Intrahepatic Fat Accumulation and Alterations in Lipoprotein Composition in Obese Adolescents

A perfect proatherogenic state

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OBJECTIVE — Among other metabolic consequences, a dyslipidemic profile often accompanies childhood obesity. In adults, type 2 diabetes and hepatic steatosis have been shown to alter lipoprotein subclass distribution and size; however, these alterations have not yet been shown in children or adolescents. Therefore, our objective was to determine the effect of hepatic steatosis on lipoprotein concentration and size in obese adolescents.

RESEARCH DESIGN AND METHODS — Using fast magnetic resonance imaging, we measured intrahepatic fat content in 49 obese adolescents with normal glucose tolerance. The presence or absence of hepatic steatosis was determined by a threshold value for hepatic fat fraction (HFF) of 5.5%; therefore, the cohort was divided into two groups (HFF $>$ or $<$5.5%). Fasting lipoprotein subclasses were determined using nuclear magnetic resonance spectroscopy.

RESULTS — Overall, the high-HFF group had 88% higher concentrations of large VLDL compared with the low-HFF group ($P < 0.001$). Likewise, the high-HFF group had significantly higher concentrations of small dense LDL ($P < 0.007$); however, the low-HFF group had significantly higher concentrations of large HDL ($P < 0.001$). Stepwise multiple regression analysis revealed that high HFF was the strongest single correlate, accounting for 32.6% of the variance in large VLDL concentrations ($P < 0.002$).

CONCLUSIONS — The presence of fatty liver was associated with a pronounced dyslipidemic profile characterized by large VLDL, small dense LDL, and decreased large HDL concentrations. This proatherogenic phenotype was strongly related to the intrahepatic lipid content.

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Studies from autopsies on 742 children (aged 2–19 years) reported fatty liver prevalence at 9.6%, and in obese children this rate increased to an alarming 38% (1). An imbalance between fatty acid flux and utilization and VLDL secretion leads to an accumulation of triglycerides within the hepatocytes and ultimately to hepatic steatosis (2). It is becoming increasingly clear that fat accumulation in the liver, per se, is not a benign condition (3). Indeed, it is frequently associated with type 2 diabetes in both adults and children (4,5) and has been labeled as the hepatic component of the metabolic syndrome (2,3).

Worsening of the dyslipidemic profile has been described in adults in association with insulin resistance and type 2 diabetes (6–8). Garvey et al. (7) have shown that subjects with type 2 diabetes have larger VLDL and smaller LDL and HDL particles compared with insulin-sensitive subjects. The insulin-resistant and type 2 diabetic groups also had greater concentrations of these atherogenic particles. Further studies by Toledo et al. (8) reported that the presence of hepatic steatosis in obese subjects with type 2 diabetes further altered lipoprotein composition compared with type 2 diabetic subjects without fatty liver. Type 2 diabetic subjects with fatty liver had larger triglyceride-rich VLDL particles, smaller LDL and HDL particles, and reduced concentrations of large LDL compared with type 2 diabetic subjects without fatty liver (8).

Obese children and adolescents are often diagnosed with dyslipidemia characterized by high triglycerides and low HDL cholesterol concentrations. In addition, the presence of small dense LDL particles has been shown in obese children (9,10). Recent studies from our group reported dyslipidemia and a deterioration in glucose metabolism in obese nondiabetic adolescents with excessive intrahepatic fat accumulation. In particular, we found rising levels of triglycerides and decreasing levels of HDL cholesterol with increasing accumulation of fat in the liver (11). Although studies in adults have shown insulin resistance, obesity, and fatty liver playing a role in the composition of lipoproteins, there are no current studies for this comprehensive phenotype in children. Therefore, our objectives were *1*) to determine whether obese normal glucose-tolerant adolescents with fatty liver had alterations in lipoprotein composition and size compared with obese normal glucose-tolerant adoles-

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Abbreviations: EMCL, extramyocellular triglyceride content; HFF, hepatic fat fraction; IMCL, intramyocellular triglyceride content; MRI, magnetic resonance imaging; NMR, nuclear magnetic resonance; WBISI, whole-body insulin sensitivity index.

