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Age-Related Decrease in Skeletal Muscle Mass Is an Independent Risk Factor for Incident Nonalcoholic Fatty Liver Disease: A 10-Year Retrospective Cohort Study

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See editorial on page 5.

Background/Aims: Sarcopenia has emerged as an important risk factor for nonalcoholic fatty liver disease (NAFLD). Although aging is the main cause of sarcopenia, the longitudinal association between age-related body composition changes and NAFLD development has not been fully investigated. Thus, we evaluated whether age-related increased fat mass or decreased muscle mass is an independent risk factor for incident NAFLD. **Methods:** We conducted a retrospective cohort study involving 4,398 initially NAFLD-free subjects who underwent routine health examinations during 2004 to 2005 and returned for a follow-up during 2014 to 2015. Their body composition was measured by bioelectrical impedance analysis, and fatty liver was diagnosed by abdominal ultrasonography. **Results:** At the 10-year follow-up, 591 out of 4,398 participants (13.4%) had developed NAFLD. In men and women, both increased fat mass and decreased appendicular skeletal muscle mass (ASM) with aging were significantly associated with incident NAFLD after adjustment. A subgroup analysis according to the baseline obesity status showed that increased fat mass was significantly associated with incident NAFLD in obese and nonobese subjects. However, decreased ASM was significantly associated with incident NAFLD in nonobese but not in obese subjects. According to ΔASM tertiles (decrease of ASM), the odds ratios for incident NAFLD in nonobese subjects were 1.38 (95% confidence interval [CI], 1.04 to 1.84) for the second tertile and 1.81 (95% CI, 1.34 to 2.45) for the third tertile after adjustment (p=0.001). **Conclusions:** A progressive increase in fat mass and a loss of ASM with aging were significantly associated with incident NAFLD. This association was more prominent in nonobese subjects. **(Gut Liver 2019;13:67-76)**

Key Words: Aging; Body composition; Sarcopenia; Non-alcoholic fatty liver disease

INTRODUCTION

Nonalcoholic fatty liver disease (NAFLD) is one of the most common metabolic liver diseases affecting 20% to 30% of adults in developed countries.[1] The clinical spectrum of NAFLD ranges from simple steatosis to severe nonalcoholic steatohepatitis (NASH).[2] Patients with NASH are at a substantial risk of developing cirrhosis, which increases the risk of hepatocellular carcinoma.[2] Furthermore, NAFLD is considered to be a hepatic component of insulin resistance and is associated with metabolic syndrome, cardiovascular morbidity and mortality.[3]

Insulin resistance is strongly associated with ectopic fat accumulation in the liver and is the main cause of NAFLD.[4] Because the skeletal muscle is the primary insulin-responsive target organ, low skeletal muscle mass reduces insulin-mediated glucose disposal and promotes insulin resistance, which may lead to the development of NAFLD.[5,6] In line with this, recent studies showed that sarcopenia, which is defined as the loss of skeletal muscle mass and strength,[7] was a newly emerging risk factor for NAFLD.[6,8,9] Although age-related progressive loss of muscle mass and function is known to be the main cause of sarcopenia,[7] most previous studies were cross-sectional in design and

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longitudinal studies were scarce.

Thus, we aimed to evaluate whether age-related increased fat mass or decreased muscle mass is an independent risk factor for incident NAFLD. We enrolled a large number of subjects without a history of significant alcohol consumption and with no apparent liver disease, and followed this cohort for 10 years in retrospective manner.

MATERIALS AND METHODS

1. Patients

We conducted our study based on subjects aged 18 years or older who underwent a routine health evaluation at the Asan Medical Center (Seoul, Republic of Korea) between September 2004 and December 2005 (baseline) and returned for a follow-up between January 2014 and December 2015 (n=9,782) (Fig. 1). Each subject completed a questionnaire on previous diseases, medication, and drinking and smoking habits. History of cardiovascular disease (CVD) was based on physician-diagnosed myocardial infarction, congestive heart failure, and/or cerebrovascular accidents. Subjects with diabetes were defined as those with fasting plasma glucose (FPG) levels ≥7.0 mmol/L or glycated hemoglobin (HbA1c) levels ≥6.5%.[10] In addition, subjects who reported the use of anti-diabetic medications on a self-report questionnaire were considered to have diabetes. Hypertension was defined as systolic and/or diastolic blood pressure (BP) ≥140/90 mm Hg and/or taking antihypertensive medication.

We excluded subjects who met the following criteria (Fig. 1): subjects with no data for bioelectrical impedance analysis (BIA), abdominal ultrasonography (US), or laboratory values at baseline or follow-up; with history of CVD; with history of cancer at baseline (2004 to 2005) or diagnosed with incident cancer between baseline and follow-up (2005 to 2014); with excessive alcohol consumption (alcohol intake ≥30 g/day for men and ≥20 g/day for women)[11] at baseline or follow-up; with positive serologic markers for hepatitis B or hepatitis C virus; with positive serology for human immunodeficiency virus; who underwent organ transplantation; with overt hyper (free thyroxine [FT4] >1.95 ng/dL) or hypothyroidism (FT4 <0.85 ng/dL);[12] and with chronic kidney disease (estimated glomerular filtration rate [eGFR] <60 mL/min per 1.73 m^2).[13] We also excluded subjects with NAFLD at baseline. After exclusion of ineligible subjects, 4,398 subjects without NAFLD at baseline were enrolled in this study. This study was approved by Asan Medical Center Ethics Committee/Institutional Review Board (IRB No. 2015-0882). Informed consent was waived by IRB because of the retrospective nature of our study.

