A greater ratio of thigh subcutaneous fat to abdominal fat is associated with protection against non-alcoholic fatty liver disease

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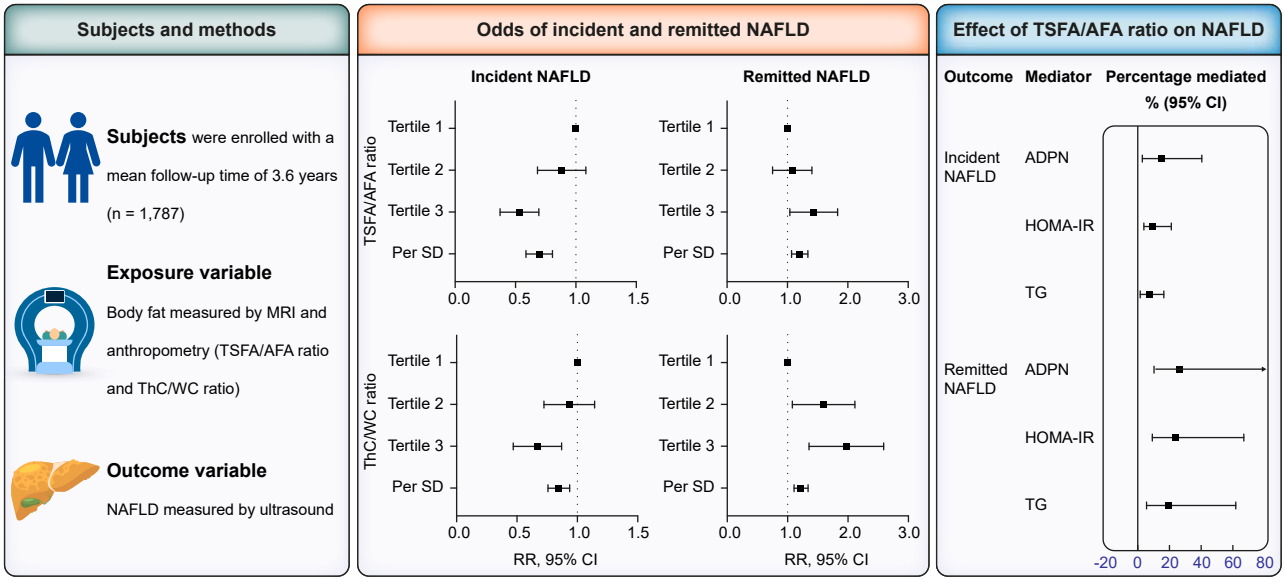
Graphical abstract



ADPN, adiponectin; AFA, abdominal fat area; CI, confidence interval; HOMA-IR, homeostasis model assessment of insulin resistance; MRI, magnetic resonance imaging; NAFLD, non-alcoholic fatty liver disease; RR, risk ratio; SD, standard deviations; TG, triglyceride; ThC, thigh circumference; TSFA, thigh subcutaneous fat area; WC, waist circumference.

Highlights

- The association of thigh subcutaneous fat with NAFLD was investigated based on a prospective cohort study.
- A higher TSFA/AFA ratio was associated with a lower risk of incident NAFLD and a higher likelihood of remitted NAFLD.
- The ThC/WC ratio was negatively associated with incident NAFLD and positively associated with remitted NAFLD.
- Adiponectin, triglyceride, and HOMA-IR mediated the effects of TSFA/AFA ratio on incident and remitted NAFLD.

Impact and implications

The associations of thigh subcutaneous fat distribution with NAFLD incidence and remission have not been prospectively examined in a community-based cohort. Our findings suggest that greater thigh subcutaneous fat relative to a given amount of abdominal fat has a protective effect against NAFLD among the middle-aged and older Chinese populations.

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Research article

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A greater ratio of thigh subcutaneous fat to abdominal fat is associated with protection against non-alcoholic fatty liver disease

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Background & Aims: No prospective studies have examined the association between thigh subcutaneous fat distribution and non-alcoholic fatty liver disease (NAFLD). We investigated the associations of thigh subcutaneous fat distribution with incidence and remission of NAFLD in a community-based prospective cohort.

Methods: We followed 1,787 subjects, who underwent abdominal ultrasonography, abdominal and femoral magnetic resonance imaging scans, and anthropometric assessments. Associations of thigh subcutaneous fat area/abdominal fat area ratio and thigh circumference/waist circumference ratio with incidence and remission of NAFLD were estimated using the modified Poisson regression model.

Results: Over a mean 3.6-year follow-up, 239 incident cases of NAFLD and 207 regressed cases of NAFLD were identified. Increasing thigh subcutaneous fat area/abdominal fat area ratio was associated with a lower risk of incident NAFLD and a higher likelihood of remission of NAFLD [risk ratio (RR) per SD: 0.69, 95% CI 0.59-0.81; 1.20, 95% CI 1.07-1.34, respectively). Each one SD increase in thigh circumference/waist circumference ratio was associated with a 16% lower risk of incident NAFLD (RR 0.84, 95% CI 0.76-0.94) and a 22% higher likelihood of remission of NAFLD (RR 1.22, 95% CI 1.11-1.34). Additionally, the effects of thigh subcutaneous fat area/abdominal fat area ratio on the incidence and remission of NAFLD were mediated through adiponectin (14.9% and 26.6%), homeostasis model assessment of insulin resistance (9.5% and 23.9%), and triglyceride (7.5% and 19.1%).

Conclusions: These results demonstrated that a favourable fat distribution, characterised by a greater ratio of thigh subcutaneous fat to abdominal fat, had a protective role against NAFLD.

Impact and implications: The associations of thigh subcutaneous fat distribution with NAFLD incidence and remission have not been prospectively examined in a community-based cohort. Our findings suggest that greater thigh subcutaneous fat relative to a given amount of abdominal fat has a protective effect against NAFLD among the middle-aged and older Chinese populations.