A table elsewhere in this issue shows conventional and Système International (SI) units and conversion factors for many substances.

cents without fatty liver, matched for the degree of overall obesity and age; and 2) to examine to what degree differences, if any, in the lipoprotein composition may be accounted for by the level of intrahepatic fat accumulation. Nuclear magnetic resonance (NMR) spectroscopy was utilized to determine lipoprotein subclass composition in fasting plasma samples, while fast magnetic resonance imaging (MRI) was used to determine the hepatic fat fraction (fatty liver).

RESEARCH DESIGN AND METHODS

— We recruited 49 obese adolescents from our Pediatric Obesity Clinic. Some subjects are part of a larger study on the prevalence of fatty liver disease in youth and thus were reported on previously (11). All subjects had a BMI greater than the 95th percentile, were taking no medications known to affect liver function or alter glucose or lipid metabolism, and all denied the use of alcohol. The Yale University School of Medicine Human Investigation Committee approved the study, and written informed consent and assent were obtained.

Metabolic studies

Oral glucose tolerance test. All subjects were invited to the Yale Center for Clinical Investigation for an oral glucose tolerance test at 8:00 A.M., following an overnight fast, as previously reported (12). The subjects were instructed to consume a diet containing at least 250 g carbohydrate the day before the study and to refrain from vigorous physical activity. Baseline blood samples were obtained from subjects while they were fasting, with the use of an indwelling venous line for measurement of glucose, insulin, lipid profile, lipoprotein concentration and size, free fatty acids, adiponectin, and leptin. A standard 3-h oral glucose tolerance test was then performed with the administration of 1.75 g glucose/kg body wt (maximum dose: 75 g), and blood samples were obtained every 30 min for plasma glucose, insulin, and C-peptide measurements.

Lipoprotein analysis

Fasting plasma samples were obtained to determine lipoprotein particle concentration and size. The analysis utilized a 400 MHz proton NMR analyzer at Liposcience (Raleigh, NC). The methodology has been described in detail (13,14). In brief, each lipoprotein subclass concentration was determined by the measured amplitudes of the characteristic lipid methyl group NMR signals they emit (15). The intensity of each signal is proportional to the quantity of the subclass, which is reported in particle concentration units (nanomoles of particles per liter for VLDL and LDL and micromoles per liter for HDL). VLDL, LDL, and HDL were separated into 10 subclass categories: large VLDL (including chylomicrons) (>60 nm), medium VLDL (35–60 nm), small VLDL (27–35 nm), intermediate-density lipoprotein (23–27 nm), large LDL (21.2–23 nm), medium-small LDL (19.8–21.2 nm), very small LDL (18–19.8 nm), large HDL (8.8–13 nm), medium HDL (8.2–8.8 nm), and small HDL (7.3–8.2 nm). Average particle sizes were computed as the sum of the diameter of each subclass multiplied by its relative mass percentage as estimated from the amplitude of its methyl NMR signal.

Imaging studies

Fast MRI: liver fat content. Measurement of hepatic fat accumulation was performed using MRI along with the Dixon method as modified by Fishbein et al. (16). The description of the method has been reported recently by our group (11). Following the analysis of hepatic fat fraction (HFF), subjects were stratified into two groups: HFF <5.5% (n = 37) and >5.5% (n = 12). This cutoff has been used previously by our research group and is a threshold to denote steatosis in this population (11,17). Secondary causes of fatty liver, such as autoimmune hepatitis, Wilson disease, α_1-antitripsin deficiency, and hepatitis B and C, were excluded with appropriate tests.

Abdominal fat distribution: MRI

Abdominal MRI studies were performed on a Siemens Sonata 1.5 Tesla system, as previously described (12).

^{1}H-NMR spectroscopy: intramyocellular triglyceride content

Muscle triglyceride content was measured using a 4.0T Biospec system (Bruker Biospin MRI, Ettlingen, Germany), as previously described (18).

Limitations of the imaging techniques

Liver MRI. This method is limited to pixels with an HFF of <50%; however, other studies (17) using various methods have shown that values >50% are rare. In addition, we measured only a single slice of the liver, which in some cases may not reflect the fat content of the liver as a whole. Despite these limitations, the two-point Dixon method is the most widely used technique in clinical MRI studies. In our group, we validated the modified Dixon method against hepatic fat measured by ^{1}H-NMR in 34 subjects (lean and obese) and found a very strong correlation between the two methods (r = 0.93, $P < 0.001$) (T. Constable and S.C., personal data). Furthermore, at our institution, four obese adolescents underwent a liver biopsy—the gold standard for diagnosing nonalcoholic fatty liver disease—which confirmed fatty liver measured by fast MRI.