2. Laboratory parameters

After overnight fasting, early morning venous blood samples were taken and subsequently analyzed by a central, certified laboratory at the Asan Medical Center. Fasting total cholesterol, high-density lipoprotein cholesterol (HDL-C), low-density lipoprotein cholesterol (LDL-C), triglyceride (TG), uric acid, aspartate

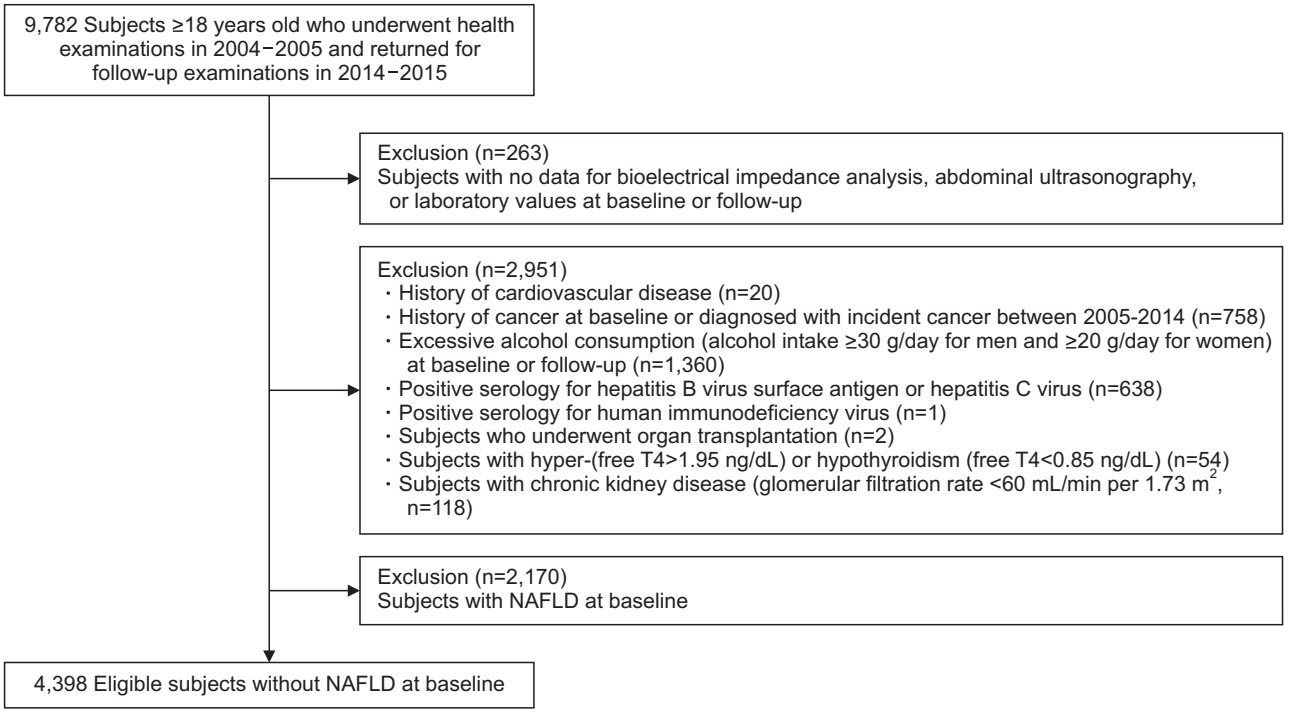

Fig. 1. Flow diagram of study subject.
NAFLD, nonalcoholic fatty liver disease.

aminotransferase (AST), and alanine aminotransferase (ALT) levels were measured by enzymatic colorimetric methods using a Toshiba 200FR Neo analyzer (Toshiba Medical System Co., Ltd., Tokyo, Japan). Gamma-glutamyltransferase (GGT) levels were measured using the L-γ-glutamyl-p-nitroanilide method (Toshiba). Creatinine levels were measured using the Jaffe method, and the eGFR was calculated using the Modification of Diet in Renal Disease study equation.[14] Serum thyroid-stimulating hormone (TSH) and FT4 levels were measured using the TSH-CTK-3 immunoradiometric assay kit (DiaSorinS.p.A, Saluggia, Italy) and FT4 RIA kit (Beckman Coulter/Immunotech, Prague, Czech Republic), respectively. FPG levels were measured using an enzymatic colorimetric method using a Toshiba 200 FR autoanalyzer (Toshiba). Serum insulin concentrations were only measured at follow-up and were determined by an immunoradiometric assay (TFB, Tokyo, Japan). Ion-exchange high-performance liquid chromatography (Bio-Rad Laboratories, Inc., Hercules, CA, USA) was used to measure HbA1c levels. Homeostasis model assessment of insulin resistance (HOMA-IR) was calculated as the product of fasting serum insulin (μU/mL) and FPG (mmol/L) levels divided by 22.5. All enzyme activities were measured at 37°C.

3. Anthropometric and body composition measurements

Height and weight were measured while the subjects wore light clothing without shoes. Body mass index (BMI) was calculated as weight in kilograms divided by the square of height in meters. Subjects with BMI ≥25 kg/m^2 were considered obese based on the criteria of the Asian Pacific region.[15] Waist circumference (WC; cm) was measured midway between the costal margin and the iliac crest at the end of a normal expiration. BP was measured on the right arm after resting for ≥5 minutes using an automatic manometer with an appropriate cuff size.

Body composition was measured by direct segmental multi-frequency BIA using InBody 3.0 at baseline or InBody 720 at follow-up (InBody CO., Ltd, Seoul, Korea). The participants were instructed to stand upright and to grasp the handles of the analyzer, thereby providing contact with a total of eight electrodes (2 for each foot and hand). The system separately measured the impedance of the participants' right arm, left arm, trunk, right leg and left leg at four (InBody 3.0; 5, 50, 250, and 500 kHz) or six (InBody 720; 1, 5, 50, 250, 500, and 1000 kHz) different frequencies for each body segment. Appendicular skeletal muscle mass (ASM) was calculated as the sum of the lean muscle mass in the bilateral arms and legs as previously described.[6,16] Percentage of body fat and ASM were calculated as fat mass and ASM in kilogram, each divided by the body weight (kg).