Keywords: Community-based prospective cohort study; Fat distribution; Incidence; Non-alcoholic fatty liver disease; Remission; Thigh circumference; Thigh subcutaneous fat.

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Introduction

Obesity, an important risk factor for non-alcoholic liver disease (NAFLD),[1] is a highly heterogeneous disease characterised by differences in the regional distribution of body fat. Emerging evidence indicates that visceral fat area (VFA) and abdominal subcutaneous fat area (ASFA) have different effects on the development and remission of NAFLD,[2,3] that is, unlike VFA, ASFA is not a fully established risk factor for NAFLD.[2,3] Based on only two published Korean cross-sectional studies, negative associations between thigh subcutaneous fat area (TSFA)[4] or leg fat to total fat ratio[5] and NAFLD are observed. The discrepancy in NAFLD risk between abdominal and thigh fat depots might originate from differences in their lipolytic activity. Femoral fat

tissue might be more likely to take up non-esterified fatty acids (NEFAs) from the circulating blood and, thus, protect the liver from high NEFA exposure, which further protects against insulin resistance.[6,7] To our knowledge, no prospective studies regarding the associations of TSFA or its relative distribution with the incidence and remission of NAFLD have been reported.

Unlike the two single adiposity indicators of waist circumference (WC) and thigh circumference (ThC), combined indicators of WC and ThC, such as waist-to-thigh ratio and its reciprocal, can reflect body shape and fat distribution to some extent. The waist-to-thigh ratio has been identified as a significant predictor of diabetes[8] and all-cause mortality.[9] However, little is known about the impacts of the ThC/WC ratio on the incidence and remission of NAFLD.

To fill these knowledge gaps, we evaluated the associations of incidence and remission of NAFLD with the ratio of TSFA/abdominal fat area (AFA), a precise measurement, and with the ThC/WC ratio, a simple surrogate for the former, among middle-aged and older Chinese populations based on a prospective cohort data set. We used the TSFA/AFA and ThC/WC ratios to more intuitively reflect the effects of a higher proportion of favourable fat depots on the incidence and remission of NAFLD.

Materials and methods

Subjects and study design

We analysed data from the previously described cohort from the Shanghai Nicheng Cohort Study.[10,11] Briefly, 17,212 subjects aged 45-70 years completed the baseline survey from 2013 to 2014. Of these, 2,849 subjects, aged 55-70 years, who had complete baseline data on abdominal ultrasonography, TSFA, ASFA, VFA, ThC, and WC, were invited to participate in the follow-up survey in 2018, and 2,008 subjects attended (follow-up rate: 70.5%).

We excluded 221 subjects according to the following criteria: (1) missing data on abdominal ultrasonography (n = 39) at follow-up or on alcohol intake (n = 3) at baseline and follow-up; (2) excessive drinking (daily alcohol consumption >30 g/day in men and >20 g/day in women; n = 138) at baseline and follow-up; and (3) positive hepatitis B surface antigen (n = 41) at baseline. Finally, 1,787 subjects with a mean follow-up time of 3.6 years (SD 0.32) were included in this study (Fig. S1).

This study conformed to the principles of the Declaration of Helsinki and was approved by the ethics committee of the Shanghai Sixth People's Hospital (Approval No: 2015-27). Written informed consent was obtained from each subject.

Clinical data collection and laboratory measurements

Data involving demographics, education level, lifestyle (smoking status, drinking status, and leisure-time exercise), medication usage, and medical history (e.g., diabetes and hypertension) were obtained via a standardised questionnaire. Height, weight, and blood pressure were measured using an established standard protocol.[12] ThC was measured at the mid-thigh between the inguinal crease and the proximal border of the patella. WC was measured along the midline between the lower margin of the costal arch and the upper margin of the iliac crest on the mid-axillary line. ThC/WC ratio was calculated as ThC (cm) divided by WC (cm). BMI was calculated as weight in kilograms divided by the square of height in metres.

Fasting plasma glucose (FPG), glycated haemoglobin (HbA_{1c}), fasting insulin (FINS), triglyceride (TG), total cholesterol, high-density lipoprotein cholesterol (HDL-C), low-density lipoprotein cholesterol, alanine aminotransferase, aspartate aminotransferase, gamma-glutamyl transpeptidase, adiponectin, fibroblast growth factor 21 (FGF21), and retinol-binding protein-4 (RBP4) were measured using overnight fasting (at least 10 h) venous blood samples. The laboratory measurement methods are described in Table S1. The homeostasis model assessment of insulin resistance (HOMA-IR) was used to quantify insulin resistance, calculated as FPG (mmol/L) × FINS (μU/ml)/22.5.[13]

Ultrasonographic examinations

Abdominal ultrasonography (Z.One Ultra, Zonare Medical Systems Inc., Mountain View, CA, USA) was performed by experienced ultrasonographists who were blinded to the study design and clinical data. Fatty liver was defined as present when at least two of the following three abdominal ultrasonographic features were found: diffusely increased echogenicity ('bright') liver with liver echogenicity greater than that of kidney or spleen, vascular blurring, and/or deep attenuation of ultrasound signal.[14]

Measurement of adipose tissue areas

Abdominal and femoral magnetic resonance imaging (MRI) scans were conducted on subjects in a supine position, using a 3.0 T General Electric scanner (GE Healthcare, Milwaukee, WI, USA). Eight slices of T1 axial images centred at the navel and the mid-thigh were obtained. Each slice thickness was 10.0 mm. Cross-sectional TSFA, ASFA, and VFA were measured in cm^2 using the mid-thigh and umbilical slices, based on an area of 2D pixels meeting the adipose shading threshold from the Digital Imaging and Communications in Medicine (DICOM) images. Two trained investigators segmented the images into different fat districts and calculated fat areas using the sliceOmatic image analysis software (version 5; Tomovision Inc., Montreal, QC, Canada). When the results differed by more than 10%, a third investigator reanalysed the images. AFA was calculated as the sum of ASFA and VFA, and the TSFA/AFA ratio was created by dividing TSFA by AFA.