Abdominal MRI. This technique gives an estimate of abdominal fat content, which is dependent on the sensitivity of the images, the intensity threshold, and the reader's ability; this method has been validated against dissection in human cadavers (19). As with the liver MRI, we only measure one slice (at the level of the L4/L5 disc space); however, single-slice methods have been shown to correlate well with multislice methods (20).

^{1}H-NMR spectroscopy. Single-voxel magnetic resonance spectroscopy is the most accurate way to determine muscle lipids, and it can distinguish intra- versus extramyocellular lipids. The disadvantage is that it only measures a single site and therefore cannot give information on heterogeneity across a tissue. Moreover, to ensure good separation of the extramyocellular triglyceride content (EMCL) intramyocellular triglyceride content (IMCL) resonances, the muscle fibers must be aligned along the Z direction of the magnet to avoid contamination of the IMCL peak with the EMCL signal. This requires the use of a smaller voxel to obtain a better fiber alignment. Overall, the imaging techniques have the advantage of being noninvasive without the use of ionizing radiation; however, they are relatively expensive and therefore not appropriate for routine screening.

Analytical methods

Plasma glucose levels were measured using the YSI 2700 STAT Analyzer (Yellow Springs Instruments) and lipid levels using an autoanalyzer (model 747-200; Roche-Hitachi). Plasma insulin, leptin, and adiponectin levels were measured using an radioimmunoassay from Millipore (St. Charles, MO). Free fatty acids were measured using a Wako Diagnostics assay. Estimated insulin sensitivity was calculated using the Matsuda index (whole-

Table 1—*Demographic and anthropometric characteristics*

	Total cohort	HFF <5.5%	HFF >5.5%	Unadjusted	Adjusted*
n	49	37	12		
Age (years)	15.3 ± 0.33	15.32 ± 0.4	15.1 ± 0.7	0.738	—
Sex					
Male	17	10	7	0.467	—
Female	32	27	5	<0.001	—
Race					
White	22	16	6	0.033	—
Hispanic	18	12	6	0.439	—
Black	9	9	0		—
BMI (kg/m^2)	35.7 ± 0.9	35.63 ± 1.12	36.0 ± 1.17	0.862	—
BMI *Z* score	2.24 ± 0.05	2.21 ± 0.06	2.35 ± 0.06	0.191	—
% fat	43.0 ± 1.0	42.0 ± 1.3	43.3 ± 2.0	0.510	—
% HFF	6.83 ± 1.61	1.15 ± 0.23	24.31 ± 3.0	<0.001	—
Visceral fat (cm^2)	64.32 ± 3.5	56.0 ± 4.3	87.3 ± 6.6	<0.001	<0.001
Subcutaneous fat (cm^2)	558.0 ± 28.0	568.0 ± 39.6	531.1 ± 61.0	0.415	0.616
Visceral-to-subcutaneous fat ratio	0.124 ± 0.01	0.106 ± 0.01	0.171 ± 0.014	<0.001	<0.001
IMCL (%)	1.33 ± 0.12	1.1 ± 0.15	1.8 ± 0.23	0.01	0.01
EMCL (%)	1.6 ± 0.14	1.42 ± 0.18	1.93 ± 0.27	0.118	0.126

Data are means ± SE. *Adjusted for sex and race; $P < 0.05$.

body insulin sensitivity index [WBISI]), which has been validated by comparison with hyperinsulinemic-euglycemic clamp studies in obese children and adolescents (21).

Statistics

Data are represented as means ± SE. Parameters that were not normally distributed were log transformed for analysis. Independent *t* tests were used to analyze differences between groups. Univariate analysis was utilized to adjust for potential confounders (race and sex). To further examine the independent association between large VLDL particle concentrations and HFF, we used a stepwise forward multiple regression analysis in the total cohort. In step 1, we entered sex and race; in step 2, visceral fat was added; in step 3, insulin sensitivity (WBISI) was added; and finally in step 4, HFF was added to the model. A *P* value of <0.05 was considered statistically significant. All analyses were performed using SPSS 14.0 for Windows.

