Title

Sex influences the association between appendicular skeletal muscle mass to visceral fat area ratio and nonalcoholic steatohepatitis in patients with biopsy-proven NAFLD

Running title

Body composition and NASH

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Abbreviations:

ALT: alanine aminotransferase; AST: aspartate aminotransferase; ALP: alkaline phosphatase; GGT: gamma-glutamyl transferase; HDL-C: high-density lipoprotein cholesterol; LDL-C: low-density lipoprotein cholesterol; HOMA-IR: homeostasis model assessment for insulin resistance; SVR: appendicular skeletal muscle mass to visceral fat area ratio; NAFLD: non-alcoholic fatty liver disease; NAS: NAFLD Active Score; NASH: nonalcoholic steatohepatitis; VFA: visceral fat area.

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All authors have nothing to declare.

Author's contributions

Study concept and design: Gang Li and Ming-Hua Zheng

Acquisition of data: Gang Li, Yue Yu, Xin-Xin Wang, Hong-Lei Ma, Liang-Jie Tang, Ou-Yang Huang, Xiao-Yan Pan

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All authors contributed to the manuscript for important intellectual content and approved the submission.

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Written informed consent was obtained from participants and personal information and records were omitted and de-identified prior to statistical analysis.

Abstract

Background: Sarcopenic obesity is regarded as a risky factor for the progression and development of non-alcoholic fatty liver disease (NAFLD). Since male sex is a risk factor for NAFLD and skeletal muscle mass markedly varies between the sexes, we examined whether sex influences the association between appendicular skeletal muscle mass to visceral fat area ratio (SVR), i.e., an index of skeletal muscle mass combined with abdominal obesity, and the histological severity of NAFLD.

Methods: SVR was measured by bioelectrical impedance in a cohort of 613(M/F=443/170) Chinese middle-aged individuals with biopsy-proven NAFLD. Multivariable logistic regression as well as subgroup analyses were used to test the association between SVR and the severity of NAFLD (i.e., nonalcoholic steatohepatitis (NASH) or NASH with presence of any stage of liver fibrosis). NASH was identified by a NAFLD activity score≥5, with a minimum score of 1 for each of its categories. Presence of fibrosis was classified as having a histological stage ≥1.

Results: SVR was inversely associated with NASH in men (adjusted-odds ratio 0.62; 95%CI 0.42-0.92, P=0.017 for NASH, adjusted-odds ratio 0.65; 95%CI 0.43-0.99, P=0.043 for NASH with presence of fibrosis); but not in women 1.47 (0.76, 2.83), P=0.25 for NASH, and 1.45 (0.74, 2.83), P=0.28 for NASH with presence of fibrosis. There was a significant interaction for sex and SVR ($P_{interaction}$=0.017 for NASH and $P_{interaction}$=0.033 for NASH with presence of fibrosis).

Conclusion: Our findings show that lower skeletal muscle mass combined with abdominal obesity is strongly associated with the presence of NASH only in men.

Keywords

Non-alcoholic fatty liver disease; non-alcoholic steatohepatitis; presence of fibrosis; appendicular skeletal muscle mass; sarcopenic obesity; visceral fat area.

Introduction

Non-alcoholic fatty liver disease (NAFLD) is a common metabolic liver disease which influences over ¼ of the world's adults[1; 2]. NAFLD includes a range of liver conditions spanning from simple steatosis to non-alcoholic steatohepatitis (NASH), advanced fibrosis or cirrhosis. Higher energy food intake and physical inactivity may contribute to the development and progression of NAFLD in genetically predisposed individuals. Patients with NAFLD often have more than one of the individual features of metabolic syndrome, and insulin resistance (IR) provides a common pathogenetic link between both conditions[3; 4]. It is known that male sex is a risk factor for NAFLD, but the explanation for this association is not entirely understood[5; 6; 7].

Abnormal body composition, typically characterized by reduced appendicular skeletal muscle mass as well as increased visceral fat mass, may also adversely affect the risk of NAFLD development and progression[8; 9]. As one of the principal sites of insulin-mediated glucose uptake, a low skeletal muscle mass results in increased whole-body IR, thereby promoting the possibility of metabolic syndrome as well as NAFLD[10; 11]. Visceral adipose tissue accumulation also leads to increased whole-body IR and low-grade inflammation, making it an additional risk factor not only for NAFLD and metabolic syndrome, but also for cardiovascular disease (CVD)[12; 13]. The phenomenon of excess adiposity combined with reduced skeletal muscle mass is called sarcopenic obesity [14; 15]. Using bioelectrical impedance, a measurement of the appendicular skeletal muscle mass and the intra-abdominal visceral fat area can be