Outcome definitions

NAFLD was defined based on ultrasound evidence of fatty liver, in the absence of excessive drinking (alcohol consumption >30 g/day in men and >20 g/day in women) and other known causes of chronic liver diseases (viral hepatitis, hepatolenticular degeneration, etc.).[15] NAFLD absent at baseline but present at follow-up was defined as incidence of NAFLD, and the reverse was defined as NAFLD remission. To assess the severity of NAFLD, two non-invasive indices, Fibrosis-4 (FIB-4)[16,17] and the Fibrosis Nonalcoholic Steatohepatitis Index (FNI),[18,19] were used. Advanced fibrosis was defined as FIB-4 ≥1.30 or FNI >0.10.

Statistical analysis

Descriptive data were expressed as means (SD), medians (25th-75th percentiles), or numbers (proportions) as appropriate. The differences between two groups were compared using Student's *t* tests or Mann-Whitney *U* test for continuous variables and the Chi-squared test for categorical variables. Correlations of TSFA/AFA ratio and ThC/WC ratio with the other baseline characteristics were assessed using the Pearson and partial correlation coefficients adjusted for sex, age, and BMI.

The modified Poisson regression model with robust error variance was used to estimate the risk ratios (RRs) and 95% CIs for the incidence and remission of NAFLD. TSFA/AFA ratio and ThC/WC ratio were entered into the models as per one SD or sex-

Table 1. Baseline characteristics of subjects by incidence and remission of NAFLD after a 3.6-year follow-up.

		Non-NAFLD at baseline (n = 981)			NAFLD at baseline (n = 806)		
Characteristics	Total (n = 1787)	No incidence of NAFLD (n = 742, 75.6%)	Incidence of NAFLD (n = 239, 24.4%)	*p* value	Remission of NAFLD (n = 207, 25.7%)	No remission of NAFLD (n = 599, 74.3%)	*p* value
Demographics							
Women, n (%)	1,131 (63.3)	420 (56.6)	155 (64.9)	0.024	123 (59.4)	433 (72.3)	0.001
Age, years	62.2 (3.8)	62.2 (3.8)	62.7 (3.9)	0.064	61.9 (3.9)	62.2 (3.9)	0.498
Clinical							
SBP, mmHg	129.9 (11.7)	127.8 (11.9)	129.9 (12.0)	0.018	131.8 (12.5)	131.7 (10.6)	0.915
DBP, mmHg	82.8 (6.0)	81.6 (6.0)	83.0 (5.6)	0.001	83.5 (5.9)	83.9 (5.8)	0.360
FPG, mmol/L	6.0 (5.6-6.6)	5.9 (5.5-6.4)	6.0 (5.7-6.5)	0.017	6.0 (5.7-6.6)	6.3 (5.8-7.2)	<0.001
HbA1c, %	5.9 (1.0)	5.6 (0.9)	5.8 (0.7)	0.037	5.8 (0.8)	6.1 (1.1)	<0.001
FINS, μU/ml	6.4 (4.6-9.1)	5.0 (3.5-6.5)	6.7 (5.0-8.7)	<0.001	6.5 (4.8-9.2)	8.9 (6.5-12.7)	<0.001
HOMA-IR	1.8 (1.2-2.6)	1.3 (0.9-1.8)	1.8 (1.3-2.4)	<0.001	1.9 (1.3-2.6)	2.6 (1.8-3.8)	<0.001
TG, mmol/L	1.3 (0.9-1.9)	1.1 (0.8-1.5)	1.3 (0.9-1.8)	<0.001	1.4 (1.0-2.0)	1.8 (1.3-2.6)	<0.001
TC, mmol/L	5.2 (1.0)	5.1 (1.0)	5.1 (0.8)	0.711	5.3 (1.0)	5.3 (1.0)	0.277
HDL-C, mmol/L	1.4 (0.3)	1.5 (0.4)	1.4 (0.3)	<0.001	1.3 (0.3)	1.3 (0.3)	0.101
LDL-C, mmol/L	3.1 (0.8)	3.0 (0.8)	3.1 (0.7)	0.101	3.2 (0.8)	3.3 (0.8)	0.469
ALT, U/L	18.9 (10.1)	16.7 (8.6)	17.2 (6.6)	0.378	18.0 (8.1)	22.6 (12.3)	<0.001
AST, U/L	23.1 (6.8)	22.9 (6.8)	21.8 (4.6)	0.003	22.0 (5.5)	24.3 (7.8)	<0.001
GGT, U/L	21.0 (16.0-33.0)	18.0 (14.0-27.0)	20.0 (15.0-27.0)	0.021	23.0 (18.0-33.0)	27.0 (19.0-39.0)	0.001
Anthropometric							
TSFA/AFA ratio	0.47 (0.21)	0.52 (0.24)	0.45 (0.18)	<0.001	0.43 (0.18)	0.41 (0.17)	0.328
ThC/WC ratio	0.59 (0.06)	0.62 (0.06)	0.59 (0.05)	<0.001	0.59 (0.05)	0.57 (0.05)	<0.001
TSFA, cm^2	117.4 (53.7)	104.0 (48.4)	119.4 (50.6)	<0.001	116.9 (50.9)	133.3 (57.6)	<0.001
AFA, cm^2	264.6 (87.8)	207.9 (66.5)	271.4 (68.6)	<0.001	279.3 (71.3)	327.0 (77.1)	<0.001
ASFA, cm^2	143.9 (55.4)	116.3 (43.4)	154.4 (50.9)	<0.001	149.6 (49.1)	172.1 (56.3)	<0.001
VFA, cm^2	120.6 (48.5)	91.6 (34.6)	116.9 (37.0)	<0.001	129.7 (40.7)	155.0 (46.5)	<0.001
ThC, cm	48.9 (3.8)	47.5 (3.4)	49.2 (3.3)	<0.001	49.5 (3.8)	50.4 (3.9)	0.005
WC, cm	82.8 (8.8)	77.5 (7.4)	83.3 (6.9)	<0.001	84.1 (7.0)	88.8 (7.3)	<0.001
BMI, kg/m^2	24.9 (3.2)	22.9 (2.4)	25.1 (2.2)	<0.001	25.3 (2.3)	27.2 (3.0)	<0.001
Adipokine							
Adiponectin, μg/ml	4.5 (1.8)	5.1 (1.9)	4.6 (1.7)	<0.001	4.2 (1.7)	3.7 (1.3)	0.001
FGF21, pg/ml	222.6 (129.5-332.8)	172.3 (103.1-290.3)	204.4 (120.1-307.0)	0.029	238.5 (159.0-343.6)	264.1 (171.5-391.6)	0.020
RBP4, mg/L	57.3 (16.3)	55.2 (17.1)	56.0 (14.8)	0.448	61.7 (14.4)	58.8 (16.0)	0.022