RESULTS

Demographic and anthropometric characteristics

As shown in Table 1, a total of 49 male and female obese adolescents were included in the study. It is evident that there were large discrepancies in race and sex in the cohort, and HFF varied widely, from undetectable to 37.3%. The wide range in HFF allowed for stratification into two groups: HFF <5.5% ($n = 37$) and HFF >5.5% ($n = 12$). There were no differences in age, BMI, or BMI *z* scores between the two groups. Although male subjects were equally represented, ~85% of the female subjects were included in the group with the low HFF ($P < 0.001$). Likewise, there were significantly more Caucasians and no African Americans in the high-HFF group ($P = 0.033$). Due to these significant differences, all analyses were statistically adjusted for race and sex.

Although there were no differences in percent body fat, visceral adiposity was significantly higher in the high-HFF group compared with the low-HFF group (87.3 ± 6.6 vs. 56.0 ± 4.3 cm^2; $P < 0.001$). There were no differences in subcutaneous fat between groups; however, due to the differences in visceral adiposity, there were also significant differences in the ratio of visceral to subcutaneous fat depots. In addition, the high-HFF group had elevated IMCL concentrations compared with the low-HFF group (1.8 ± 0.23 vs. 1.1 ± 0.15; $P < 0.01$).

Metabolic characteristics

Metabolic characteristics are shown in Table 2. All subjects included in the study had normal glucose tolerance (2 h glucose <7.7 mmol/l [140 mg/dl]). Despite comparable fasting glucose concentrations, the high-HFF group had significantly higher concentrations of fasting insulin (41.4 ± 1.13 vs. 26.3 ± 1.07; $P = 0.002$) compared with the low-HFF group. Both groups were insulin resistant, as illustrated by both homeostasis model assessment of insulin resistance and WBISI calculations; however, the high-HFF group had significantly higher homeostasis model assessment of insulin resistance levels and lower WBISI levels compared with the low-HFF group. Leptin concentrations were similar among the groups, whereas the low-HFF group had higher concentrations of adiponectin compared with the high-HFF group. This difference remained after controlling for age and sex differences.

Plasma lipids and lipoprotein composition and size

A standard lipid panel revealed plasma lipid alterations in the high-HFF group (Table 2). There were no differences between groups with regards to total cholesterol and LDL cholesterol concentrations. As expected, the high-HFF group had significantly higher triglyceride concentrations than the low-HFF group ($P < 0.001$). HDL cholesterol concentrations were also significantly lower in the high-HFF group ($P = 0.006$). Free fatty acid concentrations were not different between groups.

A more complete lipoprotein analysis revealed significant alterations in both li-

Table 2—*Metabolic, plasma lipids, and lipoprotein profile in the entire cohort stratified by HFF (%)*

	HFF <5.5%	HFF >5.5%	Unadjusted	Adjusted*
n	37	12		
Fasting glucose (mmol/l)	5.12 ± 0.07	5.01 ± 0.12	0.946	0.449
Fasting insulin (pmol/l)	150.0 ± 6.6	246.0 ± 6.78	0.002	0.002
Homeostasis model assessment of insulin resistance	5.65 ± 1.1	9.02 ± 1.13	0.003	0.003
WBISI	2.05 ± 1.1	1.19 ± 1.15	0.002	0.002
Adiponectin (μg/ml)	9.94 ± 0.67	6.5 ± 1.1	0.004	0.01
Leptin (ng/ml)	27.9 ± 2.5	27.4	0.276	0.937
Free fatty acids (mmol/l)	0.463 ± 0.028	0.517 ± 0.055	0.337	0.397
Plasma lipids (mmol/l)				
Total cholesterol	3.76 ± 0.026	3.84 ± 0.03	0.788	0.909
LDL cholesterol	2.24 ± 0.16	2.00 ± 0.24	0.438	0.425
HDL cholesterol	1.19 ± 0.04	0.95 ± 0.07	0.006	0.006
Triglycerides	0.73 ± 0.021	1.62 ± 0.015	<0.001	<0.001
VLDL particles (nmol/l)				
Total VLDL and chylomicrons	41.1 ± 3.2	55.03 ± 4.9	0.012	0.021
Large VLDL and chylomicrons	0.77 ± 0.749	6.25 ± 1.15	<0.001	<0.001
Medium VLDL	8.7 ± 1.3	18.7 ± 2.0	<0.001	<0.001
Small VLDL	32.0 ± 2.6	30.1 ± 4.0	0.821	0.754
IDL particles (nmol/l)	24.0 ± 5.43	39.4 ± 8.34	0.058	0.126
LDL particles (nmol/l)				
Total LDL	938.4 ± 51.0	1,076.46 ± 78.0	0.084	0.146
Large LDL	344.5 ± 31.0	255.4 ± 47.3	0.062	0.123
Total small LDL	570.2 ± 41.0	781.4 ± 63.0	0.002	0.007
Medium-small LDL	119.8 ± 9.52	150.4 ± 14.6	0.032	0.087
Very small LDL	450.2 ± 32.65	631.1 ± 50.15	0.001	0.004
HDL particles (μmol/l)				
Total HDL	27.0 ± 0.7	25.4 ± 1.1	0.642	0.271
Large HDL	5.2 ± 0.34	2.8 ± 0.52	<0.001	<0.001
Medium HDL	2.2 ± 0.54	5.2 ± 0.84	0.004	0.005
Small HDL	19.4 ± 0.6	17.3 ± 0.851	0.220	0.052