4. Liver ultrasound

Diagnosis of fatty liver was based on abdominal US. Abdominal US studies were performed by seven experienced radiologists with expertise in liver imaging. Diagnosis of fatty liver was based on the four standard US criteria (parenchymal brightness, hepatorenal echo contrast, deep beam attenuation, and bright vessel walls).[17]

5. Statistical analysis

Continuous variables with normal distributions are expressed as the mean±standard deviation, whereas continuous variables with skewed distributions are expressed as the median (and interquartile range). Categorical variables are expressed as percentages. Changes in study parameters were calculated as the difference between baseline and follow-up. Characteristics of the study population with respect to incident NAFLD were compared using an independent t-test or Mann-Whitney U-test for continuous variables and chi-square test for categorical variables.

Body composition changes were represented by changes in fat mass (Δfat mass) and appendicular skeletal muscle mass (ΔASM) for follow-up periods and multivariable logistic regression analysis was applied to evaluate the independent association between Δfat mass or ΔASM and incident NAFLD. Statistical analyses were performed separately for men and women or for non-obese and obese subjects because of differences in the body composition. Each subgroup (men or women and obese or non-obese) was categorized into three tertile groups according to specific cutoff values for Δfat mass or ΔASM. Considering the body composition change with aging, Δfat mass tertile 1 represented the lowest values (i.e., T1 ≤0.70 kg, T2 0.71 to 2.90 kg, and T3 ≥2.91 kg in men) whereas ΔASM tertiles 1 represented the highest values (i.e., T1 ≥–0.05 kg, T2 –0.06 to –1.12 kg, and T3 ≤–1.13 kg in men) so that increasing each tertile reflected the increase in fat mass and decrease in muscle mass as time passed. Model 1 was adjusted for age, ΔBMI, and ΔWC. Model 2 was adjusted further for smoking (current and none), diabetes, hypertension, and use of lipid-lowering drugs (none, only used at baseline, only used at follow-up, and used at baseline and follow-up). Model 3 was adjusted further for changes in metabolic risk factors including Δsystolic BP, ΔHbA1c, ΔTG, ΔLDL-C, ΔHDL-C, ΔAST, ΔALT, ΔGGT, Δuric acid, ΔTSH, and ΔFT4. All statistical analyses were performed using SPSS version 19.0 for Windows (IBM Corp., Armonk, NY, USA). A p-value of <0.05 was considered statistically significant.

RESULTS

1. Baseline clinical characteristics according to development of NAFLD

At baseline, the mean age of the 4,398 subjects was 46.3±8.3 years and BMI was 22.7±2.4 kg/m^2. The median follow-up duration was 10 years 5 months (range, 8 years 1 month to 12 years 1 month). Among the 4,398 subjects without NAFLD at baseline, 13.4% (591 of 4,398) developed NAFLD. Table 1 shows the baseline characteristics of the subjects according to

Table 1. Comparison of Baseline Characteristics between Subjects with and without Incident NAFLD

Characteristic	All (n=4,398)	Developed NAFLD over 10-year follow-up		p-value
		No (n=3,807)	Yes (n=591)	
Age, yr	46.3±8.3	46.2±8.4	46.7±8.1	0.242
Male sex	2,174 (49.4)	1,812 (47.6)	362 (61.3)	<0.001
Systolic BP, mm Hg	116.0±14.7	115.5±14.5	119.7±15.1	<0.001
Diastolic BP, mm Hg	71.8±9.3	71.4±9.2	74.3±9.6	<0.001
Current smoker	736 (16.7)	581 (15.3)	155 (26.2)	<0.001
Diabetes	266 (6.0)	215 (5.6)	51 (8.6)	0.005
Hypertension	870 (19.8)	712 (18.7)	158 (26.7)	<0.001
Lipid-lowering drugs	111 (2.5)	89 (2.3)	22 (3.7)	0.046
FPG, mg/dL	92.5±12.7	92.3±12.6	94.3±13.1	<0.001
HbA1c, %	5.41±0.47	5.40±0.46	5.50±0.53	0.001
Total cholesterol, mg/dL	187.0±32.0	186.1±31.9	192.6±31.9	<0.001
TG, mg/dL	90 (66–125)	87 (64–119)	114 (85–155)	<0.001
LDL-C, mg/dL	118.3±28.2	117.3±28.0	124.6±28.1	<0.001
HDL-C, mg/dL	57.3±13.5	60.0±13.6	53.3±12.2	<0.001
Uric acid, mg/dL	5.0 (4.2–6.0)	4.9 (4.1–5.9)	5.5 (4.6–6.3)	<0.001
AST, U/L	21 (18–25)	21 (18–25)	22 (19–26)	<0.001
ALT, U/L	16 (12–21)	15 (12–20)	18 (14–24)	<0.001
GGT, U/L	18 (14–26)	17 (13–25)	23 (16–34)	<0.001
TSH, mIU/L	1.9 (1.3–3.0)	2.0 (1.3–3.0)	1.9 (1.3–2.9)	0.128
FT4, ng/dL	1.3 (1.1–1.4)	1.3 (1.1–1.4)	1.3 (1.2–1.4)	0.062
BMI, kg/m^2	22.7±2.4	22.5±2.4	23.9±2.3	<0.001
WC, cm	79.0 (73.0–84.0)	78.0 (72.5–83.5)	82.1 (77.2–86.7)	<0.001
Body fat mass, kg	13.0 (10.7–15.6)	12.8 (10.4–15.3)	14.2 (12.1–17.2)	<0.001
Body fat percent, %	21.3 (17.6–25.5)	21.1 (17.4–25.4)	22.0 (18.6–26.5)	<0.001
ASM, kg	21.0 (18.1–25.1)	20.7 (18.0–24.9)	23.4 (19.1–26.2)	<0.001
ASM/weight, %	35.3 (32.9–37.3)	35.3 (32.9–37.4)	35.0 (32.6–37.1)	0.013

