Rashid &

Roberts 2000). The darkening of the skin is usually at the nape of the neck, in the axillae, groin and sometimes in other flexural areas. Often subtle, it is generally easier to identify in children with increased skin pigmentation. It is due to hyperplasia of pigmented skin cells bearing receptors for insulin and insulin-like growth factors (Torley & Munro 2002). In an unselected cohort of adolescents aged 12–15 years 128 (19%) had acanthosis nigricans; of these 39% had hyperinsulinaemia and 49% were obese. Multiple linear regression revealed that only acanthosis nigricans, obesity and physical activity were statistically significant predictors of hyperinsulinaemia. Children with acanthosis nigricans and obesity early in life may have the earliest clinical features of the metabolic syndrome X (Kerem *et al.* 2001).

Acanthosis nigricans is also a feature of several of the paediatric metabolic/genetic diseases in which there is a quantitative or functional abnormality of insulin receptors (Kahn *et al.* 1976; Yanovski & Arioglu 2001). Many of these disorders such as Alström syndrome, Bardet–Biedl syndrome, and polycystic ovary syndrome are associated with NAFLD (Boor *et al.* 1993), while acanthosis nigricans was present in 92% of children with type 2 diabetes mellitus (Zuhri-Yafi *et al.* 2002).

Leptin

Leptin, which is a satiety factor synthesized in white adipose tissue, binds to hypothalamic receptors to reduce appetite and increase energy expenditure. It has recently been implicated in the pathogenesis of NASH (Chitturi & Farrell 2001). Genetically obese, diabetic mice (*ob/ob* mice) have a mutation that prevents the synthesis of leptin and thus they overeat, becoming obese. They also have insulin resistance, hyperlipidaemia and fatty livers (Pelleymounter *et al.* 1995). Leptin levels are also low in lipoatrophic diabetic mice, which have insulin resistance and fatty livers due to over-expression of SREBP-1 (Shimano *et al.* 1996). Treatment with leptin reduces both insulin resistance and the fatty livers in these mice (Shimomura *et al.* 1998).

Although leptin levels are low in humans with lipodystrophy syndromes, they are high in obese humans, suggesting resistance or dysfunctional receptors (Chitturi & Farrell 2001). Leptin resistance has also been identified as a feature of syndrome X (Kennedy *et al.* 1997). Thus treatment of childhood NASH with leptin is unlikely to be of benefit in most individuals.

Genetic factors

Attempts to identify a genetic basis include examining the genes involved in formation of adipose tissue, the development of insulin resistance, of alcoholic liver disease or related metabolic diseases. The gene encoding 11 β -

hydroxysteroid dehydrogenase 1, which converts cortisone to cortisol, may be important in metabolic syndrome X (Masuzaki *et al.* 2001), while the genes responsible for the lipodystrophy syndromes and other metabolic diseases with insulin receptor abnormalities may also be involved.

Apolipoprotein E, which is polymorphic and functions as a modifier protein for other hepatic disorders, may function as a modifier gene in NAFLD (Mensenkamp *et al.* 2000). Preliminary data implicate a low-activity promoter polymorphism in the MTP gene (Bernard *et al.* 2000), *PPARA*3* encoding PPAR- α , and the mitochondrial manganese superoxide dismutase (MnSOD). Genetic variability in the immune response may also be important: a polymorphism in the promoter region of the TNF- α gene has been identified associated with NAFLD, but its mechanistic role requires further clarification (Grove *et al.* 1997; Valenti *et al.* 2002). Underlying metabolic abnormalities may act as disease modifiers. For instance, heterozygosity for *HFE*, the gene abnormal in hereditary haemochromatosis, is associated with more extensive hepatic fibrosis (George *et al.* 1998). Partial deficiency of α_1 -antitrypsin is prevalent among patients with NAFLD but its pathogenic role is unclear (Czaja 1998).