Data are means ± SE. *Adjusted for sex and race; $P < 0.05$.

poprotein subclass particle concentration and size in the high-HFF group. With regard to VLDL, the high-HFF group had ~25% more VLDL particles and 88% higher concentrations of large VLDL compared with the low-HFF group ($P = 0.021$ and $P < 0.001$, respectively). The medium VLDL particle concentration was >50% higher in the high-HFF group ($P < 0.001$). No differences were observed in the small VLDL particles. Although there was no difference in the total number of LDL particles between groups, the high-HFF group had significantly higher concentrations of small dense LDL ($P = 0.007$). Likewise, total HDL particle concentration was not different between groups, but the low-HFF group had significantly higher concentrations of large HDL and lower concentrations of medium HDL particles compared with the high-HFF group ($P < 0.001$ and $P = 0.005$, respectively). Overall size of the lipoproteins followed a similar trend. As expected, the high-HFF group had a larger VLDL particle size and smaller LDL and HDL particle sizes compared with the low-HFF group.

Relationship between HFF, insulin sensitivity, body fat distribution, and large VLDL concentrations

To assess the relationships between large VLDL and HFF, insulin sensitivity, and body fat distribution, stepwise multiple regression analysis was used. From our model, it is evident that large VLDL concentrations are independently associated with fatty liver. Furthermore, HFF explains ~32.6% of the variance in large VLDL concentrations ($P = 0.002$). No other parameter was significantly associated with large VLDL concentrations.

CONCLUSIONS

— By combining NMR spectroscopy to assess lipoprotein composition with fast MRI to quantify liver fat content, we demonstrate, in the present study, that obese adolescents with normal glucose tolerance and fatty liver have the prototypic proatherogenic lipoprotein phenotype. In particular, we found that the presence of hepatic steatosis was associated with *1*) an increase in VLDL particle size and number, *2*) an increase in small dense LDL concentrations, and *3*) a decrease in the number of large HDL particles. These alterations were reflected by an increase in triglyceride concentrations and decreased HDL cholesterol. Of note, hepatic steatosis was found to predict the concentration of the large VLDL particles, independent of overall adiposity, insulin sensitivity, and visceral adiposity, thereby suggesting that liver steatosis is important in the early pathogenesis of insulin resistance and type 2 diabetes in youth. Hence, the atherogenic profile is already fully established at this very young age.

It is widely appreciated that hepatic overproduction of VLDL constitutes the metabolic basis of various hyperlipidemic states in humans, such as the familial combined hyperlipidemia and the dyslip-