Data are presented as mean±SD, number (%), or median (interquartile ranges).
NAFLD, nonalcoholic fatty liver disease; BP, blood pressures; FPG, fasting plasma glucose; HbA1c, hemoglobin A1c; TG, triglyceride; LDL-C, low-density lipoprotein cholesterol; HDL-C, high-density lipoprotein cholesterol; AST, aspartate aminotransferase; ALT, alanine aminotransferase; GGT, gamma-glutamyltransferase; TSH, thyroid-stimulating hormone; FT4, free thyroxine; BMI, body mass index; WC, waist circumference; ASM, appendicular skeletal muscle mass.

incident NAFLD. Subjects who developed NAFLD had unfavorable metabolic parameters and included more men and current smokers than those who did not. Subjects with incident NAFLD showed a higher prevalence of diabetes and hypertension and were likely to use lipid-lowering drugs more frequently than those who did not at baseline. In terms of the body composition status, subjects who developed NAFLD showed higher BMI, WC, fat mass, fat percentage and ASM at baseline but lower ASM/weight than those who did not.

2. Comparison of changes in characteristics according to development of NAFLD

Table 2 shows changes in metabolic and body composition variables between subjects who developed NAFLD and who did not. During the 10-year period, overall, subjects showed an increase in WC and the fat mass but a decrease in ASM with aging. Those who developed NAFLD showed worsening of metabolic parameters than those who did not. Regarding body composition changes, subjects who developed NAFLD showed a greater increase in the body weight, BMI, WC, fat mass and fat percentage but a greater decrease in ASM/weight than those who did not.

3. Body composition changes and risk of incident NAFLD according to sex

Considering the sex-based differences in body composition changes, we divided subjects according to their sex and performed multivariable logistic regression analysis to evaluate the association between body composition changes and incident NAFLD. Over the 10-year follow-up period, women showed a

Table 2. Comparison of Changes (Δ) in Metabolic and Body Composition Variables between Subjects with and without Incident NAFLD

Variable	All (n=4,398)	Developed NAFLD over 10-year follow-up		p-value
		No (n=3,807)	Yes (n=591)	
ΔSystolic BP, mm Hg	1.02±13.75	0.78±13.42	2.55±15.64	0.009
ΔDiastolic BP, mm Hg	2.38±9.87	2.22±9.67	3.46±11.02	0.010
ΔFPG, mg/dL	3.37±12.88	2.58±12.15	8.47±15.94	<0.001
ΔHbA1c, %	0.18±0.39	0.15±0.34	0.34±0.58	<0.001
ΔTotal cholesterol, mg/dL	1.78±36.90	2.37±36.59	−1.97±38.64	0.011
ΔTG, mg/dL	−3 (−31 to 23)	−5 (−31 to 20)	7 (−30 to 47)	<0.001
ΔLDL-C, mg/dL	5.10±33.25	5.35±32.88	3.47±35.56	0.227
ΔHDL-C, mg/dL	2.70±10.64	3.37±10.72	−1.63±8.98	<0.001
ΔLipid-lowering drugs				<0.001
None	3,634 (82.6)	3,183 (83.6)	451 (76.3)	
Only use at baseline	19 (0.4)	14 (0.4)	5 (0.8)	
Only use at follow-up	653 (14.8)	535 (14.1)	118 (20.0)	
Use at baseline and follow-up	92 (2.1)	75 (2.0)	17 (2.9)	
ΔUric acid, mg/dL	−0.2 (−0.7 to 0.3)	−0.2 (−0.7 to 0.2)	0 (−0.6 to 0.5)	<0.001
ΔAST, U/L	2 (−2 to 6)	2 (−2 to 6)	3 (−2 to 7)	0.017
ΔALT, U/L	2 (−2 to 8)	2 (−2 to 7)	6 (0 to 12)	<0.001
ΔGGT, U/L	0 (−3 to 4)	0 (−3 to 4)	3 (−2 to 11)	<0.001
ΔTSH, mIU/L	0.4 (−0.3 to 1.2)	0.4 (−0.3 to 1.2)	0.5 (−0.3 to 1.2)	0.823
ΔFT4, ng/dL	0.1 (−0.1 to 0.2)	0.1 (−0.1 to 0.2)	0.1 (0 to 0.2)	0.600
ΔWeight, kg	−0.1 (−2.1 to 2.1)	−0.4 (−2.4 to 1.7)	2.1 (0 to 4.5)	<0.001
ΔBMI, kg/m^2	−0.05±1.30	−0.18±1.24	0.83±1.36	<0.001
ΔWC, cm	2.6 (−1.0 to 6.8)	2.0 (−1.7 to 6.1)	5.0 (2.0 to 9.0)	<0.001
ΔBody fat mass, kg	2.1 (0.4 to 3.9)	1.8 (0.1 to 3.6)	3.8 (2.3 to 5.8)	<0.001
ΔBody fat percent, %	3.49 (1.12 to 5.93)	3.22 (0.88 to 5.62)	4.98 (3.25 to 7.43)	<0.001
ΔASM, kg	−0.77 (−1.50 to 0.01)	−0.76 (−1.49 to 0)	−0.77 (−1.55 to 0.08)	0.642
ΔASM/weight, %	−1.24 (−2.60 to 0.18)	−1.07 (−2.44 to 0.32)	−2.24 (−3.51 to −0.97)	<0.001