Childhood obesity

Childhood obesity has become a major public health problem (Strauss & Pollack 2001). Current estimates of the prevalence of overweight and obesity in children depend on the definitions used. Definitions may use absolute weight, percent of ideal weight-for-height or age- and gender-normative data for body mass index (BMI), in which it is accepted that a BMI of >25 is considered overweight and a BMI of >30 is considered obese (Power *et al.* 1997; Kiess *et al.* 2001). These definitions hold only for adolescents over 16 years old and have to be redefined on an age-related basis for younger children. Approximately 12–25% of children are considered obese. A recent study indicates that nearly 40% of Canadian children aged 2–13 years are either overweight or obese. In the USA, the prevalence of obesity has increased since 1986, especially in certain ethnic groups: to 21.5% for Afro-American children, 21.8% for Hispanic children, and 12.3% for non-Hispanic Caucasian children. Similar data are available from Europe (Livingstone 2000, 2001). Current dietary patterns, including high carbohydrate snacks and beverages, less physical activity with more time spent watching television or working at a computer, are important factors for this epidemic of childhood obesity. Although the exact relationship between childhood obesity and type 2 diabetes mellitus is uncertain, the incidence of both primary hypertension and type 2 diabetes mellitus, both of which are within the spectrum of the metabolic syndrome and NASH in adults, has risen dramatically in

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children in the past few years (Power *et al.* 1997; Fagot-Campagna *et al.* 2000).

Whether obesity in childhood and adolescence inevitably leads to obesity in adulthood is a complex question (Power *et al.* 1997; Dietz 1998), which has not been fully proven. Recent studies indicate that childhood obesity is associated with greater morbidity in adulthood due to a variety of diseases, whether or not the individual remains obese (Kieiss *et al.* 2001).

Epidemiology of NASH/NAFLD in children

Several large series of childhood NAFLD have been reported, most of which involve some selection, either referral bias or previous liver histology making it difficult to determine the prevalence of NAFLD in children.

The first reports of childhood NASH appeared in the 1980s. Moran and colleagues reported three obese American children with fatty liver and steatohepatitis (Moran *et al.* 1983). These children, two boys and a girl, were 10–13 years old and had BMIs ranging from 28.5 to 33.2 at presentation. Serum aminotransferases were elevated (ALT higher than AST), and liver biopsy revealed macrovesicular steatosis with variable degrees of inflammation and fibrosis. Extensive clinical assessment revealed no other liver disease and the patients had biochemical improvement of hepatic indices when they lost weight.

Since then a number of small studies have documented the relationship between obesity, elevated aminotransferases, hypertriglyceridaemia, hyperlipidaemia (Vajro *et al.* 1994; Kocak *et al.* 2000) and ultrasonographic evidence of fatty liver (Tazaura *et al.* 1997) while others confirmed the presence of steatohepatitis (Baldrige *et al.* 1995), fibrosis and even cirrhosis in one child who had long-standing obesity, maturity-onset diabetes mellitus and hyperlipidaemia (Kinugasa *et al.* 1984).

The largest paediatric NAFLD series published to date is of 36 patients from a variety of ethnic backgrounds collected prospectively between 1985 and 1995 at the Hospital for Sick Children in Toronto (Rashid & Roberts 2000). The male:female ratio was 3:2, and patients were 4–16 years old at time of diagnosis, 20% of whom were less than 10 years old at diagnosis. Most patients were obese (weight >20% above ideal weight for height): the range was 114–192% of ideal weight for height. Approximately 30% of the cohort had acanthosis nigricans; two brothers had Bardet–Biedl syndrome; one adolescent female had polycystic ovary syndrome; one child had severe hypothyroidism, and another was heterozygous for α_1 -antitrypsin (PI MZ). Non-specific autoantibodies, most often low-titre antismooth muscle antibodies (a feature now being reported in adults with NAFLD), were present in a few patients. Four children developed diabetes mellitus after the diagnosis of NAFLD was established. Liver biopsy was performed in 24 patients, 71% of whom had

some fibrosis. One child had cirrhosis at 9 years old. Weight loss was associated with improvement in serum aminotransferases.

Series of 17 patients reported from Adelaide (Manton *et al.* 2000) and 27 from Montreal (Sathya *et al.* 2002) had similar findings. Most of the children were obese and asymptomatic, although 30% had either abdominal pain or fatigue. Hypertriglyceridaemia was found in 56% of the patients and hyperuricaemia in 18% (Sathya *et al.* 2002). Liver biopsies showed fibrosis in nine of 17 biopsies, with 'bridging' or 'early bridging' in three of these and 'evolving cirrhosis' in another from an 11-year-old male (Manton *et al.* 2000). Weight loss was the most effective treatment, defined as normalization of serum aminotransferases.