Data are presented as mean (standard deviation) or median (25th–75th percentiles), or number (proportion) as appropriate. Differences between two groups were compared using the Student's *t* test or Mann-Whitney *U* test for continuous variables and the Chi-squared test for categorical variables.
AFA, abdominal fat area; ALT, alanine aminotransferase; ASFA, abdominal subcutaneous fat area; AST, aspartate aminotransferase; DBP, diastolic blood pressure; FGF21, fibroblast growth factor 21; FINS, fasting insulin; FPG, fasting plasma glucose; GGT, gamma-glutamyl transpeptidase; HbA_{1c}, glycated haemoglobin; HDL-C, HDL-cholesterol; HOMA-IR, homeostasis model assessment of insulin resistance; LDL-C, LDL-cholesterol; NAFLD, non-alcoholic fatty liver disease; RBP4, retinol-binding protein-4; SBP, systolic blood pressure; TC, total cholesterol; TG, triglyceride; ThC, thigh circumference; TSFA, thigh subcutaneous fat area; VFA, visceral fat area; WC, waist circumference.

specific per tertile increments. The regression model was used to test the linear trend of incidence or remission of NAFLD across the tertile groups of TSFA/AFA ratio or ThC/WC ratio, using the median values of each tertile to reflect the group levels. Potential interactions between TSFA/AFA ratio or ThC/WC ratio and the other analysis variables on the incidence and remission of NAFLD were tested using the Wald test by adding their product terms to the regression models. In Model 1, adjustment variables included sex, age, education levels (primary school and below or middle school and above), smoking status (never, past, or current smokers), drinking status (never, past, or current drinkers), and leisure-time exercise (never, 1-<30 min/day, ≥30 min/day); in Model 2, BMI was additionally adjusted; in Model 3, hypertension (yes or no), diabetes (yes or no), TG, and HDL-C were further adjusted. In addition, the associations of TSFA/AFA ratio and ThC/WC ratio with the incidence and remission of NAFLD were depicted using the restricted cubic splines with 4 knots at the 5th, 35th, 65th, and 95th percentiles. The associations between TSFA/AFA ratio and incident NAFLD at different stages (non-NAFLD, NAFLD without or with advanced fibrosis) were analysed by multinomial logistic regression. The 'mediation' R package (R Foundation for Statistical Computing, Vienna, Austria)[20] was used to estimate the average causal mediation effect (ACME) and average direct effect (ADE), reflecting indirect and direct effects of the TSFA/AFA ratio on the incidence and remission of NAFLD. The mediated portion was calculated as the ratio of the ACME to the total effect (ACME plus ADE). The mediation effects were estimated using non-parametric bootstrapping (1,000 simulations).

Statistical analyses were carried out using SPSS, version 26.0 (SPSS Inc., Chicago, IL, USA), StataMP version 14.0 (StataCorp LP, TX, USA), or R version 4.0.2 (R Foundation for Statistical Computing, Vienna, Austria). A two-tailed *p* value <0.05 was considered statistically significant.

Results

Characteristics of the study population

Among 1,787 participants at baseline, 981 (54.9%) without and 806 (45.1%) with NAFLD were identified. Over a 3.6-year follow-up, 239 subjects (24.4% of 981) without NAFLD progressed to NAFLD and 207 patients (25.7% of 806) with NAFLD regressed to non-NAFLD (Table 1). Compared with subjects without incident NAFLD, those with incident NAFLD had more unfavourable

Table 2. Associations of TSFA/AFA ratio and ThC/WC ratio with incident NAFLD after a 3.6-year follow-up.[*,†]

Variable	No. of subjects	No. of cases	Incidence rate (%)	Model 1 (RR, 95% CI)[‡]	*p* value	Model 2 (RR, 95% CI)[§]	*p* value	Model 3 (RR, 95% CI)[¶]	*p* value
TSFA/AFA ratio									
Tertile 1	327	113	34.6	Reference	<0.001**	Reference	<0.001**	Reference	<0.001**
Tertile 2	327	84	25.7	0.76 (0.60-0.96)		0.87 (0.69-1.09)		0.90 (0.72-1.13)	
Tertile 3	327	42	12.8	0.38 (0.28-0.52)		0.52 (0.38-0.70)		0.55 (0.41-0.76)	
TSFA/AFA ratio (per SD)				0.61 (0.52-0.71)	<0.001	0.69 (0.59-0.81)	<0.001	0.72 (0.62-0.84)	<0.001
ThC/WC ratio									
Tertile 1	330	107	32.4	Reference	<0.001**	Reference	0.005**	Reference	0.045**
Tertile 2	322	85	26.4	0.81 (0.64-1.04)		0.92 (0.73-1.15)		0.97 (0.76-1.22)	
Tertile 3	329	47	14.3	0.45 (0.33-0.61)		0.65 (0.48-0.88)		0.72 (0.53-0.98)	
ThC/WC ratio (per SD)				0.72 (0.65-0.80)	<0.001	0.84 (0.76-0.94)	0.002	0.87 (0.78-0.97)	0.016