Data are mean±SD, median (interquartile ranges), or number (%).
NAFLD, nonalcoholic fatty liver disease; BP, blood pressures; FPG, fasting plasma glucose; HbA1c, hemoglobin A1c; TG, triglyceride; LDL-C, low-density lipoprotein cholesterol; HDL-C, high-density lipoprotein cholesterol; AST, aspartate aminotransferase; ALT, alanine aminotransferase; GGT, gamma-glutamyltransferase; TSH, thyroid-stimulating hormone; FT4, free thyroxine; BMI, body mass index; WC, waist circumference; ASM, appendicular skeletal muscle mass.

more apparent increase in fat mass and decrease in ASM compared with men. The median values (interquartile ranges) for Δfat mass were 1.8 (0, 3.6) in men and 2.4 (0.6, 4.3) in women and for ΔASM were –0.59 (–1.40, 0.22) in men and –0.89 (–1.57, –0.21) in women (p<0.001 for all). We calculated sex-specific cutoff points for Δfat mass tertiles and ΔASM tertiles and divided subjects according to those values (Table 3). According to the increase in Δfat mass tertiles, both men and women showed significantly increased odds ratios (ORs) of incident NAFLD (Table 3, Fig. 2) in unadjusted or various adjusted models (models 1–3). In addition, according to the increase in ΔASM tertiles (decreased ASM), both men and women showed significantly increasing ORs for incident NAFLD in adjusted models (models 1–3). In particular, the association between the decrease in ASM and incident NAFLD was more prominent in women compared with that in men in unadjusted or various adjusted models (models 1–3).

4. Body composition changes and risk of NAFLD development according to obesity status

Because obesity is a well-established independent risk factor for NAFLD,[18] we performed further subgroup analysis according to the baseline obesity status (Table 4). Subjects who were obese at baseline showed a lower increase in Δfat mass but a greater decrease in ΔASM compared with those who were not. The median values (interquartile ranges) for Δfat mass were 1.7 (–0.5, 3.9) in obese and 2.1 (0.5, 3.9) in non-obese subjects (p=0.001) and for ΔASM were –0.99 (–1.77, –0.23) in obese and –0.73 (–1.46, 0.05) in non-obese subjects (p<0.001). We recalculated specific cutoff points of Δfat mass tertiles and ΔASM tertiles for

Table 3. ORs and 95% CI for Incident NAFLD Based on Changes in the Body Composition Tertile Categories among Men (n=2,174) and Women (n=2,224) without NAFLD at Baseline

Subgroup	ΔFat mass tertiles*			p-value	ΔASM tertiles†			p-value
	T1	T2	T3		T1	T2	T3	
Men with incident NAFLD	54 (7.3)	92 (12.7)	216 (30.3)	<0.001	131 (18.1)	123 (17.0)	108 (14.9)	0.263
Unadjusted	1	1.85 (1.30–2.63)	5.52 (4.01–7.61)	<0.001	1	0.93 (0.71–1.22)	0.80 (0.60–1.05)	0.264
Model 1	1	1.16 (0.80–1.69)	2.05 (1.36–3.10)	<0.001	1	1.41 (1.05–1.90)	1.64 (1.19–2.26)	0.007
Model 2	1	1.16 (0.79–1.69)	2.04 (1.35–3.10)	<0.001	1	1.43 (1.06–1.94)	1.59 (1.15–2.20)	0.012
Model 3	1	1.12 (0.76–1.67)	1.89 (1.22–2.94)	0.002	1	1.41 (1.04–1.93)	1.61 (1.15–2.26)	0.016
Women with incident NAFLD	18 (2.3)	72 (9.8)	139 (19.3)	<0.001	58 (7.8)	79 (10.6)	92 (12.4)	0.014
Unadjusted	1	4.52 (2.67–7.66)	10.00 (6.05–16.53)	<0.001	1	1.40 (0.98–2.00)	1.67 (1.18–2.36)	0.014
Model 1	1	3.07 (1.77–5.31)	3.98 (2.18–7.26)	<0.001	1	1.62 (1.10–2.40)	2.23 (1.50–3.31)	<0.001
Model 2	1	3.16 (1.82–5.50)	4.14 (2.26–7.58)	<0.001	1	1.60 (1.08–2.37)	2.24 (1.50–3.35)	<0.001
Model 3	1	2.93 (1.66–5.14)	3.34 (1.79–6.24)	<0.001	1	1.47 (0.98–2.20)	2.10 (1.38–3.18)	0.002

Data are presented as number (%) or OR (95% CI). Model 1: adjusted for age, ΔBMI, and ΔWC. Model 2: Model 1 + smoking, diabetes status, hypertension, and use of lipid-lowering drugs. Model 3: Model 2 + Δsystolic BP, ΔHbA1c, ΔTG, ΔLDL-C, ΔHDL-C, ΔAST, ΔALT, ΔGGT, Δuric acid, ΔTSH, and ΔFT4.

OR, odds ratio; CI, confidence interval; ASM, appendicular skeletal muscle mass; NAFLD, nonalcoholic fatty liver disease; BMI, body mass index; WC, waist circumference; BP, blood pressure; HbA1c, hemoglobin A1c; TG, triglyceride; LDL-C, low-density lipoprotein cholesterol; HDL-C, high-density lipoprotein cholesterol; AST, aspartate aminotransferase; ALT, alanine aminotransferase; GGT, gamma-glutamyltransferase; TSH, thyroid-stimulating hormone; FT4, free thyroxine.

