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Original article

Association of adiposity, measures of metabolic dysregulation, and elevated alanine aminotransferase in subjects with normal body mass index

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Background: Differences in body fat (BF) distribution in patients with normal body mass index (BMI) with elevated alanine aminotransferase (ALT) remains poorly described.

Objective: To determine the relationship between total BF, waist circumference (WC), insulin resistance (IR), and cardiometabolic risk profile in subjects with elevated ALT and normal BMI.

Methods: We analyzed cross-sectional data from 4,914 US participants in the third National Health and Nutrition Examination Survey database, who were ≥20 years of age, had normal BMI, and had body composition assessed by bioimpedance.

Results: Mean ± SD age was 41.4 ± 0.3 years, and 58% participants were women. BF was 20 ± 0.1% in men and 29.9 ± 0.1% in women. As total BF increased by tertiles, there was a tendency towards a higher prevalence of nonalcoholic fatty liver disease in men (6.1%, 6.5%, 9.5%, $P = 0.13$), but not in women (8.7%, 8.2%, 10.7%, $P = 0.71$). As WC increased by tertiles, there was a higher prevalence of elevated ALT in men (2.6%, 8.6%, 6.6%, $P < 0.0001$), but not in women. As ALT increased, men had significantly higher levels of nonhigh density lipoprotein cholesterol (HDL-C), increased apolipoprotein B, increased IR, and lower levels of C-reactive protein, whereas, women had higher levels of non-HDL-C and increased IR.

Conclusion: In subjects with normal BMI, increased WC is associated with a higher prevalence of elevated ALT in men, but not in women. Higher levels of ALT correlated with a poor cardiometabolic risk profile.

Keywords: Body fat, body mass index, elevated ALT, waist circumference

Abbreviations

ALT = alanine aminotransferase
Anti-HCV = hepatitis C antibody
AST = aspartate aminotransferase
BF = body fat
BMI = body mass index
CAD = coronary artery disease
CRP = C-reactive protein
DM = diabetes mellitus
GGT = γ-glutamyltransferase
HBsAg = hepatitis B surface antigen
HDL-C = high density lipoprotein cholesterol
MetS = metabolic syndrome
IR = insulin resistance
NAFLD = non-alcoholic fatty liver disease
NHANES III = Third National Health and Nutrition Examination Survey
VAT = visceral adipose tissue
WC = waist circumference

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The association of nonalcoholic fatty liver disease (NAFLD) and increased body mass index (BMI) is well documented and BMI is proven to be an independent indicator of advanced liver fibrosis [1, 2]. Overweight Korean subjects with insulin resistance (IR) were at higher risk of NAFLD than those subjects with less IR [3].

Excessive adiposity may contribute to liver damage in patients with NAFLD, was proposed as a first step in the pathogenesis of fatty liver, and is considered to be a consequence of IR [4]. The second steps of oxidative stress resulting from mitochondrial fatty acid oxidation, inflammatory cytokine expression, and adipokines are considered to be potential causes of progression of liver injury [4]. Obesity alters adipose tissue by changing both the cellular composition and function of fat [5]. However, some patients with normal BMI may develop NALFD. The prevalence of NAFLD in Chinese children with normal BMI of 0.6%, while NAFLD prevalence increased to approximately 3% in overweight and 14% in obese subjects [6]. The prevalence of NAFLD in nonobese and nondiabetic Korean adults mean BMI of 25.6 ± 2.3 kg/m^2 was 23.4% [7]. NAFLD was also found to be a significant predictor of IR and other metabolic disorders such as hypertriglyceridemia [7]. Thus, the prevalence of NAFLD in patients with normal BMI may be underestimated.

The role of body fat distribution in NAFLD patients with normal weight remains poorly described. Waist circumference (WC) and waist–hip ratio are simple, indirect anthropometric parameters commonly used to identify overweight populations [8]. Actual measures of total body fat are now used in both clinical and epidemiological studies [9]. There are several methods by which to evaluate body fat, including bioelectrical impedance analysis (BIA), dual X-ray absorptiometry, computed tomography (CT), or magnetic resonance imaging (MRI) [10]. All of these tools have advantages and limitations when applied to obese subjects; however, there are few data for body fat (BF) assessment in subjects with normal weight. BIA is a simple and noninvasive method [10] used to estimate total body water, fat-free mass, total body fat, and percentage body fat (%BF). BIA was measured in a nationally representative sample of the United States population. We sought to determine the relationship between total BF (determined by BIA), WC, IR, and cardiometabolic risk profile with development of NAFLD in subjects with normal BMI, representative of the general US population.

Materials and methods

Study design and subject selection

The third National Health and Nutrition Examination Survey (NHANES III) examined a representative sample of the noninstitutionalized US civilian population from 1988 to 1994. NHANES is a periodic survey using a stratified multistage probability sampling design to produce a generalized health estimate of the United States population [11]. Of a sample of 39,695 people were selected for NHANES III, 33,994 subjects were interviewed and 30,818 submitted to an examination by a physician at mobile examination centers that included extensive anthropometric, physiological, and laboratory testing. For this study we included data from 6,164 subjects aged >20 years, with a normal BMI (18.5–24.9 kg/m^2), as defined by the US National Institutes of Health, who had bioelectrical impedance analysis to estimate body composition. From those, we excluded 629 subjects with >20 grams of alcohol consumption per day, 184 subjects who had a positive antigen for hepatitis B (HBsAg), and/or positive anti-HCV, and 437 subjects who had a transferrin saturation >45% (total = 1,250), resulting in a final sample of 4,914 subjects. IRB approval for this study was waived because it involved only secondary analyses using the NHANES III database with deidentification of survey participants.