AFA, abdominal fat area; NAFLD, non-alcoholic fatty liver disease; RR, risk ratio; ThC, thigh circumference; TSFA, thigh subcutaneous fat area; WC, waist circumference.
**A *p* value for trend (statistically significant at *p* <0.05).
* RR (95% CI) was calculated using the modified Poisson regression model with robust error variance.
† TSFA/AFA ratio: men: tertile 1, <0.23; tertile 2, 0.23-<0.28; tertile 3, ≥0.28; women: tertile 1, <0.32; tertile 2, 0.32-<0.40; tertile 3, ≥0.40. ThC/WC ratio: men: tertile 1, <0.58; tertile 2, 0.58-<0.62; tertile 3, ≥0.62; women: tertile 1, <0.60; tertile 2, 0.60-<0.64; tertile 3, ≥0.64.
‡ Adjusted for sex, age, education levels, smoking status, drinking status, and leisure-time exercise.
§ Adjusted for variables in Model 1 and also for BMI.
¶ Adjusted for variables in Model 2 and also for hypertension, diabetes, triglyceride, and high-density lipoprotein cholesterol.

metabolic profiles, higher adiposity indicators and FGF21 levels, but lower TSFA/AFA ratios [mean (SD): 0.45 (0.18) *vs.* 0.52 (0.24), *p* <0.001), ThC/WC ratio [mean (SD): 0.59 (0.05) *vs.* 0.62 (0.06), *p* <0.001], and adiponectin. Meanwhile, subjects with regressed NAFLD had more favourable metabolic profiles, lower adiposity indicators and FGF21 levels, but a higher ThC/WC ratio [mean (SD): 0.59 (0.05) *vs.* 0.57 (0.05), *p* <0.001], adiponectin, and RBP4, and a similar TSFA/AFA ratio [mean (SD): 0.43 (0.18) *vs.* 0.41 (0.17), *p* = 0.328] compared with those without regressed NAFLD.

Correlations of TSFA/AFA and ThC/WC ratios with other baseline factors

Correlations of TSFA/AFA and ThC/WC ratios with other baseline characteristics are detailed in Table S2. After adjustment for sex, age, and BMI, the TSFA/AFA and ThC/WC ratios were negatively correlated with worse metabolic profiles, ASFA, VFA, FGF21, and RBP4 (r = -0.05 - -0.43, all *p* <0.05), and were positively correlated with HDL-C and adiponectin (r = 0.11-0.30, both *p* <0.001). In addition, there was a moderate correlation between the TSFA/AFA ratio and ThC/WC ratio (r = 0.42, *p* <0.001).

Associations of TSFA/AFA and ThC/WC ratios with incident NAFLD

The TSFA/AFA ratio was negatively associated with incident NAFLD with a multivariable-adjusted RR of 0.52 (95% CI 0.38-0.70) for the highest tertile *vs.* the lowest tertile and of 0.69 (95% CI 0.59-0.81) for each one SD increase (Model 2) (Table 2). The negative association was slightly attenuated after further adjustment for hypertension, diabetes, TG, and HDL-C (RR per SD 0.72, 95% CI 0.62-0.84; Model 3). Similar to the associations between the TSFA/AFA ratio and NAFLD, the ThC/WC ratio was negatively associated with incident NAFLD with an adjusted RR of 0.65 (95% CI 0.48-0.88) for the highest tertile *vs.* the lowest tertile and 0.84 (95% CI 0.76-0.94) for each one SD increase (Model 2). There were linearly inverse associations of the TSFA/AFA ratio and ThC/WC ratio with incident NAFLD (Fig. S2A,B). Furthermore, we assessed the associations between the TSFA/AFA ratio and incident NAFLD at different stages, with or without advanced fibrosis. With non-NAFLD as the reference, in terms of FIB-4, each one SD increase in TSFA/AFA ratio was associated with a 55% lower risk of incident NAFLD without advanced fibrosis and a 35% lower risk of incident NAFLD with advanced fibrosis [odds ratio (OR) 0.45, 95% CI 0.30-0.69; OR 0.65, 95% CI 0.50-0.85, respectively; Table S3]; while, according to FNI, each one SD increase in TSFA/AFA ratio was associated with a 30% lower risk of incident NAFLD without advanced fibrosis and a 65% lower risk of incident NAFLD with advanced fibrosis (OR 0.70, 95% CI 0.55-0.90; OR 0.35, 95% CI 0.22-0.55, respectively; Table S3).

Associations of TSFA/AFA and ThC/WC ratios with remission of NAFLD

The TSFA/AFA ratio was positively associated with remission of NAFLD with a multivariable-adjusted RR of 1.40 (95% CI 1.06-1.85) for the highest tertile *vs.* the lowest tertile and of 1.20 (95% CI 1.07-1.34) for each one SD increase (Model 2; Table 3). The association was attenuated but remained in the same direction after further adjustment for metabolic risk factors (RR per SD 1.18; 95% CI 1.05-1.33; Model 3). Consistent with the associations between the TSFA/AFA ratio and remission of NAFLD, the ThC/WC ratio was also positively associated with remission of NAFLD, with RR per SD increase ratio of 1.22 (95% CI 1.11-1.34) and 1.91 (95% CI 1.39-2.62) for the highest tertile *vs.* the lowest tertile (Model 2). There were linearly positive associations of TSFA/AFA ratio and ThC/WC ratio with remission of NAFLD (Fig. S2C,D).