*Cutoff values for Δfat mass tertiles were T1 ≤0.70 kg, T2 0.71 to 2.90 kg, T3 ≥2.91 kg in men and T1 ≤1.30 kg, T2 1.31 to 3.60 kg, T3 ≥3.61 kg in women; †Cutoff values for ΔASM tertiles were T1 ≥ −0.05 kg, T2 −0.06 to −1.12 kg, T3 ≤ −1.13 kg in men and T1 ≥ −0.46 kg, T2 −0.47 to −1.31 kg, T3 ≥ −1.32 kg in women.

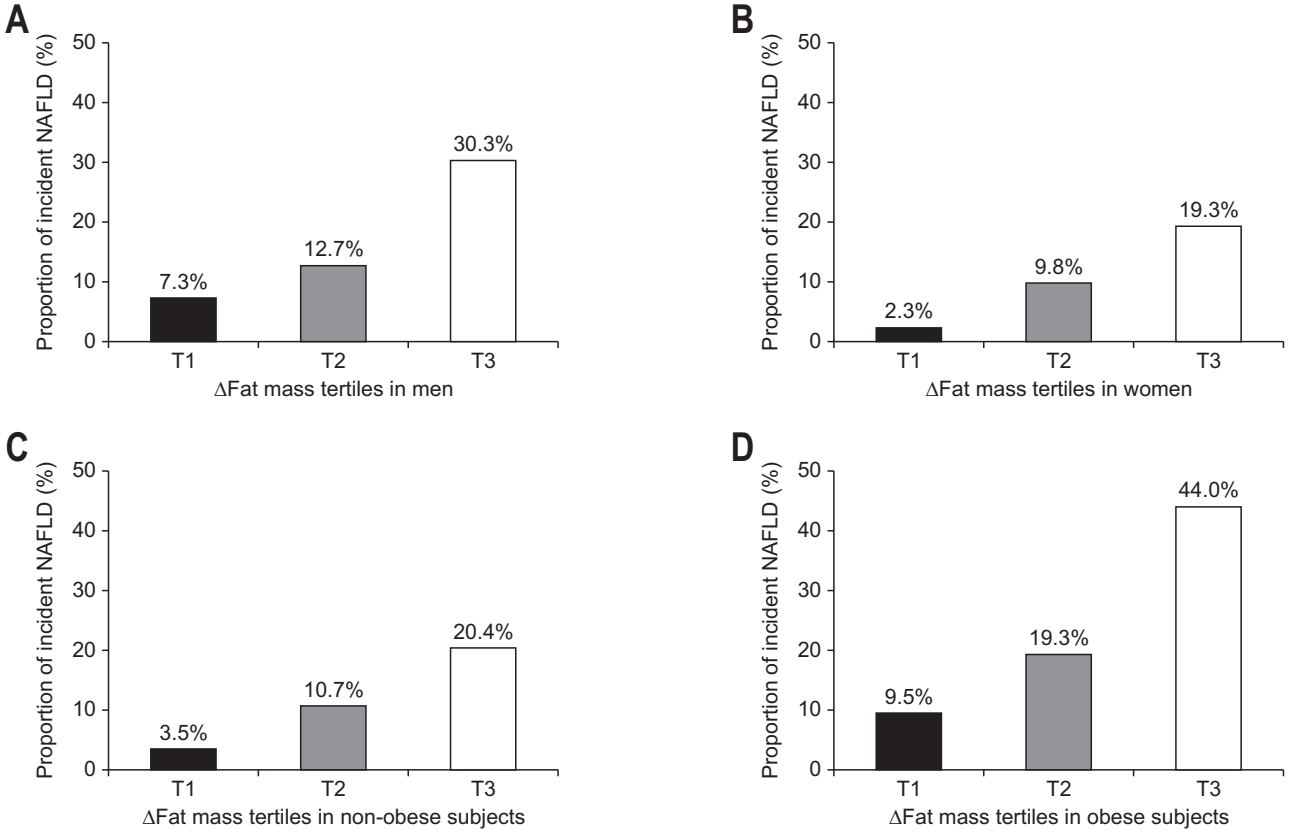

Fig. 2. Proportion of incident nonalcoholic fatty liver disease (NAFLD) according to Δfat mass tertiles in men (A), women (B), non-obese (C) and obese subjects (D).

Table 4. ORs and 95% CI for Incident NAFLD Based on Changes in the Body Composition Tertile Categories among Non-obese (n=3,703) and Obese (n=695) Subjects without NAFLD at Baseline

Subgroup	ΔFat mass tertiles*			p-value	ΔASM tertiles†			p-value
	T1	T2	T3		T1	T2	T3	
Non-obese subjects with incident NAFLD	44 (3.5)	134 (10.7)	246 (20.4)	<0.001	146 (11.8)	138 (11.2)	140 (11.3)	0.868
Unadjusted	1	3.25 (2.29–4.61)	6.96 (4.99–9.70)	<0.001	1	0.94 (0.73–1.20)	0.95 (0.75–1.22)	0.868
Model 1	1	2.14 (1.49–3.09)	2.83 (1.88–4.24)	<0.001	1	1.45 (1.10–1.90)	1.89 (1.42–2.53)	<0.001
Model 2	1	2.15 (1.49–3.10)	2.90 (1.93–4.37)	<0.001	1	1.45 (1.10–1.90)	1.87 (1.40–2.51)	<0.001
Model 3	1	1.96 (1.35–2.85)	2.48 (1.62–3.77)	<0.001	1	1.38 (1.04–1.84)	1.81 (1.34–2.45)	0.001
Obese subjects with incident NAFLD	22 (9.5)	46 (19.3)	99 (44)	<0.001	58 (25.0)	53 (22.8)	56 (24.2)	0.859
Unadjusted	1	2.29 (1.33–3.94)	7.50 (4.49–12.52)	<0.001	1	0.89 (0.58–1.36)	0.96 (0.63–1.47)	0.859
Model 1	1	1.25 (0.69–2.26)	2.22 (1.11–4.46)	0.040	1	1.18 (0.73–1.89)	1.82 (1.11–2.98)	0.048
Model 2	1	1.30 (0.71–2.36)	2.28 (1.13–4.63)	0.041	1	1.17 (0.72–1.90)	1.79 (1.08–2.97)	0.065
Model 3	1	1.42 (0.74–2.71)	2.72 (1.27–5.86)	0.020	1	1.23 (0.73–2.07)	1.91 (1.11–3.31)	0.060