117 estimated and the appendicular skeletal muscle mass to visceral fat area ratio, known
118 as the SVR index, has been used as a marker that is suggestive of sarcopenic
119 obesity[16; 17; 18; 19].
120
121 Interestingly, body composition and fat distribution are markedly different between
122 the sexes, with women having more fat around the buttocks and thighs, and men
123 having more fat around the abdomen[20]. Women also have approximately two thirds
124 less skeletal muscle mass and twice as much adipose tissue than men[21]. It is well
125 known that male sex is a risk factor for NAFLD, but the explanation for this is
126 uncertain[5; 6; 7]. It is currently not known whether the aforementioned sex-related
127 differences in body fat and muscle mass distribution may also differentially impact on
128 the association between the SVR index [8; 16; 17] and the histological severity of
129 NAFLD.
130
131 Therefore, our study tends to estimate whether there are gender difference in the
132 effect of exposure (SVR) on an outcome (histological level of NAFLD) in a huge
133 cohort of Chinese middle-aged individuals with biopsy-proven NAFLD. If differences
134 in this association do exist between the sexes, improvements in abnormal body
135 composition could provide a new focus for ameliorating NAFLD development and
136 progression.

Materials and Methods

Study subjects and design

A total of 1067 adults with presumed NAFLD (on the basis of high values of serum liver enzymes and/or proof of liver steatosis upon imaging tests) were prospectively enrolled at our hospital over three years consecutively (2016.12-2020.09). As shown in **Figure 1**, Among them, 454 subjects were excluded (including 114 with excessive alcohol intake [>70 g/week in women and >140 g/week in men], 1 with viral hepatitis A, 216 with viral hepatitis B, 10 with viral hepatitis C, 1 with viral hepatitis D, 20 with autoimmune hepatitis,3 with drug induced liver injury;58 with histological evidence of ⩽5% of hepatocyte steatosis and 31 with missing bioimpedence data from InBody 720). As a consequence, a total of 613 middle-aged individuals with biopsy-proven NAFLD were involved to this research.

The research protocol was approved by the First Affiliated Hospital of Wenzhou Medical University ethical committee (2016-246, 1 December 2016) and recorded in the Chinese Clinical Trial Registry (ChiCTR-EOC-17013562). All procedures conformed with the ethical requirements of the Institutional Research Committee and were in accordance with the 1964 Helsinki declaration. Signed written informed consent was collected from every patient ahead of participation within the research.

Clinical and laboratory data

Anthropometric and laboratory data were collected within 1 day of the liver biopsy

procedure and all of the samples of blood were collected in fasting treating. Standing height as well as body weight was measured, in a condition of the subjects barefoot and wearing light clothing. Samples of venous blood were collected post overnight fasting, with a minimum of 8 hours and up to 12 hours, followed by analysis at the Clinical Sample Test Room in the hospital. All biochemical parameters should be analyzed via an automated laboratory analyzer (Abbott AxSYM) with standard methods. Body mass index (BMI) was measured as kilograms over the square of height in meters. BMI ≥25 kg/m² was defined as overweight. Fasting glucose coupled with insulin concentrations were employed for the calculation of homeostasis model assessment of insulin resistance (HOMA-IR) like following: fasting glucose (mmol/l) × fasting insulin (mU/l) / 22.5. Presence of diabetes mellitus was diagnosed by fasting glucose level ≥7.0 mmol/L or hemoglobin A1c (HbA1c) level ≥6.5% (≥48 mmol/mol) and/or use of any glucose-lowering drugs. Subjects were considered to have hypertension if their blood pressure was ≥130/85 mmHg or if they were taking any anti-hypertensive drugs. A dual bioelectrical impedance analyzer (BIA) (InBody 720; Biospace, land Seoul, Korea) was employed to measure lean body mass of patient's limbs and to calculate visceral fat area (VFA). According to Heymsfield et al[(22)], the addition of the lean soft tissues of arms as well as legs results in appendicular skeletal muscle (ASM). We calculated the ASM (kg) adjusted by VFA (mm^2), or the skeletal-to-visceral ratio (SVR, an index of sarcopenia obesity expressed as g/mm^2).

Liver biopsy

Liver biopsy examination has been described previously[23; 24]. Briefly, histological evidence of > 5% of steatotic hepatocytes was used as a diagnostic criterion to define NAFLD. Subjects with a NAFLD Activity Score (NAS) of 5 or greater, and a score of 1 for every one of its three histological components (lobular inflammation, hepatic steatosis and ballooning) were diagnosed as having definite NASH. Stages of hepatic fibrosis were graded from zero to 4, in accordance with the Brunt's histological criteria[25]. The presence of liver fibrosis was defined as having a histological stage of 1 or greater.