Mediation by HOMA-IR, TG, and adiponectin

The effect of the TSFA/AFA ratio on incident NAFLD was 9.5% (95% CI 3.9-21.3%) mediated through HOMA-IR; 7.5% (95% CI 1.6-16.4%) mediated through TG; and 14.9% (95% CI 2.9-40.5%) mediated through adiponectin (Table 4). By contrast, the effect of the TSFA/AFA ratio on remission of NAFLD was 23.9% (95% CI 9.2-67.0%) mediated through HOMA-IR; 19.1% (95% CI 5.6-61.9%) mediated through TG; and 26.6% (95% CI 10.3-84.6%) mediated through adiponectin (Table 5).

Associations of TSFA/AFA and ThC/WC ratios with incidence or remission of NAFLD among subgroups

We further divided the subjects into different subgroups according to sex, hypertension, diabetes, and obesity. The negative associations of the TSFA/AFA ratio (RR per SD 0.65-0.76) and ThC/WC ratio (RR per SD 0.77-0.86) with incident NAFLD were all observed in women, in the subgroup with hypertension, in the subgroup without diabetes, and in both non-obese and obese subgroups (Fig. S3A). The positive associations of TSFA/AFA ratio (RR per SD 1.14-1.34) and ThC/WC ratio (RR per SD 1.14-1.27) with remission of NAFLD were observed in both sexes, in the

Table 3. Associations of TSFA/AFA and ThC/WC ratios with NAFLD remission after a 3.6-year follow-up.[*,†]

Variable	No. of subjects	No. of cases	Remission rate (%)	Model 1 (RR, 95% CI)[‡]	*p* value	Model 2 (RR, 95% CI)[§]	*p* value	Model 3 (RR, 95% CI)[¶]	*p* value
TSFA/AFA ratio									
Tertile 1	268	58	21.6	Reference	0.004**	Reference	0.016**	Reference	0.150**
Tertile 2	270	63	23.3	1.09 (0.80-1.49)		1.05 (0.77-1.42)		0.98 (0.72-1.33)	
Tertile 3	268	86	32.1	1.52 (1.15-2.02)		1.40 (1.06-1.85)		1.23 (0.92-1.64)	
TSFA/AFA ratio (per SD)				1.26 (1.11-1.42)	<0.001	1.20 (1.07-1.34)	0.001	1.18 (1.05-1.33)	0.006
ThC/WC ratio									
Tertile 1	267	42	15.7	Reference	<0.001**	Reference	<0.001**	Reference	<0.001**
Tertile 2	272	69	25.4	1.67 (1.19-2.34)		1.54 (1.11-2.14)		1.47 (1.06-2.04)	
Tertile 3	267	96	36.0	2.35 (1.71-3.23)		1.91 (1.39-2.62)		1.79 (1.30-2.46)	
ThC/WC ratio (per SD)				1.29 (1.16-1.43)	<0.001	1.22 (1.11-1.34)	<0.001	1.20 (1.09-1.32)	<0.001

AFA, abdominal fat area; NAFLD, non-alcoholic fatty liver disease; RR, risk ratio; ThC, thigh circumference; TSFA, thigh subcutaneous fat area; WC, waist circumference.
**A *p* value for trend (statistically significant at $p < 0.05$).
* RR (95% CI) was calculated using the modified Poisson regression model with robust error variance.
† TSFA/AFA ratio: men: tertile 1, <0.19; tertile 2, 0.19-<0.23; tertile 3, ≥0.23; women: tertile 1, <0.28; tertile 2, 0.28-<0.34; tertile 3, ≥0.34. ThC/WC ratio: men: tertile 1, <0.55; tertile 2, 0.55-<0.59; tertile 3, ≥0.59; women: tertile 1, <0.55; tertile 2, 0.55-<0.60; tertile 3, ≥0.60.
‡ Adjusted for sex, age, education levels, smoking status, drinking status, and leisure-time exercise.
§ Adjusted for variables in Model 1 and also for BMI.
¶ Adjusted for variables in Model 2 and also for hypertension, diabetes, triglyceride, and high-density lipoprotein cholesterol.

subgroup with hypertension, in the subgroup without diabetes, as well as non-obese and obese subgroups, but not in the subgroup with diabetes (Fig. S3B). Additionally, a positive association between TSFA/AFA ratio and remission of NAFLD was observed in subjects with advanced fibrosis (FIB-4: RR per SD 1.24, 95% CI 1.08-1.42; FNI: RR per SD 1.28, 95% CI 1.07-1.53) but not in those without advanced fibrosis (Table S4). Moreover, there were no significant interactions between TSFA/AFA ratio or ThC/WC ratio and any one of these subgroups on incidence or remission of NAFLD ($p > 0.05$ for all interactions; Fig. S3).

Discussion

Major findings

Our study demonstrated for the first time that the TSFA/AFA ratio was negatively associated with incident NAFLD (RR per SD 0.69, 95% CI 0.59-0.81) and positively associated with remission of NAFLD (RR per SD 1.20, 95% CI 1.07-1.34). We also found a protective role for a simple surrogate anthropometric indicator, ThC/WC ratio, against NAFLD, independent of multiple metabolic risks. Both higher TSFA/AFA ratio and ThC/WC ratio were adversely associated with worse metabolic profiles. Our findings stressed that more attention should be paid to this fat distribution and not only gross weight when considering weight control for a given individual. Our findings highlight that greater thigh subcutaneous fat relative to a given amount of abdominal fat had a protective effect against NAFLD among the middle-aged and older Chinese populations.

TSFA/AFA ratio and metabolic risks

Several studies have reported an inverse association of lower body adiposity with glucose and lipid levels.[21,22] For example, a study of 2,106 Americans indicated that higher TSFA [measured by computed tomography (CT)] was associated with lower levels of glucose in men and lipids in both sexes, independently of abdominal fat depots;[21] another study of 108 Americans showed that leg fat mass [measured by dual-energy X-ray absorptiometry (DXA)] was related to reduced insulin resistance and dyslipidemia independent of the increased risk attributable to trunk fat mass.[22] In line with these previous studies, our study showed that a higher TSFA/AFA ratio and its surrogate indicator (ThC/WC ratio) were associated with favourable metabolic profiles.