Thirty-nine children, predominantly male, with NAFLD have been described from Texas (Squires RH, Jr. and Lopez MJ, personal communication) which confirmed the pattern already described, but highlighted the importance of ethnicity: 20 were Hispanic, 14 Caucasian, four Asian, and one Afro-American. Five children had recovered from childhood cancer. Acanthosis nigricans was present in more than 40%, fasting serum insulin was three times the upper limit of normal in 17 patients. Thirty-one children underwent liver biopsy, which revealed steatohepatitis in all 31, fibrosis in 21 and cirrhosis in two children. In another report two boys, aged 10 and 14 years, were described with NAFLD, which progressed rapidly to cirrhosis; the older patient had had a craniopharyngioma resected (Molleston *et al.* 2002).

Familial clustering of NAFLD was prominent in a large American study (Willner *et al.* 2001), which highlighted a 14-year-old male with cirrhosis whose mother also had NAFLD with less severe fibrosis.

Unselected studies of schoolchildren give a better picture of the prevalence of NASH and obesity. An ultrasonographic study of 810 school children from northern Japan demonstrated an overall prevalence of fatty liver in 2.6%, which had a strong correlation to indices of obesity such as BMI (Tomimaga *et al.* 1995). In the National Health and Examination Survey, cycle III (NHANES III) in the USA serum ALT and gamma-glutamyl transpeptidase were measured in 2450 children, aged 12–18 years, who were classified as 'obese' if the BMI was >95th percentile for age and gender or 'overweight' if it was between the 85th and 95th percentiles. In this relatively unselected study, 6% of overweight, and 10% of obese, adolescents had an elevated ALT, but alcohol use could not be excluded (Strauss *et al.* 2000). Elevated ALT and fatty liver on sonography was 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 studies in which obese children were screened for abnormal serum aminotransferases the prevalence was

10–25% (Bergomi *et al.* 1998), but sonographic features of fatty liver were also found in five of 27 (19%) children who had normal ALT (Tazawa *et al.* 1997).

Clinical features

The most common clinical presentation is the incidental finding of isolated hepatomegaly or slightly elevated hepatic aminotransferases in a child who may have:

- Obesity (BMI >25). It is essential to use an age- and gender-adjusted guide to BMI for children since the adult thresholds of 'overweight' and 'obesity' only apply to individuals over 16 years old. Thus the degree of adiposity may be greatly underestimated in young children. The utility of waist circumference or waist-hip ratio for tracking adiposity has not been established throughout the paediatric age bracket and may not be relevant prior to puberty.
- Type 1 or 2 diabetes mellitus. The association of fatty liver and insulin resistance in adults with type 2 diabetes has been well defined and occurs in 10–75%. It is now thought to be a significant factor for mortality (de Marco *et al.* 1999). The prevalence in children has not been as well established. Fatty liver has also been described in children with type 1 diabetes mellitus, particularly if it is not well controlled (Mauriac's syndrome).
- Previous chemotherapy. Obesity with or without the metabolic syndrome of obesity, hyperinsulinaemia, and hyperlipidaemia (Talvensaari *et al.* 1996a,b) in survivors of childhood neoplasia is a well-recognized problem, particularly following acute lymphoblastic leukaemia (Odame *et al.* 1994; Mayer *et al.* 2000; Reilly *et al.* 2000). This may explain a higher incidence of NAFLD, although other factors, such as the drug treatment for childhood neoplasia, may play a role.

Hypothalamic surgery

Fatty liver has been reported following neurosurgery for various suprasellar tumours (Evans *et al.* 2002). Neurosurgical excision of these rare tumours is followed by pituitary dysfunction, hyperphagia and rapid weight gain. In all the four children studied, fatty liver developed rapidly and persisted long term, with the development of cirrhosis and portal hypertension in one.

Metabolic disease

Fatty liver is associated with a number of metabolic or genetic diseases, which include the following.