idemia of type 2 diabetes (22). Although LDL subclasses have received the most attention, subclasses of VLDL may also differ in atherogenicity. It is possible that large VLDL particles may be selectively retained in the intima of the arterial wall or may be a marker of delayed chylomicron clearance, a metabolic condition that has been related to disease severity (23). In the high-HFF group, we found a marked increase in large VLDL and, to a lesser extent, medium VLDL and no differences regarding small VLDL compared with the low-HFF group. Large VLDL particles are triglyceride rich and are excellent substrates for cholesterol ester transfer protein. Cholesterol ester transfer protein is a key enzyme in the reverse cholesterol transport system, whose activity is mediated by substrate availability (24). In these instances of hypertriglyceridemia, there is an increase in the exchange of cholesterol ester and triglycerides via cholesterol ester transfer protein between triglyceride-rich lipoproteins and HDL or LDL. This interaction yields triglyceride-rich LDL and HDL particles that can be hydrolyzed by hepatic lipase, thus promoting the formation of small dense LDL and decreased large HDL (22). The importance of triglyceride levels likely reflects the exchange of triglyceride and cholesterol esters between VLDL and LDL particles, with the subsequent hydrolysis of triglycerides. Triglyceride levels are strongly related to the size of VLDL particles and to the relative amount of each VLDL subclass, and the relative proportion of large VLDL increases rapidly at higher triglyceride levels (24). It is well known that the LDL receptor has a decreased affinity for smaller particles, and, therefore, particles are left in circulation (25). However, triglyceride-enriched HDL has been shown to be cleared more rapidly from circulation (26). This may be the case in the high-HFF group, where there was an ~50% reduction in large HDL subclasses and a 27% increase in total small LDL particles compared with the low-HFF group.

It is noteworthy that had the lipoprotein subclasses not been measured by the NMR technique, we would have missed the important finding regarding the pattern of changes in the LDL subclasses present in these youngsters with fatty liver. Indeed, the traditional fasting lipid profile revealed normal LDL cholesterol concentrations in both groups. In contrast, we found a significant increase in small LDL particles with increasing liver fat content. Small dense LDL is known to be proatherogenic; they are more susceptible to oxidation and may be taken up by macrophages, which eventually leads to the development of atherosclerotic plaque formation in the arterial wall.

The obese adolescents with fatty liver also had a greater visceral fat depot and higher IMCL and were more insulin resistant than their matched controls. In adults, a strong association between fatty liver and visceral adiposity has been reported, but no associations have been reported with IMCL content (27–29). Petersen et al. (30) reported both intrahepatic triglyceride and IMCL content to be increased in Asian-Indian men compared with Caucasian men. However, after adjusting for insulin sensitivity, the Asian-Indian men had more than a twofold increase in hepatic triglyceride content compared with the Caucasian men, whereas the differences in the amount of IMCL between the groups did not persist. In the present study, no significant relation between HFF (fatty liver) and visceral fat ($r^2 = 0.232$, $P = 0.108$ data not shown) was found. This, however, may be due to the small sample size and rather homogeneous group of obese adolescents. In an attempt to discern the relationships between large VLDL concentrations and fatty liver, we performed a stepwise regression analysis. HFF was the strongest single correlate, accounting for 32.6% of the variance in the VLDL concentration.

The disturbances in triglyceride metabolism may, in part, explain the risk of future cardiovascular disease. Recently, Godsland et al. (18) showed that in adults, triglyceride concentrations are a strong correlate of ethnic differences in ischemic heart disease risk. In particular, they showed that both medium and large VLDL levels were significantly higher in Caucasian compared with African-American men and women. Triglyceride concentrations predict ischemic heart disease, even though triglycerides per se do not seem to be directly involved in the atherogenic process (30). Moreover, Herd et al. (31) found that African-American children have lower triglyceride concentrations than Caucasian children, but differences in visceral fat did not explain this result, and VLDL concentrations rose more slowly with increasing waist circumference in African-American compared with Caucasian children.

The marked differences in the lipoprotein composition between the groups with and without steatosis cannot be accounted for by unequal sex and ethnic distribution, since we have adjusted for these variables during the analysis.

Triglyceride levels can quantitatively and qualitatively affect circulation. Hypertriglyceridemia results in the accumulation of excess triglyceride-rich lipoproteins including chylomicrons, VLDL, and their remnants. Interestingly, the use of thiazolidinediones has been associated with changes in the lipoprotein subclass particles, which to some extent may be related to their increased risk of coronary artery disease (32).

In summary, among a small group of obese adolescents with normal glucose tolerance, the presence of fatty liver was associated with a pronounced dyslipidemic profile characterized by large VLDL, small LDL, and decreased large HDL concentrations. This proatherogenic phenotype was strongly related to the intrahepatic lipid content. The coexistence of fatty liver with severe insulin resistance and dyslipidemia may represent the underlying metabolic defects that could precede the onset of type 2 diabetes in these youngsters.

