

Intrauterine Growth Retardation, Insulin Resistance, and Nonalcoholic Fatty Liver Disease in Children

VALERIO NOBILI, MD¹
MATILDE MARCELLINI, MD¹
GIULIO MARCHESINI, MD²
ESTER VANNI, MD³

MELANIA MANCO, MD¹
ALBERTO VILLANI, MD⁴
ELISABETTA BUGIANESI, MD³

Intrauterine growth retardation is associated with the development of abnormalities in glucose tolerance in adulthood (1,2). Studies in adults and children born small for gestational age (SGA) (3–7) indicate that insulin resistance is the earliest component associated with low birth weight, irrespective of confounding factors, including obesity (8) and a family history of type 2 diabetes.

In SGA children, the typical central fat accumulation may actively contribute to insulin resistance (9). Visceral fat and fatty liver represent special depots of ectopic fat, independently associated with insulin resistance (10–12). In the liver, hepatic triglyceride accumulation characterizes nonalcoholic fatty liver disease (NAFLD), a highly prevalent and potentially progressive condition in adults, now considered the hepatic expression of the metabolic syndrome (13). In the pediatric population, the prevalence of NAFLD is only 2–3% but increases to 53% in the presence of obesity (14,15).

We studied the association of low birth weight with histologically assessed pediatric NAFLD to test the hypothesis that intrauterine growth retardation might be an additional factor responsible for metabolic liver disease in children via insulin resistance.

RESEARCH DESIGN AND

METHODS

— We studied 90 children with NAFLD, consecutively ob-

served in the Liver Unit, Bambino Gesù Children's Hospital, Rome, Italy, from June 2001 to April 2003. Part of this cohort group was reported elsewhere (16). All had a complete anthropometric and laboratory investigation, including a 2-h oral glucose tolerance test, within 2 months of a liver biopsy confirming the diagnosis of NAFLD. To compare BMI across different ages and sexes, we calculated the BMI Z score (17). Obesity was defined as BMI above the 97th percentile and overweight as BMI from the 85th to 97th percentile. The control group consisted of 90 children pair matched by age and sex, with normal liver scanning and liver function tests, selected among 200 consecutive subjects observed in the general pediatric department.

All children were born at term (≥ 37 weeks). They were defined SGA or appropriate for gestational age when their weight at birth was, respectively, ≤ 10 th and > 10 th percentile, corrected for gestational age, sex, and the local standard growth curve (18). The study was approved by the ethics committee of the Bambino Gesù Children's Hospital.

RESULTS — Of 90 NAFLD children, 35 (38.9%) were classified as SGA, compared with 6.7% of control subjects ($P < 0.0001$). The prevalence of SGA in NAFLD was also approximately fourfold higher compared with the average SGA prevalence of children admitted to our

pediatric department in the last decade (10%). Obesity and altered glucose regulation (American Diabetes Association criteria [19]) were more common in NAFLD patients (Table 1). Insulin resistance (20), assessed by homeostasis model assessment of insulin resistance (21) and by the insulin sensitivity index derived from the oral glucose tolerance test (22), was very common in children with NAFLD, independent of obesity. SGA children with NAFLD had higher basal glucose and insulin levels and were more insulin resistant. The family history of metabolic diseases was not systematically different between groups, with the notable exception of type 2 diabetes in second-degree relatives.

In multivariate regression, SGA was independently associated with NAFLD (odds ratio [OR] 7.94 [95% CI 2.71–23.24]) after correction for age, sex, BMI Z score, and glucose regulation. Among children with NAFLD, SGA was significantly associated with both homeostasis model assessment of insulin resistance (1.75 [1.17–2.62]) and insulin sensitivity index (0.73 [0.56–0.95]) after correction for age, sex, and BMI Z score, and the association was maintained after exclusion of type 2 diabetes cases.

The main histological features of NALFD (steatosis, necro-inflammation, and fibrosis) were scored according to the Non-Alcoholic Steatohepatitis (NASH) Clinical Research Network proposal (23) and combined into the NAFLD activity score (NAS) (NAS ≥ 5 , "NASH"; NAS ≤ 2 , "non-NASH"; and NAS 3–4, "borderline"). Thirty-six patients (40%) met the criteria for NASH. After correction for age, sex, BMI, insulin resistance (both basal and postload), and the presence of pre-diabetes/diabetes, the risk of NAS > 5 associated with SGA was highly significant (OR 3.45 [95% CI 1.20–9.91]). Fibrosis was present in 56 cases (62%), but its presence (1.28 [0.53–3.09]) and severity (stage 3: 1.61 [0.22–11.96]) was not associated with SGA.