Anthropometric measurements, body fat and body composition analyses

All personnel performing NHANES III anthropometric and body composition measurements were trained and followed a strict protocol. Body weight was measured with an electronic load cell scale to the nearest 0.01 kg. Participants wore only undershorts and disposable paper shirts, pants, and foam slippers. Stature was measured to the nearest 0.1 cm using a fixed stadiometer. Participants were positioned with heels, buttocks, back and head against the upright surface of the stadiometer with their head positioned in the Frankfort horizontal plane. Waist circumference (WC) was measured by a trained examiner and determined using a measuring tape positioned at the high point of the iliac crest. The measurement was made with minimal respiration to the nearest 0.1 cm, with the tape snug but not compressing the skin. BMI was calculated as weight in kilograms divided by height in meters squared (kg/m^2). Pregnant women and subjects with

pacemakers were ineligible for bioelectrical impedance analysis. All subjects were requested to avoid eating or drinking anything except water during the fasting period. The prediction equations for total body water and fat free mass use resistance measured with data from RJL bioelectrical impedance analyzers. NHANES III resistance data were obtained using Valhalla impedance analyzers. Therefore, bioimpedance resistance was converted to RJL Res values (Ω) and was used to calculate BF as previously described by Chumlea et al. [10]. The prediction equations used to estimate lean mass are as follows:

Men: Lean mass = –10.678 + 0.262 kg + 0.652 S^2/Res + 0.015 Res
Women: Lean mass = –9.529 + 0.168 kg + 0.696 S^2/Res + 0.016 Res

Where S^2/Res represents the stature squared divided by resistance (cm^2/Ω). We then calculated BF as follows: BF % = [(weight – lean mass) / weight] × 100

Detailed information on the BIA procedure is presented elsewhere [12, 13].

Adiposity was based on standard clinical definitions for BMI (normal weight, 18.5–24.9; overweight, 25.0–29.9; obese 30.0–34.9; and morbid obese, ≥35.0 kg/m^2). WC was classified into normal WC, <88.0 cm for women and <102.0 cm for men, while abdominal obesity is defined by WC 88.0 cm for women and ≥102.0 cm for men. Percentage of BF is classified into normal %BF, <30% for women and <25% for men while obesity is defined by a %BF ≥30% for women and ≥25% for men [14].

Laboratory measurements

NHANES III serum biochemistries were performed using a Hitachi 737 automated multichannel chemistry analyzer (Boehringer Mannheim Diagnostics, Indianapolis, IN) at a central laboratory (White Sands Research Center, Alamogordo, NM). Serum levels of alanine aminotransferase (ALT), glucose, insulin, lipids, apolipoprotein B, and C-reactive protein (CRP) were measured, and the degree of IR was calculated by the updated Homeostasis Model Assessment (HOMA2). A detailed description of the laboratory assays and quality control procedures is available elsewhere. Lipids were measured enzymatically with the use of commercially available reagents. Glucose was measured using standard assay (Sigma, St Louis, MO), and plasma insulin was measured using a Pharmacia insulin radioimmunoassay kit (Pharmacia Diagnostics, Sweden). We determined the insulin sensitivity index using the updated computer homeostatic model assessment (HOMA2) index, which is a method for assessing β-cell function and IR from fasting glucose and insulin or C-peptide concentrations [15]. The HOMA2 model is an updated version of HOMA1. HOMA2 is a curvilinear model accounting for variations in liver and peripheral glucose and insulin resistance [16]. Apolipoprotein B was measured by radial immunodiffusion in the first 8.2% of the specimens during the first 5 months of the survey and by rate immunonephelometry for the remaining specimens. Serum leptin concentrations were measured by radioimmunoassay at Linco Research (St. Charles, MO) [17]. C-reactive protein (CRP) was measured using a modification of the Behring latex-enhanced CRP assay (Behring Diagnostics, Westwood, MA), as previously described. Detailed methodologies on laboratory procedures of NHANES III are published elsewhere [18].

Definitions of elevated liver transaminases, alcohol consumption, hepatitis B and C, hemato-chromatosis and non-alcoholic fatty liver disease

On the basis of the NHANES III laboratory cutoff values for normal levels, we defined elevated liver test results as AST levels higher than 37 U/L for men and 31 U/L for women, γ-glutamyltransferase (GGT) levels higher than 51 U/L for men and 33 U/L for women. Elevated ALT levels used the most recently proposed values (>30 IU/L in men and >19 IU/L in women) [19].

The frequency of alcoholic beverages (beer, wine, and liquor) during the past month was assessed using a food-frequency questionnaire during the interview. We categorized subjects who drank up to once per week, more than once per week, but less than once per day, and those who drank alcohol once a day or more. During the physical examination at the mobile examination center a 24 h dietary recall was administered, which assessed the amount of alcohol consumed during the previous day. From these data, the daily intake of alcohol (in grams) was calculated [20]. The presence of viral hepatitis C antibody (anti-HCV) was tested using a second-generation enzyme

immunoassay test (Abbott Laboratories, Chicago, IL) and confirmed with the MATRIX assay (Abbott Laboratories), a third-generation anti-HCV test. The presence of HBsAg was tested by a solid-phase competitive immunoassay (Abbott Laboratories). Hemochromatosis was defined as a subject who had a transferrin saturation >45%. Elevated ALT levels using the most recently proposed values (>30 IU/L in men and >19 IU/L in women), with all the above etiologies excluded, was used as a surrogate for NAFLD [19].