Alström syndrome Alström syndrome is a rare autosomal recessive disorder which is similar to Bardet-Biedl syndrome. The genetic basis of Alström syndrome is being determined (Collin *et al.* 2002; Hearn *et al.* 2002), but it is

thought that modifier genes may play a role. It is characterized by pigmentary retinopathy with infantile cone-rod dystrophy, obesity, sensorineural deafness, dilated cardiomyopathy, diabetes mellitus with insulin resistance, and normal intelligence (Michaud *et al.* 1996; Russell-Eggitt *et al.* 1998). There are numerous reports of associated hepatic dysfunction with Alström syndrome (Marshall *et al.* 1997; Chang *et al.* 2000; Chen *et al.* 2000; Chou *et al.* 2000; Hung *et al.* 2001). The liver disease has been attributed to alcoholic liver disease (Sebag *et al.* 1984), with mild steatosis, portal inflammation and moderate fibrosis (Connolly *et al.* 1991), or hepatic steatosis with cirrhosis (Awazu *et al.* 1995, 1997). An 8-year-old girl with Alström syndrome who was obese with hyperinsulinaemia with non-insulin dependent (type 2) diabetes mellitus developed progressive liver disease. She had periportal and sinusoidal fibrosis, with ballooned hepatocytes on liver biopsy at 5 years old and decompensated cirrhosis at 8 years old (Quiros-Tejeira *et al.* 2001). Development of type 2 diabetes mellitus and hepatic dysfunction with steatosis has also been observed in adults with Alström syndrome (Satman *et al.* 2002).

Bardet-Biedl syndrome Bardet-Biedl syndrome is characterized by progressive loss of visual acuity due to 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 in only a few patients (Green *et al.* 1989). Non-insulin dependent (type 2) diabetes mellitus may develop in these patients because of defective insulin receptor function (Escallon *et al.* 1989; Green *et al.* 1989; Iannello *et al.* 2002). Bardet-Biedl syndrome is genetically heterogeneous with at least six different loci associated with the phenotype (Katsanis *et al.* 2001a,b); candidate gene products may be responsible for the functional disorder or act as gene modifiers (Hamacher *et al.* 2001; Mykytyn *et al.* 2001). Cirrhosis has been reported in one patient previously (Pagon *et al.* 1982).

Alström/Bardet-Biedl like Occasional patients cannot be categorized as either syndrome. A brother and sister both had a multisystemic disease with some features of each syndrome as well as polycystic ovaries in the girl: one had hepatic steatosis and both had decreased insulin receptor binding (Boor *et al.* 1993).

Polycystic ovary syndrome (PCOS) PCOS is a multisystem endocrine disorder of adolescent (Lewy *et al.* 2001) and young adult women characterized mainly by disorders of ovulation with menstrual disorders, features of androgen excess including hirsutism and acne, and structurally abnormal ovaries. Central obesity occurs in half the patients. Acanthosis nigricans is frequently present

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(Conway & Jacobs 1990). Insulin resistance appears to be due to insulin receptor dysfunction or to postreceptor mechanisms (Dunaif 1997; Parsanezhad *et al.* 2001). Hyperinsulinaemia intensifies the adverse effects of androgen excess. Hypertriglyceridaemia is often present. To date few paediatric cases of PCOS with NAFLD have yet been reported but this may be because PCOS is a disease of adolescence. Modest weight loss (5–10% overall) or metformin improves ovarian function and diminishes other features of androgen excess (Batukan & Baysal 2001; De Conciliis *et al.* 2001; Nardo & Rai 2001).

Turner syndrome Girls with Turner syndrome (XO) are often obese. Abnormal liver biochemistry has been attributed to hormonal treatment, including administration of growth hormone and oestrogen, as in two girls, 13 and 14 years old, who had steatosis and fibrosis on liver biopsy (Salerno *et al.* 1999). Another 4-year-old with Turner syndrome and severe obesity (body weight 159% of ideal weight) had elevated ALT years prior to hormone treatment (Sato *et al.* 2001).

Lipodystrophy Lipodystrophy/lipoatrophy syndromes are primary disorders of insulin action, and hyperinsulinaemia is associated with relative insulin resistance. They are genetically heterogeneous. AGPAT2 is mutated in congenital generalized lipodystrophy (Agarwal *et al.* 2002). The gene product, 1-acylglycerol-3-phosphate O-acyltransferase, is involved in the synthesis of triacylglycerol and glycerophospholipids. In a different form of congenital generalized lipodystrophy, mutations are found in BSCL2, whose gene product is a novel human protein called seipin, a protein of unknown function, although homologous to G-protein (Magre *et al.* 2001). Some autosomal familial partial lipodystrophies are associated with mutations in LMNA, which encodes lamin A/C, a nuclear envelope protein (Shackleton *et al.* 2000; De Sandre-Giovannoli *et al.* 2002). Lamin A has been shown to interact with SREBP 1 and 2, which is at least a candidate mechanism for fatty liver (Lloyd *et al.* 2002). Other autosomal familial partial lipodystrophies are associated with mutations in PPAR γ , which encodes the peroxisome proliferator-activated receptor- γ (Agarwal & Garg 2002). These observations in the lipodystrophy syndromes not only support the contention that hyperinsulinaemia associated with insulin resistance is critical in the pathogenesis of NAFLD, but also indicate some genes and gene products which may be important in the disease mechanism of NAFLD.