Statistical analysis

In both men and women, clinical and biochemical data were stratified by tertiles (T) of SVR as follows: T1, <2.18 g/mm^2; T2, 2.18–2.63 g/mm^2; and T3, >2.63 g/mm^2 for men; and T1, <1.50 g/mm^2; T2, 1.50–1.88 g/mm^2; and T3, >1.88 g/mm^2 for women, respectively. Categorical variables and continuous strings of data were shown as percentages and means ± SD or medians (1st quartile, 3rd quartile), respectively. The one-way ANOVA and the Pearson's chi-squared test were employed to test significant differences in clinical and biochemical variables among the patient groups. Multivariable logistic regression analyses were used to examine the association between SVR (as the exposure variable) and the histological severity of NAFLD (i.e. presence of NASH or NASH with any stage of liver fibrosis, as the outcome measures) within both women and men. Subgroup and interaction analyses were also employed to test the association between decreasing SVR (as the exposure variable)

and presence of NASH or NASH with any stage of liver fibrosis (as the outcome measures). The likelihood ratio test was used to examine the interactions as well as modifications of different patient subgroups. Statistical significance was examined at two-sided p-value of 0.05. All statistical tests were performed with R software (version 3.5.2, R Foundation for Statistical Computing, Vienna, Austria).

Results

Baseline characteristics of participants

Six hundred and thirteen biopsy-confirmed NAFLD were included, among which 72.3% (n=443) were men. NASH was confirmed in 257 subjects (men: 176, 68.5%). The major biochemical as well as clinical features and the liver histology features of participants, stratified by sex as well as SVR tertiles, are illustrated in **Table 1**. Men in the 1st tertile of SVR (i.e. reflecting a greater reduction in appendicular skeletal muscle mass to visceral fat area ratio) were more likely to be older and overweight/obese, and to have type 2 diabetes than those belonging to the 2nd and 3rd tertiles of SVR. Notably, the former also had a greater proportion of definite NASH, as well as liver fibrosis, lobular inflammation and hepatocyte ballooning on histology. Conversely, women in the 1st tertile of SVR tend to be older and overweight/obese, and to have hypertension than those belonging to the 2nd and 3rd tertiles of SVR. However, no significant differences were found in definite NASH and all its individual histologic scores across SVR tertiles in women.

Association between SVR and severity of NAFLD

Multivariable logistic regression analyses were used to estimate the effect of SVR (as the exposure variable) on the severity of NAFLD (as the outcome measures). As shown in **Table 2**, SVR showed a significant inverse association with either NASH (OR 0.77, 95% CI 0.61-0.91; P=0.022) or NASH with presence of any stage of liver fibrosis (OR 0.72, 95% CI 0.56-0.91; P=0.006) in unadjusted logistic regression models. However, these associations were no longer significant after adjustment by sex, age, BMI, hypertension, pre-existing type 2 diabetes and other potential confounding factors (adjusted models 2).

Subgroup analyses for the relevance of SVR with severity of NAFLD

As detailed in **Table 3**, there were significant interactions of SVR with sex ($P_{interaction}$ =0.017 for NASH, $P_{interaction}$ =0.033 for NASH with presence of fibrosis) and age ($P_{interaction}$ =0.009 for NASH, $P_{interaction}$ =0.033 for NASH with presence of fibrosis), but not with overweight/obesity, hypertension or pre-existing diabetes (all $P_{interaction}$ >0.05). In stratified analyses, after adjustment for potential confounders, SVR remained inversely relevant with the severity of NAFLD only within men (adjusted-OR 0.62, 95% CI: 0.42-0.92, p=0.017 for NASH; adjusted-OR 0.65, 95% CI: 0.43-0.99, p=0.043 for NASH with presence of fibrosis), and in older individuals (adjusted-OR 0.40, 95% CI: 0.22-0.74, p=0.003 for NASH; adjusted-OR 0.46, 95% CI: 0.25-0.86, p=0.014 for NASH with presence of fibrosis).

Discussion

We found that there are clear sex-related and age-related associations between a progressive reduction in SVR (i.e. reflecting a reduction in appendicular skeletal muscle mass to visceral fat area ratio) and the severity of NAFLD histology. In particular, we found that decreasing SVR is closely associated with NASH in both men and older individuals, but not in women or younger subjects. Notably, the interaction test between SVR and sex was significant for either definite NASH or NASH with presence of any stage of liver fibrosis. According to our knowledge, this is the largest and first research to date that has investigated the impact of sex on the association of SVR as well as disease severity of liver for patients having NAFLD.