Statistical analysis

Data were summarized by calculating means and standard errors for continuous variables and number and percentages for categorical variables. We divided our sample of normal BMI subjects into sex-specific tertiles of BF and WC. All analyses were stratified by sex and controlled for age and race/ethnicity. Only subjects with fasting and morning samples (n = 2,269) were used for analyses of IR for HOMA2. We performed log transformation to reduce the positive skewness of liver enzymes, HOMA2, triglycerides, CRP, and leptin. We calculated the prevalence and *P*-values for trend adjusted for age and race for NAFLD across tertiles of BF and WC groups. We assessed metabolic profile, including non-HDL cholesterol (non HDL-C), leptin, CRP, apolipoprotein B and IR, across quartiles of ALT with the hypothesis that subjects with an elevated ALT will have higher levels of IR, non-HDL-C, leptin, CRP and apolipoprotein B. Finally, to assess the generalizability of our results, we examined whether our selected population of subjects with normal BMI with body composition analyses and blood measurement were similar to those without these measurements. All analyses were weighted according to NHANES methodology and were performed using SAS version 9.1 and SUDAAN version 9.0.3.

Results

The baseline characteristics of 4,914 subjects are shown in **Table 1**. Most of the subjects (77.5%) were non-Hispanic whites, 9.2% were non-Hispanic blacks, 4.2% were Mexican Americans and 9.1% were from a different ethnicity. Mean ± SD age was 41.4 ± 0.3 years, and 58% were women. Mean BMI was 22.2 ± 0.02 kg/m^2 and the mean value of WC was 84 ± 0.2 cm in men and 77.7 ± 0.1 cm in women. The average percentage of BF was within normal limits with results of 20.0 ± 0.1% in men and 29.9 ± 0.1% in women.

Elevated ALT, GGT, and prevalence of NAFLD according to BF tertiles

Increasing age was associated with increased %BF in both sexes. After controlling for age and race, increased %BF content in men was significantly associated with higher levels of ALT, but no association between ALT levels and the increased %BF was found in women (**Tables 2 and 3**). Increased %BF content in men was also significantly associated with higher levels of GGT, whereas there was no relationship between %BF content and levels of GGT in women (**Tables 2 and 3**). The proportions of subjects with NAFLD accounted for 6.1%–9.5% of men and 8.2%–10.7% of women by tertiles of BF, even though they all had normal BMI and WC.

Table 1. Baseline characteristics of 4,914 subjects with normal body mass index

Variable; mean ± SD or number (%)	Total (N = 4,914)
Age (years)	41.4 ± 0.26
Female sex (%)	2,706 (58.5)
Race (%)	
Non-Hispanic white	2,174 (77.5)
Non-Hispanic black	1,291 (9.2)
Mexican-Americans	1,194 (4.2)
Other ethnicity	255 (9.1)
Body mass index (kg/m^2)	22.2 ± 0.02
Waist circumference (cm)	80.3 ± 0.11
Waist-to-hip ratio	0.86 ± 0.001
Body fat	25.8 ± 0.10
ALT (U/L)	14.1 ± 0.13
AST (U/L)	19.5 ± 0.11
GGT (U/L)	20.9 ± 0.39
Transferrin saturation (%)	25.2 ± 0.14

Table 2. Anthropometric and metabolic measures in men with a normal body mass index by %body fat tertiles

Variable; mean ± SE or number (%) (n = 2,208)	1st Tertile BF% (n = 741)	2nd Tertile BF% (n = 736)	3rd Tertile BF% (n = 731)	Age + race adj. *P* for trend
Age (years)	37.5 ± 0.62	40.6 ± 0.60*	45.5 ± 0.58*	<0.0001
Race (%)				
Non-Hispanic white	818 (82.8)	695 (74.4)	661 (73.7)	
Non-Hispanic black	460 (8.1)	431 (9.2)*	400 (10.7)*	<0.0001
Mexican-American	293 (2.7)	418 (4.6)	483 (5.6)	
Other ethnicity	78 (6.3)	101 (11.7)	76 (10.0)	
Body mass index (kg/m^2)	21.1 ± 0.04	22.5 ± 0.03*	23.5 ± 0.03*	<0.0001
Waist circumference (cm)	79.9 ± 0.23	84.8 ± 0.23*	88.7 ± 0.24*	<0.0001
Waist-to-hip ratio	0.88 ± 0.002	0.91 ± 0.002*	0.94 ± 0.002*	<0.0001
Body fat (%)	14.9 ± 0.12	20.9 ± 0.05*	25.9 ± 0.08*	<0.0001
ALT	13.6 ± 0.19	14.0 ± 0.25	14.9 ± 0.24†	0.0001
ALT > 30,U/L	41 (6.1)	48 (6.5)	56 (9.5)	0.13
AST	19.7 ± 0.16	19.3 ± 0.22	19.6 ± 0.16	0.54
AST/ALT <1	108 (8.9)	128 (8.8)	163 (14.6)†	0.0004
GGT	18.6 ± 0.51	21.3 ± 0.72	23.9 ± 0.80*	<0.0001

*P < 0.0001, †P < 0.001, when compared with the lowest tertile

Table 3. Anthropometric and metabolic measures in women with a normal body mass index by %body fat tertiles