CONCLUSIONS — The major finding of the study is the association of pediatric NAFLD with intrauterine growth

From the ¹Liver Unit, Bambino Gesù Children's Hospital, Rome, Italy; the ²Unit of Clinical Dietetics, University of Bologna, Bologna, Italy; ³Gastroenterology, University of Turin, Turin, Italy; and ⁴Pediatrics, Bambino Gesù Children's Hospital, Rome, Italy.

Address correspondence and reprint requests to Valerio Nobili, MD, Liver Unit, Research Institute, Bambino Gesù Children's Hospital, Piazza S. Onofrio 4, 00165 Rome, Italy. E-mail: nobili66@yahoo.it.

Received for publication 10 February 2007 and accepted in revised form 22 May 2007.

Published ahead of print at <http://care.diabetesjournals.org> on 29 May 2007. DOI: 10.2337/dc07-0281.

Abbreviations: NAFLD, nonalcoholic fatty liver disease; NAS, NAFLD activity score; NASH, nonalcoholic steatohepatitis; SGA, small for gestational age.

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

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Table 1—Anthropometric, clinical, and biochemical characteristics of children with NAFLD, subgrouped according to weight at birth, and of control subjects, matched for age and sex

Variables	Control subjects	NAFLD	AGA NAFLD	SGA NAFLD
n	90	90	55	35
Age (years)	11.3 ± 3.8	11.7 ± 3.2	11.3 ± 3.3	12.3 ± 3.0
Sex (male/female)	63/27	63/27	37/18	26/9
Birth weight (kg)	3.38 ± 0.42	3.12 ± 0.66*	3.57 ± 0.36	2.41 ± 0.29†
Gestational age (weeks)	39.3 ± 1.0	39.3 ± 1.1	39.1 ± 1.2	39.6 ± 0.7
BMI (kg/m ²)	19.7 ± 2.7	26.3 ± 3.6‡	26.5 ± 3.6	26.0 ± 3.5
BMI Z score	1.14 ± 0.38	1.78 ± 0.72‡	1.82 ± 0.85	1.73 ± 0.43
Obesity	6 (2–13)	44 (34–54)‡	44 (31–56)	46 (29–60)
NGT/IFG-IGT/diabetes (%)	99/1/0	84/9/7‡	89/9/2	78/19/3
Family history of diabetes				
First degree (%)	NA	8 (3–15)	10 (4–20)	3 (1–14)
Second degree (%)	NA	21 (13–30)	29 (18–41)	9 (2–20)§
AST (IU/l)	28 ± 7	48 ± 25‡	49 ± 26	47 ± 23
ALT (IU/l)	28 ± 5	74 ± 61‡	77 ± 60	71 ± 63
gGT (IU/l)	22 ± 6	26 ± 21	26 ± 22	26 ± 18
Cholesterol (mg/dl)	138 ± 28	154 ± 34*	148 ± 34	164 ± 34§
Triglycerides (mg/dl)	79 ± 22	95 ± 53*	93 ± 36	99 ± 73
Fasting glucose (mg/dl)	81 ± 6	82 ± 10	81 ± 9	85 ± 11§
Fasting insulin (μU/l)	6.7 ± 2.3	11.4 ± 6.0‡	10.5 ± 5.1	13.5 ± 6.7†
HOMA-IR	1.34 ± 0.46	2.32 ± 1.25‡	1.99 ± 0.98	2.85 ± 1.46†
ISI	NA	4.42 ± 1.98	4.86 ± 1.87	3.73 ± 1.98†
HOMA-IR >2.6¶	11 (6–19)	48 (37–54)‡	38 (25–50)	63 (45–76)§
ISI <6.0¶	NA	73 (63–81)	65 (51–76)	86 (69–93)§
Liver histology				
NAS index	NA	4.41 ± 2.06	4.29 ± 2.11	4.60 ± 1.99
Fibrosis stage	NA	0.74 ± 0.73	0.73 ± 0.73	0.77 ± 0.73

Data are means ± SD and percent cases (95% CI) unless otherwise indicated. **P* vs. control subjects <0.01; †*P* vs. NAFLD appropriate for gestational age (AGA) <0.01; ‡*P* vs. control subjects <0.001; §*P* vs. NAFLD AGA <0.05. ¶These cutoffs, representing the upper (homeostasis model assessment of insulin resistance [HOMA-IR]) and lower (insulin sensitivity index derived from oral glucose load [ISI]) quartiles of a control population, respectively (ref. 20), identify subjects considered insulin resistant. ALT, alanine aminotransferase; AST, aspartate aminotransferase; IFG, impaired fasting glucose; IGT, impaired glucose tolerance; NGT, normal glucose tolerance; γGT, γ-glutamyl transpeptidase.

retardation independent of and in addition to insulin resistance. SGA children with NAFLD represent a subset with a higher prevalence of both metabolic abnormalities and NASH, the most severe form of liver damage, independently of age, sex, BMI, and genetic inheritance.