Patients with lipodystrophy/lipoatrophy syndromes have complete or partial lack of adipose tissue, elevated insulin and low leptin levels. The most severely affected patients develop diabetes mellitus (Reitman *et al.* 2000). NAFLD has been detected in patients with congenital forms of lipodystrophy, including one patient who later

underwent liver transplantation (Powell *et al.* 1989; Cauble *et al.* 2001). The severity of the hepatic steatosis is proportional to the extent of extrahepatic fat loss.

Diagnostic approach

It is important to consider and exclude alternative aetiologies for hepatic steatosis in any child who appears to have NAFLD (Table 10.1). The main differential diagnoses include:

Table 10.1 Differential diagnosis of fatty liver in children with macrovesicular steatosis.

Nutritional

Dehydration, severe infection
Acute starvation
Protein-calorie malnutrition (kwashiorkor)
Total parenteral nutrition
Jejuno-ileal bypass; gastric reduction operations
Obesity
Non-alcoholic fatty liver disease

Systemic disease

Chronic hepatitis C
Schwachman syndrome (pancreatic insufficiency)
Coeliac disease
Inflammatory bowel disease
Diabetes mellitus
Nephrotic syndrome

Drugs

Amiodarone
Methotrexate
Prednisone/glucocorticoids
L-asparaginase
Vitamin A
Ethanol

Inherited metabolic disorders

Cystic fibrosis
Wilson's disease
 α_1 -antitrypsin deficiency
Hereditary tyrosinaemia, type I
Homocystinuria
Galactosaemia
Hereditary fructose intolerance
Glycogen storage diseases (mainly types I, VI)
Sialidosis, mannosidosis, fucosidosis
Refsum disease
Abeta- or hypobetalipoproteinaemia
Neutral lipid storage disease
Wolman disease
Cholesterol ester storage disease
Tangier disease
Familial hyperlipoproteinaemias
Citrullinaemia, argininaemia, arginosuccinic aciduria (ornithine transcarbamylase deficiency: mainly microvesicular fat)
Systemic carnitine deficiency (usually microvesicular fat)
Weber-Christian disease
Chronic granulomatous disease
Porphyria cutanea tarda

Table 10.2 Diagnostic approach to the investigation of non-alcoholic fatty liver disease/non-alcoholic steatohepatitis.*Groups to be investigated*

All children with

- type 2 diabetes mellitus
- simple obesity
- acanthosis nigricans
- history of certain interventions, e.g. cranial surgery and bone marrow transplantation in whom ALT and/or AST have been raised at least twice in a 6-month period

Routine blood tests

Full blood count and coagulation

Urea and electrolytes, uric acid

Liver function tests: Bilirubin, AST, ALT, ALP, GGT, albumin
ALT/AST ratio should be documented*Tests to exclude other causes of liver disease*

Autoantibodies, immunoglobulins and complement

Copper and caeruloplasmin

 α_1 -antitrypsin level and phenotype

Amino and organic acids

Iron, ferritin

Thyroid function tests

Serology for CMV, EBV, HAV, HBV and HCV

Fasting lipids

Cholesterol

Triglycerides

HDL-cholesterol

Low-density lipoprotein

Fasting metabolic tests

Glucose

Insulin

C-peptide

Leptin

Lactate

Free fatty acids

3-hydroxybutyrate

*Ultrasound scan of abdomen (and ovaries if female)**Liver biopsy for histopathology and frozen specimen**DEXA scan for body fat composition and distribution*

- Chronic hepatitis C which should be excluded by virological testing.
- Drug hepatotoxicity, especially methotrexate.
- Inherited metabolic disease, especially Wilson's disease which may present with prominent hepatic steatosis (Chapter 13).
- Cystic fibrosis (CF). Hepatic steatosis is a very common liver abnormality, which affects 30–40% of CF patients (Colombo *et al.* 1999; Lindblad *et al.* 1999) (Chapter 11). It should be excluded by sweat testing and mutation analysis.