Variable; mean ± SE or number (%) (n = 2,706)	1st Tertile BF% (n = 908)	2nd Tertile BF% (n = 909)	3rd Tertile BF% (n = 889)	Age + race adj. *P* for trend
Age (years)	37.5 ± 0.62	40.6 ± 0.60*	45.5 ± 0.58*	<0.0001
Race (%)				
Non-Hispanic white	818 (82.8)	695 (74.4)	661 (73.7)	
Non-Hispanic black	460 (8.1)	431 (9.2)*	400 (10.7)*	
Mexican-American	293 (2.7)	418 (4.6)	483 (5.6)	
Other ethnicity	78 (6.3)	101 (11.7)	76 (10.0)	<0.0001
Body mass index (kg/m^2)	21.1 ± 0.04	22.5 ± 0.03*	23.5 ± 0.03*	<0.0001
Waist circumference (cm)	73.4 ± 0.19	78.3 ± 0.22*	83.0 ± 0.22*	<0.0001
Waist-to-hip ratio	0.80 ± 0.001	0.83 ± 0.002*	0.85 ± 0.002*	<0.0001
Body fat (%)	24.8 ± 0.11	31.0 ± 0.04*	35.6 ± 0.06*	<0.0001
ALT	13.6 ± 0.19	14.0 ± 0.25	14.9 ± 0.24	0.52
ALT > 19, U/L	82 (8.7)	86 (8.2)	93 (10.7)	0.71
AST	19.7 ± 0.16	19.3 ± 0.22	19.6 ± 0.16	0.14
AST/ALT <1	108 (8.9)	128 (8.8)	163 (14.6)	0.35
GGT	18.6 ± 0.51	21.3 ± 0.72	23.9 ± 0.80§	0.060

P < 0.0001, §*P* < 0.05, when compared with lowest tertiles

Elevated ALT, GGT and prevalence of NAFLD according to WC tertiles

Increasing age was associated with increased WC in both sexes. After controlling for age and race, as WC increased, there was a significantly higher level of ALT in both sexes. Increased WC in both sexes was significantly associated with higher levels of GGT, and was also significantly associated with a higher proportion of subjects with abnormal GGT (>51 U/L), as shown in Table 3. Increased WC in men was significantly associated with a higher prevalence of NAFLD (**Table 4**); however, this association was not found in women (**Table 5**). Regarding the 1st, 2nd and 3rd tertiles of WC, the proportions of subjects with NAFLD in men were 2.6%, 8.6%, and 6.6%, respectively, whereas those proportions in women were 7.4%, 6.9%, and 9.0%, respectively.

Table 4. Anthropometric and metabolic measures in men with a normal body mass index by waist circumference tertiles

Variable; mean ± SE or number (%) (n = 2,208)	1st Tertile WC (n = 747)	2nd Tertile WC (n = 728)	3rd Tertile WC (n = 733)	Age + race adj. *P* for trend
Age (years)	30.2 ± 0.47	37.7 ± 0.59*	48.4 ± 0.62*	<0.0001
Race (%)				
Non-Hispanic white	169 (62.2)	265 (72.8)	418 (83.9)	
Non-Hispanic black	338 (18.2)	186 (8.4)*	115 (5.0)*	<0.0001
Mexican-American	193 (6.4)	236 (6.4)	168 (3.4)	
Other ethnicity	47 (13.3)	41 (12.4)	32 (7.8)	
Body mass index (kg/m^2)	21.3 ± 0.06	22.7 ± 0.05*	23.7 ± 0.04*	<0.0001
Waist circumference (cm)	75.8 ± 0.11	83.5 ± 0.08*	92.2 ± 0.13*	<0.0001
Waist-to-hip ratio	0.85 ± 0.001	0.90 ± 0.002*	0.97 ± 0.002*	<0.0001
Body fat (%)	16.7 ± 0.2	20.0 ± 0.15*	23.1 ± 0.16*	<0.0001
ALT	15.6 ± 0.31	17.8 ± 0.46‡	17.6 ± 0.43*	<0.0001
ALT > 30, U/L	31 (2.6)	52 (8.6)§	46 (6.6)†	0.0005
AST	21.5 ± 0.27	21.5 ± 0.45	21.0 ± 0.24	0.37
AST/ALT <1	64 (9.1)	97 (20.5)†	108 (22.0) *	<0.0001
GGT	21.0 ± 1.25	26.1 ± 0.98†	28.9 ± 1.48 *	<0.0001

*P < 0.0001, †P < 0.001, ‡P < 0.01, §P < 0.05, when compared with the lowest tertile

Table 5. Anthropometric and metabolic measures in women with a normal body mass index by waist circumference tertiles

Variable; mean ± SE or number (%) (n = 2,706)	1st Tertile WC (n = 931)	2nd Tertile WC (n = 891)	3rd Tertile WC (n = 884)	Age + race Adj. *P* for trend
Age (years)	34.7 ± 0.48	41.5 ± 0.55*	52.5 ± 0.61*	<0.0001
RaceNon-Hispanic white	419 (81.6)	420 (79.4)	483 (80.7)	
Non-Hispanic black	261 (9.0)	230 (8.6)*	161 (7.2)*	<0.0001
Mexican-American	207 (3.6)	195 (3.2)	195 (3.3)	
Other ethnicity	44 (5.8)	46 (8.8)	45 (8.8)	
Body mass index (kg/m^2)	20.8 ± 0.05	22.2 ± 0.05*	23.2 ± 0.05*	<0.0001
Waist circumference (cm)	70.2 ± 0.10	77.9 ± 0.07*	86.8 ± 0.14*	<0.0001
Waist-to-hip ratio	0.77 ± 0.001	0.82 ± 0.002*	0.90 ± 0.002*	<0.0001
Body fat (%)	26.7 ± 0.15	30.5 ± 0.14*	33.2 ± 0.13*	<0.0001
ALT	11.7 ± 0.20	11.7 ± 0.21	12.7 ± 0.24	0.016
ALT > 19, U/L	71 (7.4)	66 (6.9)	87 (9.0)	0.48
AST	17.8 ± 0.17	18.2 ± 0.18	18.9 ± 0.20	0.10
AST/ALT <1	42 (5.7)	35 (3.9)	53 (7.3)§	0.020
GGT	15.6 ± 0.46	16.5 ± 0.59	22.2 ± 0.98*	<0.0001

*P < 0.0001, §P < 0.05, when compared with the lowest tertile

Elevated ALT and metabolic profiles

As ALT increased for each quartile, men had significantly higher levels of non-HDL-C, higher levels of apolipoprotein B, increased IR, and lower levels of CRP, whereas increased ALT in women was associated only with higher levels of non-HDL-C and increased IR (**Tables 6 and 7**).