TSFA/AFA ratio and NAFLD risks

Previously, relevant research examining the associations of TSFA and its relative distribution with NAFLD were limited to two cross-sectional analyses.[4,5] One cross-sectional study involving 408 Koreans observed a negative association of TSFA (measured by CT) with NAFLD in women after adjustment for VFA and ASFA.[4] Another recent cross-sectional study involving 14,502 Koreans found that a lower leg fat to total fat ratio (measured by DXA) was associated with a higher risk of prevalent NAFLD.[5] Our prospective cohort study demonstrated that the direction of the association between TSFA (measured by MRI) and incident NAFLD reversed before and after adjustment for BMI with a RR of per SD increase in TSFA, originally being 1.27 (95% CI 1.12-1.43) and reversing to 0.83 (95% CI 0.71-0.95) (Table S5). This reflected different effects of the absolute quantity of a given fat compartment compared with the relative amount of this compartment to the total body fat on NAFLD risk. Thus, we further analysed the association of the relative distribution of TSFA (reflected by the TSFA/AFA ratio) with NAFLD and observed a negative association between the two. In addition, this negative association was observed between the TSFA/AFA ratio and both incident liver steatosis and NAFLD with advanced fibrosis.

In addition, our study demonstrated that a higher TSFA/AFA ratio was favourably associated with remission of NAFLD (RR per SD 1.20, 95% CI 1.07-1.34). Moreover, our study found that the odds of remission of NAFLD decreased with the presence of fibrosis: subjects with advanced fibrosis defined by FNI were associated with a 36% lower likelihood of remission of NAFLD compared with those without advanced fibrosis (RR 0.64, 95% CI 0.51-0.81; Table S6). However, in subjects with advanced fibrosis, a favourable effect of TSFA/AFA ratio on remission of NAFLD was still present (FIB-4: RR per SD 1.24; FNI: RR per SD 1.28, both $p < 0.05$; Table S4). This significant association was not observed in people with diabetes (Table S4), which might be because of a smaller sample size. Our study is the first to provide prospective evidence that a higher TSFA/AFA ratio, reflecting the propensity to accumulate fat in the thigh subcutaneous compartment rather than in the abdominal compartment, has a protective effect against NAFLD, even against NAFLD with advanced fibrosis.

ThC/WC ratio and NAFLD risks

Simple anthropometric measurements (WC and ThC) have been widely used in studies on cardiometabolic diseases, acting as

Table 4. Mediation analyses to estimate the indirect, direct, and total effects of TSFA/AFA ratio on incident NAFLD.*

Mediator	ACME estimate (95% CI)	ADE estimate (95% CI)	Total effect estimate (95% CI)	Percentage mediated (95% CI)	*p* value†
HOMA-IR‡	-0.007 (-0.01 to -0.003)	-0.06 (-0.09 to -0.04)	-0.07 (-0.09 to -0.05)	9.49 (3.92-21.26)	<0.001
TG‡	-0.005 (-0.01 to -0.001)	-0.07 (-0.09 to -0.04)	-0.07 (-0.09 to -0.05)	7.51 (1.59-16.45)	0.016
Adiponectin	-0.01 (-0.02 to -0.002)	-0.06 (-0.08 to -0.03)	-0.07 (-0.09 to -0.04)	14.94 (2.91-40.52)	0.014
FGF21‡	0.000 (-0.004-0.003)	-0.07 (-0.09 to -0.04)	-0.07 (-0.09 to -0.04)	0.13 (-4.90-6.70)	0.992
RBP4	-0.001 (-0.004-0.002)	-0.07 (-0.09 to -0.05)	-0.07 (-0.09 to -0.05)	0.86 (-2.53-5.24)	0.570

ACME, average causal mediation effects; ADE, average direct effects; AFA, abdominal fat area; FGF21, fibroblast growth factor 21; HOMA-IR, homeostasis model assessment of insulin resistance; NAFLD, non-alcoholic fatty liver disease; RBP4, retinol-binding protein-4; TG, triglyceride; TSFA, thigh subcutaneous fat area.
* Two multivariable-adjusted regression models (the linear regression model for the mediator and Poisson regression model for the outcome) were established to evaluate these effects. The effects of TSFA/AFA ratio on incident NAFLD were adjusted for sex, age, education levels, smoking status, drinking status, leisure-time exercise, and BMI. CIs were calculated using percentile bootstrap (replications = 1000).
† A *p* value for percentage mediated (statistically significant at $p < 0.05$).
‡ Log_e-transformed before analysis.

Table 5. Mediation analyses to estimate the indirect, direct, and total effects of TSFA/AFA ratio on NAFLD remission.*

Mediator	ACME estimate (95% CI)	ADE estimate (95% CI)	Total effect estimate (95% CI)	Percentage mediated (95% CI)	*p* value†
HOMA-IR‡	0.01 (0.005-0.02)	0.04 (0.007-0.08)	0.05 (0.02-0.10)	23.93 (9.24-67.04)	0.002
TG‡	0.01 (0.003-0.02)	0.04 (0.007-0.08)	0.05 (0.02-0.10)	19.12 (5.61-61.88)	0.002
Adiponectin	0.01 (0.006-0.02)	0.04 (0.001-0.07)	0.05 (0.01-0.09)	26.62 (10.31-84.65)	0.014
FGF21‡	0.001 (-0.001-0.003)	0.05 (0.02-0.09)	0.05 (0.02-0.09)	0.96 (-2.31-6.22)	0.524
RBP4	-0.001 (-0.004-0.001)	0.05 (0.02-0.09)	0.05 (0.02-0.09)	-1.34 (-9.36-2.93)	0.526