Data are presented as number (%) or OR (95% CI). Model 1: adjusted for age, sex, ΔBMI, and ΔWC. Model 2: Model 1 + smoking, diabetes status, hypertension, and use of lipid-lowering drugs. Model 3: Model 2 + Δsystolic BP, ΔHbA1c, ΔTG, ΔLDL-C, ΔHDL-C, ΔAST, ΔALT, ΔGGT, Δuric acid, ΔTSH, and ΔFT4.

OR, odds ratio; CI, confidence interval; NAFLD, nonalcoholic fatty liver disease; ASM, appendicular skeletal muscle mass; BMI, body mass index; WC, waist circumference; BP, blood pressure; HbA1c, hemoglobin A1c; TG, triglyceride; LDL-C, low-density lipoprotein cholesterol; HDL-C, high-density lipoprotein cholesterol; AST, aspartate aminotransferase; ALT, alanine aminotransferase; GGT, gamma-glutamyltransferase; TSH, thyroid-stimulating hormone; FT4, free thyroxine.

*Cutoff values for Δfat mass tertiles were T1 ≤1.00 kg, T2 1.01 to 3.30 kg, T3 ≥3.31 kg in non-obese and T1 ≤0.30 kg, T2 0.31 to 3.20 kg, T3 ≥3.21 kg in obese subjects; †Cutoff values for ΔASM tertiles were T1 ≥−0.23 kg, T2 −0.24 to −1.18 kg, T3 ≤ −1.19 kg in non-obese and T1 ≥−0.51 kg, T2 −0.52 to −1.37 kg, T3 ≥ −1.38 kg in obese subjects.

each subgroup (Table 4). According to the increase in Δfat mass tertiles, both non-obese and obese subjects showed significantly increased ORs for incident NAFLD (Table 4, Fig. 2) in unadjusted or various adjusted models (models 1–3). Among the subjects who were non-obese at baseline, an increase in ΔASM tertiles (decreased ASM) was significantly associated with incident NAFLD in models 1–3. However, this association was attenuated in obese subjects and was not significant after further adjustment for all the clinical variables in model 3.

5. Age-subgroup analysis and additional analysis that evaluated body composition change and regression of NAFLD

We further divided subjects into younger (≤45 years) versus older (>45 years) group. In both men and female, increase in ΔASM tertiles (decreased ASM) was significantly associated with incident NAFLD in older (>45 years) group but this association was not observed in younger (≤45 years) group (Supplementary Tables 1 and 2). However, in non-obese group, increase in ΔASM tertiles (decreased ASM) was significantly associated with incident NAFLD regardless of age (Supplementary Tables 3 and 4). In addition, we further evaluated the effect of body composition change on regression of NAFLD (Supplementary Tables 5 and 6). According to increase in Δfat mass tertiles, men or non-obese subjects showed significantly decreasing odds ratios (ORs) for regressed NAFLD after adjustment but increase in ΔASM tertiles (decreased ASM) was not significantly associated with regression of NAFLD regardless of sex and obesity status.

DISCUSSION

In this study, we found that both increased fat and decreased muscle mass were significantly associated with incident NAFLD after adjustment. In particular, decreased muscle mass was significantly associated with incident NAFLD in non-obese but not obese subjects after adjustment.

Recently, several studies showed that sarcopenia was associated with an increased risk of NAFLD,[8,9] but those studies have limitations that were cross-sectional design. To the best of our knowledge, no longitudinal study has evaluated an independent association between aging-related loss of muscle mass and incident NAFLD for 10-year follow-up. In this study, decreased muscle mass was an independent predictor of NAFLD in both men and women after adjusting for various potential confounders. These findings suggested that aging-related muscle loss was an important risk factor for incident NAFLD.

In our study, a stronger association between decreasing muscle mass and incident NAFLD was observed in women compared with men (Table 3). Although the reasons are unclear, sex-based differences in changes in muscle mass or body weight with aging might have resulted in this discrepancy. In our

analysis, women showed a greater decrease in muscle mass with aging compared with men (Supplementary Table 7). Thus, the greater loss of muscle with aging in women might exacerbate the harmful effects of sarcopenia. In addition, sex-based differences in weight change according to ΔASM tertiles might affect our outcomes. Compared with women, men showed a greater decrease in body weight according to ΔASM tertiles (Supplementary Table 7). In previous studies, even modest weight loss was significantly associated with NAFLD regression,[18,19] Regarding our finding that men with decreased muscle mass (T3) showed a greater decrease in body weight compared with women (Supplementary Table 7), the harmful effects of decreased muscle mass causing incident NAFLD might be attenuated by weight loss in this group, resulting in clinical insignificance in the unadjusted model. Further studies are needed to clarify the effect of calorie balance on the change in body composition and incident NAFLD.