The main investigations are in Table 10.2 but should include:

- Routine haematology and liver function tests. Labora-

tory studies show that serum ALT is more elevated than serum AST.

- Urea and electrolytes. Hyperuricaemia is common.
- Fasting lipids. Hypertriglyceridaemia is the typical blood lipid abnormality.
- Autoantibodies, immunoglobulins and relevant metabolic investigations.
- Viral serology.
- Abdominal ultrasound. Studies in adults show that imaging can identify patients with hepatic steatosis quite accurately, especially if the steatosis is moderately severe, but does not distinguish simple steatosis from NASH (Saadeh *et al.* 2002). Microvesicular steatosis due to inherited mitochondrial disorders, urea cycle disorders, and valproic acid hepatotoxicity may occasionally be severe enough to be identified by liver ultrasound and should be excluded.

- Liver biopsy is important for the diagnosis of NAFLD and may distinguish between NASH and other possible diagnoses. The optimal timing of liver biopsy has not been determined. Some advocate deferring liver biopsy in children with possible NASH until after a trial of weight loss for 3–6 months: if weight loss is not achieved and serum aminotransferases remain elevated, a liver biopsy is performed. Early biopsy for younger children or those with acanthosis nigricans is reasonable, but insufficient data are available to evaluate these criteria. Histological findings in childhood NASH may be slightly different from those in adults with NASH (Matteoni *et al.* 1999; Brunt 2001), but the essential findings of macrovesicular steatosis, inflammation or its residua, and fibrosis are present in the majority of cases (Rashid & Roberts 2000). Both the histological scoring and the histological features of NAFLD in children require further precision.

- Measurement of insulin resistance. These include fasting glucose, insulin and C-peptide. Formulae for estimating insulin resistance, such as HOMA-IR, the homeostasis model assessment insulin resistance index (Matthews *et al.* 1985; Radziuk 2000), have been validated for use in children, but their use is still restricted to the research setting.

It is important to have a consistent approach to evaluating children with hepatic steatosis, which expedites the diagnostic process and facilitates treatment. A model based on current practice is shown (Fig. 10.2).

Treatment

The approach to treatment is best within a multidisciplinary management team to deal with the diverse aspects of this disorder (medical, endocrinological, dietary, psychosocial). Treatment is directed towards management of obesity, insulin resistance or reducing lipid peroxidation or oxidative stress. The usual reported endpoints for