Over the past few years, the relationship between decreasing SVR and presence of cardiometabolic diseases (including NAFLD) has attracted increasing scientific interest[8; 16; 17; 18; 26]. However, no unified conclusion has been reached mainly due to different diagnostic methods of NAFLD and sarcopenic obesity. According to a cross-sectional research of Japanese individuals, Shida et al. published that SVR was inversely relevant to NAFLD for both sexes; however, in this research the determination of NAFLD was obtained by ultrasonography and not by biopsy[8]. Another cross-sectional study performed in Chinese patients with type 2 diabetes found that SVR was inversely relevant to the existance of ultrasonography-defined NAFLD only for women [18]. An independent association of sarcopenia with both NAFLD and NAFLD-related advanced fibrosis has been recently reported in a meta-analysis of three cross-sectional studies[27]. And Seo et al. found that sarcopenia, as

estimated from bioimpedance measurements, was related to a greater risk of having ultrasound-defined NAFLD only for men, in a large cross-sectional research from the Seoul Metabolic Syndrome Cohort[28]. Koo et al. claimed that sarcopenia was related to NAFLD, but this association became non-significant after adjustment for potential confounding elements. Among subjects with biopsy-proven NAFLD, sarcopenic patients tend to have NASH compared with their counterparts without sarcopenia[29]. However, these investigators did not perform separate statistical analyses stratifying by sex.

It is known that when skeletal muscle mass decreases and visceral adipose tissue increases, insulin-mediated skeletal muscle and adipose tissue's ability to use or store blood glucose decrease. Indeed, low skeletal muscle mass and increased visceral fat accumulation reduce whole-body insulin-mediated glucose uptake and may promote the development of NAFLD[8; 30]. In our study, we found that the association between decreasing SVR and NASH (with or without accompanying liver fibrosis) was observed only in men and in older individuals (age ≥44 years). A possible explanation for this observed sex-related difference in the association between decreasing SVR and the severity of NAFLD histology is that there are gender differences in muscle pathophysiology as well as body fat deposition that might confound the association between SVR and whole-body insulin resistance. Sex differences in muscle capillary density, muscle fiber type composition, and estrogen receptors expressed in skeletal muscle may affect the ability of glycolytic *vs*. oxidative substrate metabolism[31].

Additionally, women have more subcutaneous adipose tissue than men, which may affect circulating levels of adipokines. Circulating adipokines adversely affect skeletal muscle metabolism through receptor binding[31; 32]. There is also evidence showing that appendiceal muscle mass is inversely related to HOMA-estimated insulin resistance for regular-weight as well as obese men, however not for women[33], and that sarcopenic obesity occurs more frequently in older individuals, who lose muscle mass and become more centrally obese and directly affected inflammation and insulin resistance with advancing years of life[34].

Our research has several significant limitations which should be listed. First of all, sarcopenia not only includes lower muscle mass, but also includes lower hand-grip strength and gait speed. A confirmed diagnosis of sarcopenic obesity needs to include measures of both muscle mass and muscle function, but these latter were not measured in our study[14; 35]. Secondly, due to the cross-sectional design of the research, a causal and temporal relationship cannot be established. And then, the gold standard method for measuring visceral fat area, i.e. the computed tomography (CT), was not available in our study, because of its high monetary cost as well as radiation exposure to each patient. However, we used a dual bioelectrical impedance analyzer (BIA), which is a reliable, non-radioactive as well as repeatable methodology. A good correlation has been reported between CT scans and BIA for assessing abdominal visceral adiposity[36]. Finally, we did not include any detailed information about physical activities, menopausal status, sex hormone levels, or current use of estrogen

and progestogen drugs.

To conclude, the findings of our research showed that a progressive appendicular skeletal muscle mass to visceral fat area ratio (as reflected by decreasing SVR) is strongly associated with the severity of NAFLD (i.e. NASH with varying levels of fibrosis) only for men, but not for women, after adjusted for potential confounding factors. These findings provide a new focus for ameliorating NAFLD development and progression. However, future prospective and mechanistic researches are required to identify the linkage of sarcopenia obesity with the risk and progression of NAFLD better.

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Competing interests:

The authors declare that there are no conflicts of interest associated with this manuscript.

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Table Legends

Table 1. Baseline characteristics of patients with biopsy-proven NAFLD, stratified by sex and SVR tertiles.

Table 2. Associations between SVR and severity of NAFLD in the whole cohort of patients.

Table 3. Adjusted associations between SVR (as the exposure variable) and severity of NAFLD (as the outcome measure) in different patient subgroups.

Figure Legends

468 **Figure 1**. Flowchart of the study.

469