When we performed subgroup analysis, decreasing muscle mass was significantly associated with incident NAFLD in non-obese but not in obese subjects after adjustment (Table 4). Although a relatively smaller number in the obese subgroup might result in clinical insignificance in our analysis, the differences in muscle quality between the non-obese and obese subgroups could have affected our result. Obesity is associated with fat infiltration to muscles, leading to poor muscle quality, strength, and physical function.[20] Although we did not evaluate muscle quality, because of the poor muscle quality in obese subjects, the beneficial effects of preserved muscle mass in preventing incident NAFLD may not be shown in obese subjects in our analysis.

The exact mechanism underlying the association between decreased muscle mass and incident NAFLD has not been fully elucidated. It is unclear whether sarcopenia causes NAFLD or physiologic changes accompanied by NAFLD are responsible for sarcopenia. Because skeletal muscle is the primary tissue responsible for insulin-mediated glucose disposal, loss of muscle mass causes glucose intolerance and increases insulin resistance.[5] Increased insulin resistance results in lipolysis, and increased delivery of free fatty acids to the liver may enhance hepatic fat accumulation.[4] In addition, insulin resistance and accompanied hyperinsulinemia involving anabolic processes promote *de novo* lipogenesis and hepatic steatosis.[21] In line with these hypotheses, in our additional analysis, subjects with decreased muscle mass showed significantly increased HOMA-IR values despite having a lower BMI at the follow-up. The median follow-up values (interquartile ranges) for HOMA-IR according to ΔASM tertiles were 1.16 (0.69, 1.60) for T1, 1.18 (0.69, 1.61) for T2, and 1.27 (0.73, 1.70) for T3 (p=0.001).

Furthermore, because skeletal muscle is considered an active endocrine organ, myokines, a variety of peptides released by skeletal muscle, may prevent incident NAFLD. Irisin, an exercise-induced myokine, is known to promote fatty acid β-oxidation in the liver[22] and a previous study showed that serum levels of irisin were inversely associated with the hepatic TG content in obese adults.[22] In addition, interleukin-6, another myokine, was reported to downregulate lipogenic genes and upregulate fatty acid oxidation-associated genes, thereby preventing hepatic steatosis.[23]

Chronic inflammation may also be an important link between decreased muscle mass and NAFLD.[24,25] Recent study reported that growth differentiation factor (GDF-15) which is one of the inflammatory and sarcopenic biomarker, increased the risk of hepatic inflammation and fibrosis in NAFLD.[26] Thus, elevated level of GDF-15 might influence on sarcopenia and incident NAFLD. In addition, lower serum vitamin D levels might result in decreased muscle mass and incident NAFLD because vitamin D deficiency is known to contribute to both sarcopenia and NAFLD.[27,28]

Our current study has several limitations. First, we diagnosed incident NAFLD using abdominal US, not by liver biopsy and both intra- and inter-observer variability in sonographic evaluation were not assessed. Although liver biopsy is regarded as the gold standard,[29] in this large population-based study, it was impracticable to perform invasive tests such as biopsies. Second, we estimated skeletal muscle mass using BIA. To assess the body composition, computed tomography, magnetic resonance imaging, and dual energy X-ray absorptiometry (DXA) are considered to be more reliable in distinguishing fat, bone mineral, and lean tissues.[7] However, newly developed segmental multi-frequency BIA used in our study, overcame the problems encountered with single frequency BIA by measuring impedance from the various body compartments[30,31] and several studies showed that SMF-BIA provided an accurate estimate of DXA-derived fat mass and ASM.[16,30] Third, we only measured muscle mass and did not evaluate muscle function, which was associated with metabolic disorder and incident NAFLD.[32] In addition, because we did not assess physical activity affecting muscle function and there is no standard cutoff values for ΔASM, we could not discriminate decrease in muscle mass in our study was result from age-related change or sedentary lifestyle. Fourth, although increased HOMA-IR, chronic inflammation and vitamin D deficiency potentially play a role in sarcopenia and NAFLD, we did not measure these at baseline.[27,28] Lastly, because our study was based on Korean participants who voluntarily returned for follow-up routine health examinations, a selection bias may have occurred and our result may not be generalized to other ethnicities.

However, our study had several strengths. First, we performed a longitudinal analysis to assess whether aging-related changes in the body composition increased the risk of incident NAFLD over a 10-year period in a relatively large number of the general population. Several longitudinal studies have shown that increased in weight and visceral adipose tissue were significantly associated with incident NAFLD.[18,19,33] Although increased fat

mass was still a strong risk factor for incident NAFLD in our analysis, preserving muscle mass was another important factor for preventing NAFLD. Second, we excluded subjects with an abnormal thyroid function and chronic kidney disease, which were reported to be closely associated with the development of NAFLD or sarcopenia,[12,13,34] whereas previous studies included these subjects.[6,8,9] Third, in our multiple logistic regression analysis, we properly adjusted for multiple metabolic risk factors that might affect incident NAFLD, including ΔBMI, ΔWC, TSH, FT4, and uric acid, whereas previous studies did not.[6,8,9] Lastly, because all subjects visited the same medical center and underwent a detailed health examination at baseline and after a 10-year follow-up, procedure-related variability was reduced in our study compared with that in multi-center studies.

In conclusion, both a progressive increase in fat mass and loss of muscle mass with aging were significantly associated with incident NAFLD in the general population over a 10-year period. This association was more distinct in women and non-obese subjects. Our results imply that in addition to weight reduction, increasing skeletal muscle via resistance training may be a treatment option for preventing incident NAFLD. Further prospective, interventional studies assessing changes in the body composition status and NAFLD are needed to elucidate the relationship between changes in the body composition and incident NAFLD.

CONFLICTS OF INTEREST

No potential conflict of interest relevant to this article was reported.

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