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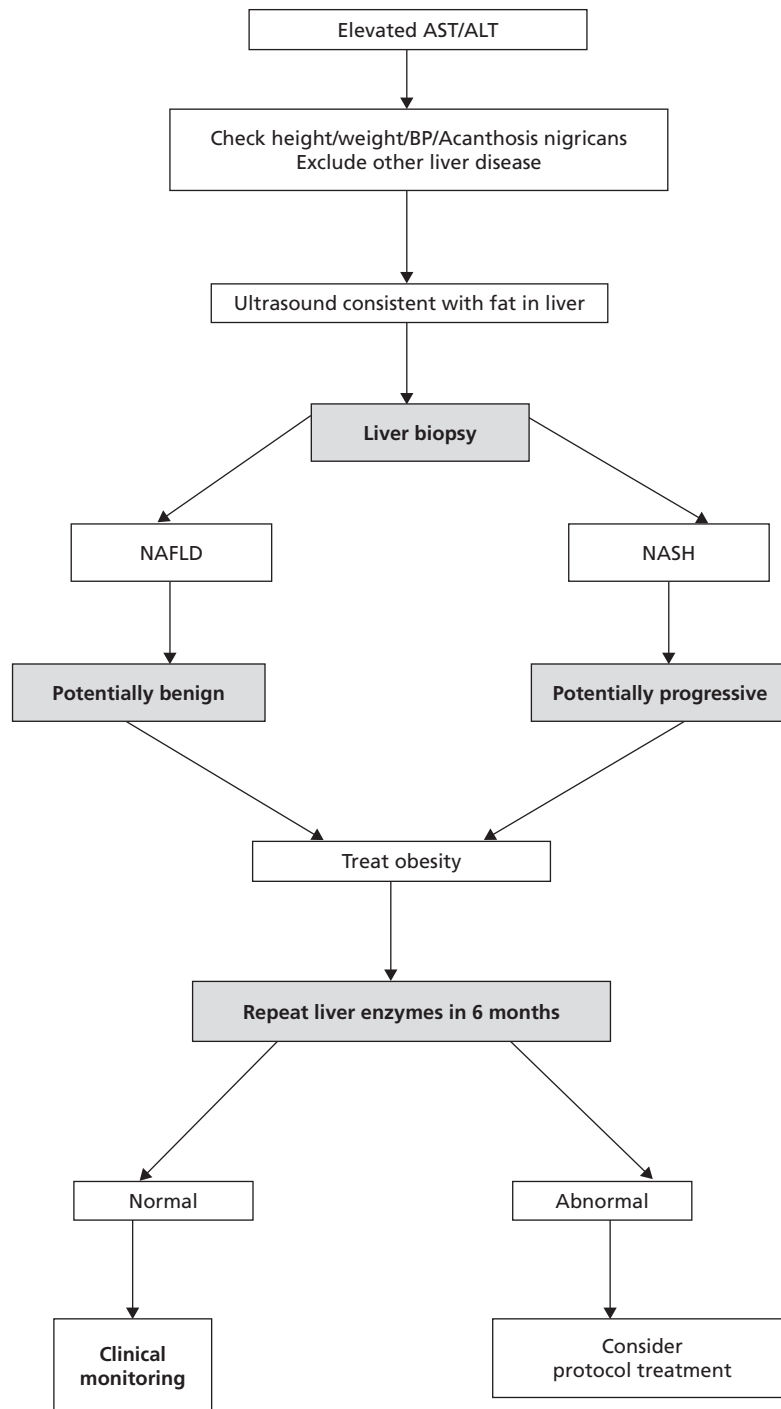


Fig. 10.2 Approach to diagnosis and treatment. Most patients are asymptomatic and are referred because of elevated serum aminotransferases or abnormal findings on liver sonography. Timing of liver biopsy remains somewhat controversial, but it is required to confirm the diagnosis of non-alcoholic steatohepatitis (NASH). Treatment modalities are not yet defined for children: only ursodiol has been tested in a clinical trial. NAFLD; non-alcoholic fatty liver disease (simple steatosis).

judging efficacy are normalization of serum aminotransferases, loss of steatosis on ultrasound, or histological improvement on liver biopsy.

Obesity

The only treatment thus far shown to be convincingly effective in childhood NAFLD is the treatment of obesity by

weight loss. Reduction in body weight has led to normalization of serum aminotransferases in several clinical series (Vajro *et al.* 1994; Manton *et al.* 2000; Rashid & Roberts 2000; Vajro *et al.* 2000), while substantial improvement in liver histology was reported in one paediatric patient (Vajro *et al.* 1994). Although it has not been established how much weight must be lost to achieve improvement, the usual strategy is to reduce caloric intake and increase

moderately intense aerobic exercise. Achieving and maintaining weight loss in children is difficult, especially as nutrition must be maintained for general growth. Well-designed studies examining treatment of childhood obesity have shown that a specific diet designed to minimize hyperinsulinaemia may be more effective than the conventional low-calorie diet (Ludwig *et al.* 1999; Spieth *et al.* 2000). This low glycaemic index diet may be easier to maintain long-term than a calorie-restricted diet and consists of comparatively straightforward food inclusions/restrictions: inclusion of fruits, vegetables, legumes, whole-grain or high fibre or traditionally processed grains and pasta, and exclusion or limited consumption of white potatoes, sugar (sucrose), and highly refined white flour (Ludwig 2002). Complementing the child's weight reduction regimen with family-based behavioural intervention may also enhance success (Goldfield *et al.* 2001). Instituting a regimen of regular physical exercise is important because exercise also reduces hyperinsulinaemia.