Intrauterine growth has a strong independent effect on insulin resistance. The relative risk of metabolic syndrome in adulthood increases by 1.72 times for each tertile decrease in birth weight (24). Insulin resistance probably appears early in the postnatal period, during the catch-up growth period, but the metabolic derangements are initially moderate (25–27).

At an average age of 11 years, most of our study's subjects (80%) were insulin resistant, despite normal BMI and a very low prevalence of metabolic abnormalities. Insulin resistance in adipose tissue develops early during fetal growth restriction (5) and is maintained during the neo-

natal period and adulthood. NAFLD has been proposed as part of a generalized abnormality of the adipose tissue (28) known as lipotoxicity, leading to fat accumulation in ectopic sites, including muscle and liver, and to an altered pattern of circulating and intracellular adipokines (29). This defect might stem from a combination of acquired and genetic factors. Notably, the family history of type 2 diabetes was less common in SGA NAFLD, suggesting that genetic factors have lower relevance in the onset of NAFLD in this cohort, counterbalanced by an adverse in utero environment favoring the future development of abnormal adipose tissue.

SGA was also associated with more severe disease activity at histology independently of age, sex, and insulin resistance. This confirms that factors known to affect insulin sensitivity may also independently contribute to liver disease progression (30). Insulin-resistant states are characterized by chronic subclinical in-

flammation, and an imbalance in cytokine activity produced by dysfunctional fat cells may be the link between metabolic and liver disorders. In conclusion, intrauterine growth retardation is an important risk factor for pediatric NAFLD; careful monitoring of SGA children may be considered.

References

1. Hales CN, Barker DJ, Clark PM, Cox LJ, Fall C, Osmond C, Winter PD: Fetal and infant growth and impaired glucose tolerance at age 64. *BMJ* 303:1019–1022, 1991
2. Lithell HO, McKeigue PM, Berglund L, Mohsen R, Lithell UB, Leon DA: Relation of size at birth to non-insulin dependent diabetes and insulin concentrations in men aged 50–60 years. *BMJ* 312:406–410, 1996
3. Flanagan DE, Moore VM, Godsland IF, Cockington RA, Robinson JS, Phillips DI: Fetal growth and the physiological control of glucose tolerance in adults: a min-