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References

1. Schwimmer JB, Deutsch R, Kahen T, Lavine JE, Stanley C, Behling C: Prevalence of fatty liver in children and adolescents. *Pediatrics* 118:1388–1393, 2006
2. Sanyal AJ: Mechanisms of disease: pathogenesis of nonalcoholic fatty liver disease. *Nat Clin Prac Gastro Hep* 2:46–53, 2005
3. Angulo P: Nonalcoholic fatty liver disease. *N Engl J Med* 346:1221–1231, 2002
4. Vozarova B, Stefan N, Lindsay RS, Saremi A, Pratley RE, Bogardus C, Tataranni PA: High alanine aminotransferase is associated with decreased hepatic insulin sensitivity and predicts the development of type 2 diabetes. *Diabetes* 51:1889–1895, 2002

5. Nadeau KJ, Klingensmith G, Zeitler P: Type 2 Diabetes in children is frequently associated with elevated alanine aminotransferase. *J Pediatr Gastroenterol Nutr* 41:94–98, 2005
6. Festa A, Williams K, Hanley AJG, Otvos JD, Goff DC, Wagenknecht LE, Haffner SM: Nuclear magnetic resonance lipoprotein abnormalities in prediabetic subjects in the Insulin Resistance Atherosclerosis Study. *Circulation* 111:3465–3472, 2005
7. Garvey WT, Kwon S, Zheng D, Shaughnessy S, Wallace P, Hutto A, Pugh K, Jenkins AJ, Klein RL, Liao Y: Effects of insulin resistance and type 2 diabetes on lipoprotein subclass particle size and concentration determined by nuclear magnetic resonance. *Diabetes* 52:453–462, 2003
8. Toledo FGS, Sniderman AD, Kelley DE: Influence of hepatic steatosis (fatty liver) on severity and composition of dyslipidemia in type 2 diabetes. *Diabetes Care* 29: 1845–1850, 2006
9. Kang H-S, Gutin B, Parbeau P, LItaker MS, Allison J, Lee N-A: Low-density lipoprotein particle size, central obesity, cardiovascular fitness, and insulin resistance syndrome markers in obese youths. *Int J Obes* 26:1030–1035, 2002
10. Miyashita M, Okada T, Kuromori Y, Harada K: LDL particle size, fat distribution, and insulin resistance in obese children. *Eur J Clin Nut* 60:416–420, 2006
11. Burgert TS, Taksali SE, Dziura J, Goodman TR, Yeckel CW, Papademetris X, Constable RT, Weiss R, Tamborlane WV, Savoye M, Seyal AA, Caprio S: Alanine aminotransferase levels and fatty liver in childhood obesity: associations with insulin resistance, adiponectin, and visceral fat. *J Clin Endocrinol Metab* 91:4287–4294, 2006
12. Weiss J, Dziura J, Burgert TS, Tamborlane WV, Taksali SE, Yeckel CW, Allen K, Lopes M, Savoye M, Morrison J, Sherwin RS, Caprio S: Obesity and metabolic syndrome in children and adolescents. *N Engl J Med* 350:3262–3274, 2004
13. Otvos JD, Jeyarajah EJ, Bennett DW, Krauss RM: Development of a proton nuclear magnetic resonance spectroscopic method for determining plasma lipoprotein concentrations and subspecies distributions from a single, rapid measurement. *Clin Chem* 38:1632–1638, 1992
14. Otovs JD: Measurement of lipoprotein subclass profiles by nuclear magnetic resonance spectroscopy. In *Handbook of Lipoprotein Testing*. Rifai N, Wainick R, Cominazak M, Eds. Washington, DC, AACC Press, 2000, p. 609–623
15. Jeyarajah EJ, Cromwell WC, Otvos JD: Lipoprotein particle analysis by nuclear magnetic resonance spectroscopy. *Clin Lab Med* 26:847–870, 2006
16. Fishbein MH, Gardner KG, Potter CJ, Schmalbrock P, Smith MA: Introduction of fast MR imaging in the assessment of hepatic steatosis. *Magn Reson Imaging* 15: 287–293, 1997