Drug treatment of insulin resistance/oxidative stress

Few pharmacological treatments have been investigated in either children or adults. Treatment directed towards reducing insulin resistance includes weight loss but some drugs have been used:

- Metformin which appears to act directly on SREBP-1c (Lin *et al.* 2000; Zhou *et al.* 2001) has been used for treatment of NASH in adults (Marchesini *et al.* 2001), but it has not been used in children with NAFLD as there are concerns about hepatotoxicity. Nevertheless, there is some experience with metformin in relevant childhood diseases. It has been used in children with acanthosis nigricans with improvement (Hermanns-Le *et al.* 2002) and in obese adolescents to induce weight loss (Kay *et al.* 2001). In these adolescents the combination of metformin plus low-calorie diet was more effective than the low-calorie diet alone. It has also been used effectively for treatment of children with type 2 diabetes mellitus (Jones *et al.* 2002; Zuhri-Yafi *et al.* 2002), polycystic ovary syndrome (Nardo & Rai 2001; Arslanian *et al.* 2002), and Prader-Willi syndrome (Chan *et al.* 1998).
- Thiazolidinediones such as troglitazone have improved both liver function tests and histology in adults (Caldwell *et al.* 2001). Troglitazone has been withdrawn because of hepatotoxicity, but trials with rosiglitazone are in progress (Angulo & Lindor 2001; Neuschwander-Tetri 2002).

Drugs used to reduce or prevent lipid peroxidation or oxidative stress include N-acetyl cysteine (used in the treatment of paracetamol toxicity and acute liver failure) for which there are no specific data in NASH. Others include:

- Ursodeoxycholic acid. A small randomized controlled

trial of ursodeoxycholic acid (10–12.5 mg/kg/day) was conducted with 31 children with NAFLD diagnosed by sonography. There was no additional benefit over weight reduction and diet, and ursodeoxycholic acid alone was ineffective in improving liver biochemistries and the sonographic appearance (Vajro *et al.* 2000).

- Vitamin E (400–1200 IU/day orally) in an open-label pilot study in 11 children with NASH was associated with improvements in serum aminotransferases, but there was no major change in BMI or in sonographic appearance of the liver. Biochemical relapse occurred in two children who discontinued vitamin E (Lavine 2000).
- Betaine is commonly used for various disorders of homocysteine metabolism in children (Ogier de Baulny *et al.* 1998). It has been used in adults with NASH, but it has not been examined in childhood NASH.

Components of the complex system regulating nutrient intake and utilization may provide opportunities for treatment of NASH. These include leptin (Uygun *et al.* 2000; Wang *et al.* 2001), adiponectin (Maeda *et al.* 2001; Haque *et al.* 2002; Maeda *et al.* 2002), resistin (Steppan & Lazar 2002) and the intracellular nutrient-sensing pathway, the hexoseamine biosynthesis pathway (Obici *et al.* 2002). Since hyperleptinaemia and leptin resistance occur in NAFLD, simple supplementation along this regulatory pathway is unlikely to be effective. By contrast, in generalized lipodystrophy syndromes characterized by defective expression of leptin, leptin supplementation has improved hepatic steatosis (Oral *et al.* 2002).

Outcome

The majority of children with non-alcoholic steatosis have childhood NAFLD. Simple hepatic steatosis appears to be benign; but there are no long-term studies as yet to determine the outcome in children and there are no data about long-term extrahepatic disease.

NASH may be more severe in certain ethnic groups, including Hispanics and Asians, or in association with metabolic disorders characterized by abnormalities in insulin receptor/signalling, such as lipodystrophy syndromes. Progression to cirrhosis is reported but is rare in children. Hepatocellular carcinoma may develop. Adult studies demonstrate that the more severe forms of NAFLD may progress to chronic liver failure necessitating liver transplantation. NASH recurs post-liver transplantation, but the prognosis is similar to other indications (Contos *et al.* 2001). The role of therapy is undetermined, as only weight loss has had a significant effect on histology.

Conclusion

NAFLD/NASH is due to disordered insulin action, with hyperinsulinaemia and relative insulin resistance. Like

type 2 diabetes in children, it is likely to become an important liver disease among children as the prevalence of childhood obesity continues to increase. It occurs in young children and there is no female predominance in the paediatric age bracket. Most children are either asymptomatic, or present with vague abdominal pain. Weight loss through dietary redesign with a low glycaemic index diet and a regimen of regular exercise is the mainstay for treatment. The efficacy of vitamin E supplementation or ursodeoxycholic acid in children is unclear, although other drug treatments, such as metformin, may be shown to be effective in the near future.

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