Supplemental Material

Metabolic Features of Nonalcoholic Fatty Liver (NAFL) in a Multiethnic Cohort of Obese Adolescents

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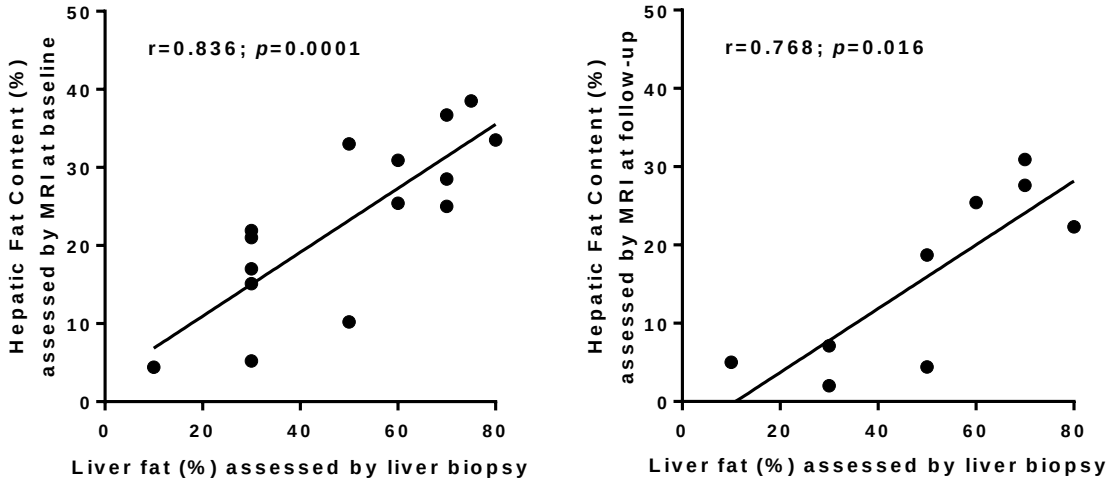
Supplemental Methods

Metabolic Studies

All metabolic studies were performed at the Yale Center for Clinical Investigation at 8.00 am following a 10 – 12 h overnight fast. A standard OGTT (1.75 g/Kg body weight, up to 75 g) was performed. Blood samples for determination of glucose, insulin, and C-peptide were drawn at −15, 0, 30, 60, 90, 120 and 180 min (1). Type 2 diabetes and conditions of increased risk for diabetes (prediabetes) were defined according to the American Diabetes Association criteria (2). Insulin sensitivity was assessed by the whole-body insulin sensitivity index (WBISI), which we have previously validated by comparison with the euglycemic-hypeinsulinemic clamp studies in obese adolescents (3). The insulinogenic index (IGI), which represents early phase insulin secretion and is a commonly used index of beta cell function, was calculated from the OGTT data as follows: IGI = Δinsulin (0 – 30 min) in microunits per milliliter divided by the glucose (0–30 min) in milligrams per deciliter. To better describe the state of insulin secretion in our population we also included the disposition index (DI), which derives from a non-linear hyperbola-like curve and provides an integrate picture of glucose tolerance including both insulin sensitivity and insulin secretion. The DI was calculated as the product of the IGI and the WBISI, based on the curvilinear relation of these OGTT derived parameters previously described by our group in obese children and adolescents (3).

Total body composition was measured by dual-energy x-ray absorptiometry with a Hologic scanner (Boston, MA).

Supplemental Figure 1



Supplemental Figure 1. Correlation between liver fat assessed by liver biopsy at baseline and hepatic fat content assessed by magnetic resonance imaging (MRI) either at baseline (*n*=15) or after a 2 year follow up (*n*=9) in obese adolescents, tested using Spearman's rank correlations.

Supplemental Table 1. Baseline characteristics of the overall study cohort and of the subgroups of subjects who underwent longitudinal assessments or liver biopsy.