Table 6. Metabolic measures in men according to aspartate aminotransferase quartiles

Variable; mean ± SE or number (%) (n = 2,208)	1st Quartile ALT (n = 499)	2nd Quartile ALT (n = 473)	3rd Quartile ALT (n = 533)	4th Quartile ALT (n = 523)	Age + race adj. *P* for trend
Age (years)	44.9 ± 0.97	41.2 ± 0.91	39.1 ± 0.68	36.6 ± 0.56	<0.0001*
Race					
Non-Hispanic white	225 (75.7)	214 (79.1)	178 (71.8)	182 (71.0)	
Non-Hispanic black	173 (13.8)	117 (9.2)	153 (10.3)	116 (6.9)	<0.0001*
Mexican-American	80 (3.5)	123 (4.6)	172 (6.5)	185 (6.1)	
Other ethnicity	21 (7.1)	19 (7.2)	30 (11.4)	40 (16.0)	
Body mass index (kg/m^2)	22.2 ± 0.08	22.6 ± 0.07	22.7 ± 0.07	22.9 ± 0.07	<0.0001*
Waist circumference (cm)	83.6 ± 0.35	83.5 ± 0.34	84.4 ± 0.31	84.8 ± 0.29	<0.0001*
Waist-to-hip ratio	0.90 ± 0.003	0.90 ± 0.003	0.90 ± 0.002	0.91 ± 0.002	<0.0001*
Body fat (%)	19.7 ± 0.24	19.6 ± 0.22	19.8 ± 0.22	20.7 ± 0.22	<0.0001*
Non-HDL cholesterol	137.8 ± 1.9	137.4 ± 1.9	141.2 ± 1.7	150.0 ± 1.7	<0.0001*
Leptin (ng/ml)	3.8 ± 0.36	3.9 ± 0.39	3.2 ± 0.16	3.4 ± 0.13	0.24
C-reactive protein (mg/dl)	0.39 ± 0.03	0.38 ± 0.03	0.29 ± 0.02	0.28 ± 0.02	0.044*
Apolipoprotein B	95.9 ± 1.5	96.6 ± 1.5	99.3 ± 1.4	104.4 ± 1.6	0.0017*
HOMA2 insulin resistance	0.81 ± 0.02	0.78 ± 0.02	0.82 ± 0.02	0.89 ± 0.02	<0.0001*

*$P < 0.05$ when compared with 1st quartile aspartate aminotransferase

Table 7. Metabolic measures in women according to aspartate aminotransferase quartiles

Variable; mean ± SE or number (%) (n = 2,544)	1st Quartile ALT (n = 654)	2nd Quartile ALT (n = 626)	3rd Quartile ALT (n = 560)	4th Quartile ALT (n = 704)	Age + race adj. *P* for trend
Age (years)	42.4 ± 0.77	41.9 ± 0.72	41.8 ± 0.71	44.9 ± 0.69	0.17
Race (%)					
Non-Hispanic white	302 (79.1)	334 (82.8)	290 (83.3)	333 (79.3)	
Non-Hispanic black	223 (13.5)	142 (7.5)	111 (5.1)	107 (5.3)	0.0002*
Mexican-American	107 (2.7)	120 (2.7)	131 (3.2)	220 (5.1)	
Other ethnicity	22 (4.7)	30 (7.0)	28 (8.4)	44 (10.3)	
Body mass index (kg/m^2)	21.8 ± 0.07	21.9 ± 0.07	22.1 ± 0.07	22.1 ± 0.07	0.0095*
Waist circumference (cm)	77.1 ± 0.29	77.3 ± 0.29	78.0 ± 0.30	78.8 ± 0.29	0.0012*
Waist-to-hip ratio	0.82 ± 0.003	0.82 ± 0.003	0.83 ± 0.003	0.84 ± 0.003	0.0014*
Body fat (%)	30.0 ± 0.19	30.1 ± 0.19	29.9 ± 0.21	29.8 ± 0.20	0.114
Non-HDL cholesterol	134.4 ± 1.6	133.8 ± 1.7	135.6 ± 1.6	140.6 ± 1.6	0.021*
Leptin (ng/ml)	9.3 ± 0.34	9.4 ± 0.30	10.0 ± 0.42	9.0 ± 0.27	0.83
C-reactive protein (mg/dl)	0.36 ± 0.02	0.32 ± 0.02	0.30 ± 0.02	0.38 ± 0.02	0.67
Apolipoprotein B	94.7 ± 1.4	92.3 ± 1.2	92.9 ± 1.4	95.8 ± 1.6	0.29
HOMA2 insulin resistance	0.77 ± 40.3	0.81 ± 0.02	0.84 ± 0.02	0.87 ± 0.02	0.0003*

*$P < 0.05$ when compared to 1st quartile aspartate aminotransferase

Discussion

We analyzed data from a relatively large number of patients in the NHANES III database that provides some insight into the relationship between total BF, WC, IR, and cardiometabolic risk profile with elevated ALT levels in subjects with normal BMI. Our results demonstrate that 3%–11% of subjects had NAFLD in this population with normal BMI, regardless of BF or WC. Even though all subjects had normal BMI, about 10% of them had a fatty liver. BMI is the most commonly used measure to diagnose overweight people, with the cut-off levels of ≥30 kg/m^2 used to define obesity in western countries, whereas cut-off levels of >25 kg/m^2 are used in Asian populations [21, 22]. The development of NAFLD in subjects with normal BMI is certainly under-recognized; however, further study to re-examine the standard definition of patients with NAFLD needs to be made to identify the association of adiposity, measures of metabolic dysregulation, and NAFLD.