17. Szczepaniak LS, Nurenberg P, Leonard D, Browning JD, Reingold JS, Grundy S, Hobbs HH, Dobbins RL: Magnetic resonance spectroscopy to measure hepatic triglyceride content: prevalence of hepatic steatosis in the general population. *Am J Physiol Endocrinol Metab* 288:E462–E468, 2005
18. Abate N, Burns D, Peshock RM, Garg A, Grundy SM: Estimation of adipose tissue mass by magnetic resonance imaging: validation against dissection in human cadavers. *J Lipid Res* 35:1490–1496, 1994
19. Ross R, Leger L, Morris D, de Guise J, Guardo R: Quantification of adipose tissue by MRI: relationship with anthropometric variables. *J Appl Physiol* 72:787–795, 1992
20. Yeckel CW, Weiss R, Dziura J, Taksali SE, Dufour S, Burgert TS, Tamborlane WV, Caprio S: Validation of insulin sensitivity indices from oral glucose tolerance test parameters in obese children and adolescents. *J Clin Endocrinol Metab* 89:1096–1101, 2004
21. Ayyobi A, Brunzell JD: Lipoprotein distribution in the metabolic syndrome, type II diabetes mellitus, and familial combined hyperlipidemia. *Am J Cardiol* 92 (Suppl. 4A):27J–33J, 2003
22. Morton RE, Zilversmit DB: Inter-relationship of lipids transferred by the lipid transfer protein isolated from human lipoprotein-deficient plasma. *J Biol Chem* 258:11751–11757, 1983
23. Freedman DS, Bowman BA, Otvos JD, Srinivasan SR, Berenson GS: Levels and correlates of LDL and VLDL particle sizes among children: the Bogalusaheart Study. *Atherosclerosis* 152:441–449, 2000
24. Nigon F, Lesnik P, Rouis M, Chapman MJ: Discrete subspecies of human low density lipoproteins are heterogeneous in their activation with the cellular LDL receptor. *J Lipid Res* 32:1741–1753, 1991
25. Lamarche B, Uffelman KD, Carpentier A, Cohn JS, Steiner G, Barrett PH, Lewis GF: Triglyceride enrichment of HDL enhances in vivo metabolic clearance of HDL apo A-1 in healthy mean. *J Cli Invest* 103: 1191–1199, 1999
26. Fencki S, Rota S, Sabir N, Akdaq B: Ultrasonographic and biochemical evaluation of visceral obesity in obese women with Non-alcoholic fatty liver disease. *Eur J Med Res* 12:68–73, 2007
27. Sabir N, Sermen Y, Kazil S, Zencir M: Correlation of abdominal fat accumulation and liver steatosis: importance of ultrasonoagraphic and anthropometric measurements. *Eur J Ultrasound* 14:121–128, 2001
28. Busetto L, Tregnaghi A, De Marchi F, Segato G, Foletto M, Sergi G, Favretti F, Lise M, Enzi G: Liver volume and visceral obesity in women with hepatic steatosis undergoing gastric banding. *Obes Res* 10:408–411, 2002
29. Petersen KF, Dufour S, Feng J, Befroy D, Dziura J, Dalla Man C, Cobelli C, Shulman GI: Increased prevalence of insulin resistance and nonalcoholic fatty liver diseasein Asian-Indian men. *Proc Natl Acad Sci U S A* 103:18273–18277, 2006
30. Godsland IF, Johnston DG, Chaturvedi N: Mechanisms of disease: lessons from ethnicity in the role of triglyceride metabolism in ischemic heart disease. *Nat Clin Pract Endocrinol Metab* 3:530–538, 2007
31. Herd SL, Gower BA, Dashti N, Goran MI: Body fat, fat distribution and serum lipids, lipoproteins and apolipoproteins in African-American and Caucasian-American prepubertal children. *Int J Obes Relat Metab Disord* 25:198–204, 2001
32. Deeg MA, Buse JB, Goldberg RB, Kendall DM, Zagar AJ, Jacober SJ, Khan MA, Perez AT, Tan MH, the GLAI Study Investigators: Pioglitazone and rosiglitazone have different effects on serum lipoprotein particle concentrations and sizes in patients with type 2 diabetes and dyslipidemia. *Diabetes Care* 30:2458–2464, 2007