	Overall Study Cohort (*n*=503)	**Longitudinal Cohort** (*n*=133)	**Subjects with Liver Biopsy** (*n*=15)
CLINICAL FEATURES			
Age (years)	13.66±2.84	13.82±2.62	13.61±2.26
Sex (M/F) [%]	206/297 [41.0/59.0]	58/75 [43.6/56.4]	8/7 [53.3/46.7]
Race (white/black/Hispanic) [%]	191/134/178 [38.0/26.6/35.4]	53/32/48 [39.8/24.1/36.1]	4/0/11 [26.7/0/73.3] [a, b]
z-score Body Mass Index	2.22±0.41	2.26±0.42	2.33±0.34
Body Mass Index (kg/m^2)	33.39±6.38	34.12±6.10	34.89±6.73
Tanner Stage (1/2/3/4/5) [%]	[9.5/15.9/17.9/18.9/37.8]	[7.5/10.5/21.8/18.8/41.4]	[0.0/6.7/20.0/20.0/53.3]
Systolic Blood Pressure (mmHg)	118.19±11.10	118.73±10.08	122.75±14.80
Diastolic Blood Pressure (mmHg)	67.76±7.96	68.98±8.21	69.25±9.00
GLUCOSE METABOLISM			
Fasting glucose (mg/dl)	91.35±8.66	94.48±8.79	95.00±15.46
Fasting insulin (μU/ml)	34.48±22.28	35.98±20.34	52.83±34.37 [a, b]
Fasting C peptide (pmol/l)	1187.9±526.2	1187.3±411.9	1556.9±532.0 [a, b]
2 h glucose (mg/dl)	124.70±31.71	129.14±25.75	161.44±37.22 [a, b]
Hemoglobin A_{1C} (%)	5.51±0.25	5.52±0.38	5.69±0.50
Whole Body Insulin Sensitivity Index (WBISI)	1.80±1.12	1.65±0.91	0.86±0.59 [a, b]
Insulinogenic index (IGI)	4.74±3.57	4.62±4.01	4.58±4.29
Disposition Index (DI)	7.34±5.99	6.32±5.34	3.29±1.82 [a, b]
LIPID PROFILE			
Total Cholesterol (mg/dL)	154.15±30.27	155.66±32.25	157.00±27.81
HDL Cholesterol (mg/dL)	43.47±10.18	44.13±11.64	38.38±6.83 [a, b]
LDL Cholesterol (mg/dL)	89.78±25.38	90.44±27.45	85.00±26.05
Triglycerides (mg/dL)	104.48±62.67	110.50±80.07	156.42±82.37 [a, b]
BODY FAT DISTRIBUTION			
Hepatic Fat Fraction (%)	8.68±11.41	9.63±12.66	23.09±10.92 [a, b]
HFF > 5.5% (no/yes) [%]	[58.4/41.6]	[57.1/42.9]	[13.3/86.7] [a, b]
Visceral Fat (cm^2)	58.41±27.67	63.87±27.63	75.54±32.2 [a]
Subcutaneous Fat (cm^2)	491.77±198.72	517.83±187.42	495.81±223.06
LIVER ENZYMES			
Alanine Transaminase (U/L)	28.87±25.27	29.03±28.24	156.21±94.48 [a, b]
Aspartate Transaminase (U/L)	25.31±12.95	25.05±13.50	91.14±59.72 [a, b]
γ-Glutamyl Transferase (U/L)	22.46±12.07	23.12±18.47	70.83±55.65 [a, b]
Alkaline Phosphatase (U/L)	191.12±100.64	175.95±98.85	205.21±113.66

There were not statistically significant differences between the overall study cohort and the longitudinal cohort.

[a] $p < 0.05$ for the difference between the overall study cohort and the subgroup of subjects with liver biopsy.

[b] $p < 0.05$ for the difference between the longitudinal cohort and the subgroup of subjects with liver biopsy.

Supplemental Table 2. Histological and genetic characteristics of the subgroup of subjects who underwent liver biopsy (*n* = 15).

Histological / Genetic Variable	***n* (%)**
Diagnosis	
Not steatohepatitis	-
Borderline	3 (20)
Steatohepatitis	12 (80)
NAS	
0-2	-
3-4	3 (20)
5-8	12 (80)
Steatosis grade	
0 - < 5%	-
1 - 5-33%	6 (40)
2 - 34-66%	4 (27)
3 - > 66%	5 (33)
Lobular inflammation	
0 - none	1 (7)
1 - < 2 foci per 200× field	12 (80)
2 - 2-4 foci per 200× field	2 (13)
3 - > 4 foci per 200× field	-
Portal inflammation	
0 - None to minimal	13 (87)
1 - Greater than minimal	2 (13)
Ballooning	
0 - none	3 (20)
1 - few balloon cells	8 (53)
2 - many cells	4 (27)
Fibrosis	
0- none	1 (7)
1 - perisinusoidal or periportal	6 (40)
2 - perisinusoidal and portal/periportal	7 (47)
3 - bridging	1 (7)
4 - cirrhosis	-
PNPLA3 rs738409 [a]	
CC	4 (29)
CG	4 (29)
GG	6 (43)
GCKR rs1260326 [a]	
CC	5 (36)
CT	2 (14)
TT	7 (50)
TM6SF2 rs58542926 [a]	
CC	6 (43)
CT	8 (57)
TT	-

Biopsies were scored according to the NASH CRN Scoring System (4-6).

[a] Genotyping was performed in 14 subjects.

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