The assessment of obesity may be better measured by BIA to detect %BF than using BMI and/or WC. The accuracy of BMI in detecting excess body adiposity in adults is limited, especially in those with intermediate range BMI (25–29.9 kg/m^2), in men and in the elderly [23]. Moreover, BMI changes may not accurately reflect changes in adiposity of children, especially those with low BMI [24]. WC is also used globally as a clinical representative of central obesity. Different WC cutoffs have been suggested and used in different ethnic groups, separately for men and women [22]. Dervaux et al. [25] performed a cross-sectional study and found that WC was the measure most strongly associated with metabolic syndrome (MetS), while total BF showed an association with high risk of coronary artery disease (CAD). BMI was a weaker predictor for MetS and high CAD risk [25].

Fat distribution is increasingly used for CAD prediction [26]. To detect visceral adipose tissue (VAT) or subcutaneous adipose tissue, CT is the criterion standard [27]. A single slice CT image with a thickness of 10 mm at the level of the 4th and 5th lumbar vertebrae was used to analyze for VAT, and shows a high correlation with adipose tissue volume [28]. WC is highly correlated with VAT in both sexes and is used as a clinical marker for abdominal obesity [39]. A limitation of measurement of fat distribution by CT is that it is a high cost procedure and increases risk of radiation exposure. By contrast, bioelectrical impedance analysis is much simpler and noninvasive [30]. It is one of the commonly used measures for determining excess VAT in both children and adults of both sexes [31]. For the relationship between cardiovascular risk factors and fat distribution, a Korean study showed that VAT was more closely associated with cardiovascular risk factors than was subcutaneous adipose tissue [32]. A cohort study of 44,702 American women aged 40 to 65 years found that waist hip ratio (WHR) and WC were strongly and independently associated with increased risk of CAD in subjects with a BMI of 25 kg/m^2 or less [33]. The cut-off value of WHR (>0.76) or WC (>76.2 cm) was associated with a 2-fold increased risk of CAD [33]. VAT is a strong independent predictor of all-cause mortality in Canadian men, with an odds ratio (OR) of 1.8 (95% CI 1.2–2.7), whereas other predictors of mortality are abdominal subcutaneous adipose tissue (OR 1.4; 95% CI 1.02–2.03) and WC (OR 1.4; 95% CI 1.01–1.9) [34]. All previous studies supported the notion that VAT accumulation in either lean or obese subjects may play an important role in increasing risk for CAD events [35]. A study using the NHANES III database showed that at least 23% men and 33% women had normal weight obesity. In addition an association was found between MetS and increasing cardiovascular mortality (hazard ratio of 2.2) in participants with normal weight obesity [36].

Our findings showed that increasing WC was significantly associated with a higher proportion of NAFLD in men, but not in women, while higher ALT levels were associated with higher levels of non-HDL-C and increased IR in both sexes. These findings are consistent with a study which found that VAT was a strong correlate of most metabolic risk factors [37]. Interestingly, a systematic review reported that VAT reduction is correlated with modest weight loss [38]. Ross et al. [39] evaluated the effects of diet and exercise-induced weight loss on VAT change and found that every 1 cm reduction in WC was associated with a 4% reduction in VAT mass for both sexes ($P < 0.01$). One of the important factors associated with VAT measurement is ethnicity [40]. Most of our study population was non-Hispanic white, while another study showed that African-American men and women had lower VAT than Hispanics and whites, despite having similar BMI and WC [41]. Therefore, ethnic differences need to be considered as a potential confounders of body composition assessment.

Our study also found a strong association between GGT levels and increased WC in both sexes. GGT is

an enzyme mainly expressed on the cell surface and may play role in catalyzing glutathione breakdown. Increasing environmental oxidative stress may induce higher levels of GGT. Recent evidence is supportive of the components of MetS such as hypertension, hyperlipidemia and diabetes mellitus (DM) being associated with increased GGT levels [42]. Serum GGT levels were significantly higher in Korean subjects with MetS than in healthy subjects, and GGT may be used as a marker of IR [43]. GGT levels higher than 40 U/L are associated with an increased incidence of DM with a hazard ratio of 2.5 [44]. Kim et al. measured GGT levels in healthy subjects who had no liver diseases, DM or hypertension and found that the OR for subjects with the highest quartile of normal GGT compared with subjects with the lowest quartile of GGT, were 3.2 (95% CI 2.2–4.7) for DM and 1.9 (95% CI 1.6–2.3) for obese subjects [45]. GGT in subjects with NAFLD may be used to predict both cellular oxidative stress and IR in the development of MetS. We therefore suggest that a combination of the diagnosis of NAFLD with other measures of VAT, especially high WC or high BF, and high levels of GGT in asymptomatic normal BMI, may be used to predict an increased risk of cardiovascular events, at least in white subjects.

A strength of our study is the inclusion of a large sample size with normal BMI, representative of the general US population. All subjects underwent anthropometric and body composition measurements following a strict protocol. Our data included important surrogate estimates of IR including HOMA2, cholesterol and leptin as well as markers tested for cardiovascular risk such as CRP. However, our study has several limitations. First, the diagnosis of NAFLD in participants was not confirmed by either ultrasonography or liver biopsy. Second, a cross-sectional study with only one set of blood liver tests may be inadequate to confirm the diagnosis of NAFLD. Finally, most of our subjects were white, and ethnic differences may affect body composition. Therefore, these results may not be representative of other populations of different ethnic groups.