- imal model analysis. *Am J Physiol Endocrinol Metab* 278:E700–E706, 2000
4. Hofman PL, Cutfield WS, Robinson EM, Bergman RN, Menon RK, Sperling MA, Gluckman PD: Insulin resistance in short children with intrauterine growth retardation. *J Clin Endocrinol Metab* 82:402–406, 1997
 5. Jaquet D, Gaboriau A, Czernichow P, Levy-Marchal C: Insulin resistance early in adulthood in subjects born with intrauterine growth retardation. *J Clin Endocrinol Metab* 85:1401–1406, 2000
 6. Phillips DI, Barker DJ, Hales CN, Hirst S, Osmond C: Thinness at birth and insulin resistance in adult life. *Diabetologia* 37:150–154, 1994
 7. Veening MA, Van Weissenbruch MM, Delemarre-Van De Waal HA: Glucose tolerance, insulin sensitivity, and insulin secretion in children born small for gestational age. *J Clin Endocrinol Metab* 87:4657–4661, 2002
 8. Hovi P, Andersson S, Eriksson JG, Jarvenpaa A-L, Strang-Karlsson S, Makitie O, Kajantie E: Glucose regulation in young adults with very low birth weight. *N Engl J Med* 356:2053–2063, 2007
 9. Rasmussen EL, Malis C, Jensen CB, Jensen JE, Storgaard H, Poulsen P, Pilgaard K, Schou JH, Madsbad S, Astrup A, Vaag A: Altered fat tissue distribution in young adult men who had low birth weight. *Diabetes Care* 28:151–153, 2005
 10. Marchesini G, Brizi M, Bianchi G, Tomassetti S, Bugianesi E, Lenzi M, McCullough AJ, Natale S, Forlani G, Melchionda N: Nonalcoholic fatty liver disease: a feature of the metabolic syndrome. *Diabetes* 50:1844–1850, 2001
 11. Gastaldelli A, Miyazaki Y, Pettiti M, Matsuda M, Mahankali S, Santini E, DeFronzo RA, Ferrannini E: Metabolic effects of visceral fat accumulation in type 2 diabetes. *J Clin Endocrinol Metab* 87:5098–5103, 2002
 12. Medina J, Fernandez-Salazar LI, Garcia-Buey L, Moreno-Otero R: Approach to the pathogenesis and treatment of nonalcoholic steatohepatitis. *Diabetes Care* 27:2057–2066, 2004
 13. Eckel RH, Grundy SM, Zimmet PZ: The metabolic syndrome. *Lancet* 365:1415–1428, 2005
 14. Franzese A, Vajro P, Argenziano A, Puzziello A, Iannucci MP, Saviano MC, Brunetti F, Rubino A: Liver involvement in obese children: ultrasonography and liver enzyme levels at diagnosis and during follow-up in an Italian population. *Dig Dis Sci* 42:1428–1432, 1997
 15. Tominaga K, Kurata JH, Chen YK, Fujimoto E, Miyagawa S, Abe I, Kusano Y: Prevalence of fatty liver in Japanese children and relationship to obesity: an epidemiological ultrasonographic survey. *Dig Dis Sci* 40:2002–2009, 1995
 16. Nobili V, Marcellini M, Devito R, Ciampalini P, Piemonte F, Comparcola D, Sartorelli MR, Angulo P: NAFLD in children: a prospective clinical-pathological study and effect of lifestyle advice. *Hepatology* 44:458–465, 2006
 17. Cole TJ, Bellizzi MC, Flegal KM, Dietz WH: Establishing a standard definition for child overweight and obesity worldwide: international survey. *BMJ* 320:1240–1243, 2000
 18. Gairdner D, Pearson J: A growth chart for premature and other infants. *Arch Dis Child* 46:783–787, 1971
 19. The Expert Committee on the Diagnosis and Classification of Diabetes Mellitus: Follow-up report on the diagnosis of diabetes mellitus. *Diabetes Care* 26:3160–3167, 2003
 20. Bugianesi E, Pagotto U, Manini R, Vanni E, Gastaldelli A, De Iasio R, Gentilcore E, Natale S, Rizzetto M, Pasquali R, Marchesini G: Plasma adiponectin in non-alcoholic fatty liver is related to hepatic insulin resistance and hepatic fat content, not to liver disease severity. *J Clin Endocrinol Metab* 90:3498–3504, 2005
 21. Matthews DR, Hosker JP, Rudenski AS, Naylor BA, Treacher DF, Turner RC: Homeostasis model assessment: insulin resistance and beta-cell function from plasma fasting glucose and insulin concentrations in man. *Diabetologia* 28:412–419, 1985
 22. Matsuda M, DeFronzo RA: Insulin sensitivity indices obtained from oral glucose tolerance testing: comparison with the euglycemic insulin clamp. *Diabetes Care* 22:1462–1470, 1999
 23. Kleiner DE, Brunt EM, Van Natta M, Behling C, Contos MJ, Cummings OW, Ferrell LD, Liu YC, Torbenson MS, Unalp-Arida A, Yeh M, McCullough AJ, Sanyal AJ: Design and validation of a histological scoring system for nonalcoholic fatty liver disease. *Hepatology* 41:1313–1321, 2005
 24. Valdez R, Athens MA, Thompson GH, Bradshaw BS, Stern MP: Birthweight and adult health outcomes in a biethnic population in the USA. *Diabetologia* 37:624–631, 1994
 25. Soto N, Bazaes RA, Pena V, Salazar T, Avila A, Iniguez G, Ong KK, Dunger DB, Mericq MV: Insulin sensitivity and secretion are related to catch-up growth in small-for-gestational-age infants at age 1 year: results from a prospective cohort. *J Clin Endocrinol Metab* 88:3645–3650, 2003
 26. Yajnik CS, Fall CH, Vaidya U, Pandit AN, Bavdekar A, Bhat DS, Osmond C, Hales CN, Barker DJ: Fetal growth and glucose and insulin metabolism in four-year-old Indian children. *Diabet Med* 12:330–336, 1995
 27. Bo S, Bertino E, Bagna R, Trapani A, Gambino R, Martano C, Mombro M, Paganò G: Insulin resistance in pre-school very-low-birth weight pre-term children. *Diabet Metab* 32:151–158, 2006
 28. Unger RH: Lipotoxic diseases. *Annu Rev Med* 53:319–336, 2002
 29. Bugianesi E, McCullough AJ, Marchesini G: Insulin resistance: a metabolic pathway to chronic liver disease. *Hepatology* 42:987–1000, 2005
 30. Marchesini G, Bugianesi E, Forlani G, Cerrelli F, Lenzi M, Manini R, Natale S, Vanni E, Villanova N, Melchionda N, Rizzetto M: Nonalcoholic fatty liver, steatohepatitis, and the metabolic syndrome. *Hepatology* 37:917–923, 2003