In conclusion, in subjects with normal weight by BMI criteria, increased WC is associated with a higher prevalence of elevated ALT in men, but not in women. Higher levels of ALT correlate with a poor cardiometabolic risk profile in subjects with normal BMI.

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References

1. Angulo P, Hui JM, Marchesini G, Bugianesi E, George J, Farrell GC, et al. The NAFLD fibrosis score: a noninvasive system that identifies liver fibrosis in patients with NAFLD. Hepatology. 2007; 45:846-54.
2. Church TS, Kuk JL, Ross R, Priest EL, Biltoft E, Blair SN. Association of cardiorespiratory fitness, body mass index, and waist circumference to nonalcoholic fatty liver disease. Gastroenterology. 2006; 130: 2023-30.
3. Park SH, Kim BI, Kim SH, Kim HJ, Park DI, Cho YK, et al. Body fat distribution and insulin resistance: beyond obesity in nonalcoholic fatty liver disease among overweight men. J Am Coll Nutr. 2007; 26:321-6.
4. Duvnjak M, Lerotic I, Barsic N, Tomasic V, Virovic Jukic L, Velagic V. Pathogenesis and management issues for non-alcoholic fatty liver disease. World J Gastroenterol. 2007; 13:4539-50.
5. Bouloumie A, Curat CA, Sengenes C, Lolmede K, Miranville A, Busse R. Role of macrophage tissue infiltration in metabolic diseases. Curr Opin Clin Nutr Metab Care. 2005; 8:347-54.
6. Wan YP, Xu RY, Fang H, Lu LP, Zhang XM, Cai W. [The prevalence of non-alcoholic fatty liver disease and its related risk factors in 1180 school children in Shanghai]. Zhonghua Gan Zang Bing Za Zhi. 2007; 15: 644-8.
7. Kim HJ, Kim HJ, Lee KE, Kim DJ, Kim SK, Ahn CW, et al. Metabolic significance of nonalcoholic fatty liver disease in nonobese, nondiabetic adults. Arch Intern Med. 2004; 164:2169-75.
8. Hsieh SD, Yoshinaga H, Muto T. Waist-to-height

ratio, a simple and practical index for assessing central fat distribution and metabolic risk in Japanese men and women. Int J Obes Relat Metab Disord. 2003; 27:610-6.
9. Chumlea WM, Guo SS. Assessment and prevalence of obesity: application of new methods to a major problem. Endocrine. 2000; 13:135-42.
10. Chumlea WC, Guo SS, Kuczmarski RJ, Flegal KM, Johnson CL, Heymsfield SB, et al. Body composition estimates from NHANES III bioelectrical impedance data. Int J Obes Relat Metab Disord. 2002; 26:1596-609.
11. Plan and operation of the Third National Health and Nutrition Examination Survey, 1988–94. Series 1: programs and collection procedures. Vital Health Stat 1. 1994;32:1-407.
12. Chumlea WC, Guo SS, Zeller CM, Reo NV, Baumgartner RN, Garry PJ, et al. Total body water reference values and prediction equations for adults. Kidney Int. 2001; 59:2250-8.
13. Sun SS, Chumlea WC, Heymsfield SB, Lukaski HC, Schoeller D, Friedl K, et al. Development of bioelectrical impedance analysis prediction equations for body composition with the use of a multicomponent model for use in epidemiologic surveys. Am J Clin Nutr. 2003; 77:331-40.
14. Sui X, LaMonte MJ, Laditka JN, Hardin JW, Chase N, Hooker SP, et al. Cardiorespiratory fitness and adiposity as mortality predictors in older adults. JAMA. 2007; 298:2507-16.
15. Matthews DR, Hosker JP, Rudenski AS, Naylor BA, Treacher DF, Turner RC. Homeostasis model assessment: insulin resistance and beta-cell function from fasting plasma glucose and insulin concentrations in man. Diabetologia. 1985; 28:412-9.
16. Wallace TM, Levy JC, Matthews DR. Use and abuse of HOMA modeling. Diabetes Care. 2004; 27:1487-95.
17. Ruhl CE, Everhart JE. Leptin concentrations in the United States: relations with demographic and anthropometric measures. Am J Clin Nutr. 2001; 74: 295-301.
18. Muntner P, He J, Chen J, Fonseca V, Whelton PK. Prevalence of non-traditional cardiovascular disease risk factors among persons with impaired fasting glucose, impaired glucose tolerance, diabetes, and the metabolic syndrome: analysis of the Third National Health and Nutrition Examination Survey (NHANES III). Ann Epidemiol. 2004; 14:686-95.
19. Prati D, Taioli E, Zanella A, Della Torre E, Butelli S, Del Vecchio E, et al. Updated definitions of healthy ranges for serum alanine aminotransferase levels. Ann Intern Med. 2002; 137:1-10.
20. Rohrmann S, Crespo CJ, Weber JR, Smit E, Giovannucci E, Platz EA. Association of cigarette smoking, alcohol consumption and physical activity with lower urinary tract symptoms in older American men: findings from the third National Health And Nutrition Examination Survey. BJU International. 2005; 96:77-82.
21. Chitturi S, Farrell GC, Hashimoto E, Saibara T, Lau GK, Sollano JD. Non-alcoholic fatty liver disease in the Asia-Pacific region: definitions and overview of proposed guidelines. J Gastroenterol Hepatol. 2007; 22:778-87.
22. Alberti KG, Zimmet P, Shaw J. The metabolic syndrome—a new worldwide definition. Lancet. 2005; 366(9491):1059-62.
23. Romero-Corral A, Somers VK, Sierra-Johnson J, Thomas RJ, Collazo-Clavell ML, Korinek J, et al. Accuracy of body mass index in diagnosing obesity in the adult general population. Int J Obes (Lond). 2008; 32:959-66.
24. Demerath EW, Schubert CM, Maynard LM, Sun SS, Chumlea WC, Pickoff A, et al. Do changes in body mass index percentile reflect changes in body composition in children? Data from the Fels Longitudinal Study. Pediatrics. 2006; 117:e487-95.
25. Dervaux N, Wubuli M, Megnien JL, Chironi G, Simon A. Comparative associations of adiposity measures with cardiometabolic risk burden in asymptomatic subjects. Atherosclerosis. 2008; 201:413-7.
26. Han TS, Lean ME, Seidell JC. Waist circumference remains useful predictor of coronary heart disease. BMJ. 1996; 312:1227-8.
27. Kvist H, Sjostrom L, Tylen U. Adipose tissue volume determinations in women by computed tomography: technical considerations. Int J Obes. 1986; 10:53-67.
28. Smith SR, Lovejoy JC, Greenway F, Ryan D, deJonge L, de la Bretonne J, et al. Contributions of total body fat, abdominal subcutaneous adipose tissue compartments, and visceral adipose tissue to the metabolic complications of obesity. Metabolism. 2001; 50:425-35.
29. Han TS, Carter R, Currall JE, Lean ME. The influence of fat free mass on prediction of densitometric body composition by bioelectrical impedance analysis and by anthropometry. Eur J Clin Nutr. 1996; 50:542-8.
30. Ryo M, Maeda K, Onda T, Katashima M, Okumiya A, Nishida M, et al. A new simple method for the measurement of visceral fat accumulation by bioelectrical impedance. Diabetes Care. 2005; 28:

451-3.
31. Nagai M, Komiya H, Mori Y, Ohta T, Kasahara Y, Ikeda Y. Development of a new method for estimating visceral fat area with multi-frequency bioelectrical impedance. Tohoku J Exp Med. 2008; 214:105-12.
32. Kim JA, Park HS. Association of abdominal fat distribution and cardiometabolic risk factors among obese Korean adolescents. Diabetes Metab. 2008; 34:126-30.
33. Rexrode KM, Carey VJ, Hennekens CH, Walters EE, Colditz GA, Stampfer MJ, et al. Abdominal adiposity and coronary heart disease in women. JAMA. 1998; 280:1843-8.
34. Kuk JL, Katzmarzyk PT, Nichaman MZ, Church TS, Blair SN, Ross R. Visceral fat is an independent predictor of all-cause mortality in men. Obesity (Silver Spring). 2006; 14:336-41.
35. Romero-Corral A, Lopez-Jimenez F, Sierra-Johnson J, Somers VK. Differentiating between body fat and lean mass-how should we measure obesity? Nat Clin Pract Endocrinol Metab. 2008; 4:322-3.
36. Romero-Corral A, Somers VK, Sierra-Johnson J, Korenfeld Y, Boarin S, Korinek J, et al. Normal weight obesity: a risk factor for cardiometabolic dysregulation and cardiovascular mortality. Eur Heart J. 2010; 31: 737-46.
37. Rosito GA, Massaro JM, Hoffmann U, Ruberg FL, Mahabadi AA, Vasan RS, et al. Pericardial fat, visceral abdominal fat, cardiovascular disease risk factors, and vascular calcification in a community-based sample: the Framingham Heart Study. Circulation. 2008; 117:605-13.
38. Chaston TB, Dixon JB. Factors associated with percent change in visceral versus subcutaneous abdominal fat during weight loss: findings from a systematic review. Int J Obes (Lond). 2008; 32:619-28.
39. Ross R, Rissanen J, Hudson R. Sensitivity associated with the identification of visceral adipose tissue levels using waist circumference in men and women: effects of weight loss. Int J Obes Relat Metab Disord. 1996; 20:533-8.
40. Lovejoy JC, Smith SR, Rood JC. Comparison of regional fat distribution and health risk factors in middle-aged white and African American women: The Healthy Transitions Study. Obes Res. 2001; 9: 10-6.
41. Carroll JF, Chiapa AL, Rodriquez M, Phelps DR, Cardarelli KM, Vishwanatha JK, et al. Visceral fat, waist circumference, and BMI: impact of race/ethnicity. Obesity (Silver Spring). 2008; 16:600-7.
42. Yokoyama H. [Gamma glutamyl transpeptidase (gammaGTP) in the era of metabolic syndrome]. Nihon Arukoru Yakubutsu Igakkai Zasshi. 2007; 42:110-24.
43. Lee MY, Koh SB, Koh JH, Nam SM, Shin JY, Shin YG, et al. Relationship between γ-glutamyltransferase and metabolic syndrome in a Korean population. Diabet Med. 2008; 25:469-75.
44. Monami M, Bardini G, Lamanna C, Pala L, Cresci B, Francesconi P, et al. Liver enzymes and risk of diabetes and cardiovascular disease: results of the Firenze Bagno a Ripoli (FIBAR) study. Metabolism. 2008; 57: 387-92.
45. Kim DJ, Noh JH, Cho NH, Lee BW, Choi YH, Jung JH, et al. Serum γ-glutamyltransferase within its normal concentration range is related to the presence of diabetes and cardiovascular risk factors. Diabet Med. 2005; 22:1134-40.