ACME, average causal mediation effects; ADE, average direct effects; AFA, abdominal fat area; FGF21, fibroblast growth factor 21; HOMA-IR, homeostasis model assessment of insulin resistance; NAFLD, non-alcoholic fatty liver disease; RBP4, retinol-binding protein-4; TG, triglyceride; TSFA, thigh subcutaneous fat area.
* Two multivariable-adjusted regression models (the linear regression model for the mediator and Poisson regression model for the outcome) were established to evaluate these effects. The effects of TSFA/AFA ratio on remission of NAFLD were adjusted for sex, age, education levels, smoking status, drinking status, leisure-time exercise, and BMI. CIs were calculated using percentile bootstrap (replications = 1000).
† A *p* value for percentage mediated (statistically significant at $p < 0.05$).
‡ Log_e-transformed before analysis.

more cost-effective and implementable substitutes for accurately measured adiposity indicators, such as AFA and TSFA. The combination of WC and ThC (*i.e.* waist-to-thigh ratio) provides an estimate of body shape and fat distribution, which have been identified as significant predictors of diabetes[8] and all-cause mortality.[9] The Hoorn Study, which included 1,357 subjects, reported that an increased waist-to-thigh ratio was associated with a higher risk of incident diabetes (OR: men 1.42; women 1.92).[8] Another study of 10,638 adults demonstrated that a larger waist-to-thigh ratio was associated with an increased risk of all-cause mortality (hazard ratio: men 1.14; women 1.21).[9] Yet, to date, no studies have examined the association of the ThC/WC ratio with NAFLD. Our study is the first to show that an increased ThC/WC ratio has a favourable effect on reducing the risk of incident NAFLD and increasing the remission probability for NAFLD. Our results suggest that the ThC/WC ratio could be measured to evaluate the possibility of onset and remission of NAFLD in clinical practice.

Evidence that supports the favourable effect of thigh subcutaneous fat

It has been suggested that subcutaneous fat acts as an 'energy sink', storing fat to buffer the energy surplus, protecting other tissues from lipid overflow.[23] The lipolytic rate is lower in lower-body fat than in upper-body fat.[24,25] The basal blood flow, which is an important determinant of local adipose tissue fatty acid trafficking, of the lower-body fat is slower than that of upper-body fat.[26] This indicates that the release of NEFA into the systemic circulation from lower-body fat is less than from upper-body fat.[25,27] Chronic exposure to NEFA is associated with both decreased insulin biosynthesis and impaired insulin secretion.[28] Thus, by trapping excess fatty acids,[25] thigh subcutaneous fat might protect our bodies from insulin resistance, which is a well-known pathophysiological hallmark of NAFLD.[29] This is supported by our study, because we found that the TSFA/AFA ratio was negatively related to insulin resistance (r = -0.16). Meanwhile, we also found that the associations between TSFA/AFA ratio and incidence and remission of NAFLD could be mediated through insulin resistance (percentage mediated 9.5% and 23.9%, respectively).

Adiponectin is exclusively secreted by adipocytes and can decrease the influx of NEFAs, increase fatty acid oxidation, and enhance insulin sensitivity in the liver.[30,31] A higher gluteofemoral fat mass has been shown to result in higher plasma adiponectin levels and increased insulin sensitivity.[32,33] Our results provide further prospective evidence in humans that the effect of the TSFA/AFA ratio on the incidence and remission of NAFLD might be partially mediated by adiponectin (percentage mediated 14.9% and 26.6%, respectively). Moreover, our results provide new insights into the pathogenetic pathway of NAFLD and suggest that adiponectin could be targeted as a future therapeutic for remission of NAFLD.

Strengths and limitations

Our study had several strengths. First, this was the first community-based prospective cohort study to examine the associations between relative body fat distribution (TSFA/AFA ratio and ThC/WC ratio) and the incidence and remission of NAFLD. Second, the adipose tissue areas were accurately measured by MRI. Third, most potential confounding factors had been considered.

However, our study had limitations. First, ultrasound has a limited sensitivity of 60-94% when used to diagnose hepatic steatosis.[34] However, ultrasound is recommended as the first-choice imaging modality to detect hepatic steatosis in clinical practice and large-scale epidemiological studies,[35,36] because it is

cheap and convenient to implement. Second, although ThC and WC are simple and cost-effective anthropometric indicators, they failed to accurately reflect body fat composition. Finally, our results are only applicable to middle-aged and older Chinese populations.

In conclusion, the TSFA/AFA and ThC/WC ratios were negatively associated with incident NAFLD but positively associated with remission of NAFLD among middle-aged and older Chinese populations. Our findings demonstrated that a favourable fat distribution, characterised by a relatively greater ratio of thigh subcutaneous fat to abdominal fat, had a protective role against NAFLD. Thus, individual obesity management should focus on not only weight, but also body shape to more effectively reduce the incidence risk of obesity-related metabolic diseases.

Abbreviations

ACME, average causal mediation effect; ADE, average direct effect; AFA, abdominal fat area; ALT, alanine aminotransferase; ASFA, abdominal subcutaneous fat area; AST, aspartate aminotransferase; CT, computed tomography; DBP, diastolic blood pressure; DICOM, Digital Imaging and Communications in Medicine; DXA, dual-energy X-ray absorptiometry; FGF21, fibroblast growth factor 21; FIB-4, Fibrosis-4; FINS, fasting insulin; FNI, Fibrosis Non-alcoholic steatohepatitis Index; FPG, fasting plasma glucose; GGT, gamma-glutamyl transpeptidase; HbA1c, glycated haemoglobin; HDL-C, HDL-cholesterol; HOMA-IR, homeostasis model assessment of insulin resistance; LDL-C, LDL-cholesterol; MRI, magnetic resonance imaging; NAFLD, non-alcoholic fatty liver disease; NEFA, non-esterified fatty acids; OR, odds ratio; RBP4, retinol-binding protein-4; RR, risk ratio; SBP, systolic blood pressure; TC, total cholesterol; TG, triglyceride; ThC, thigh circumference; TSFA, thigh subcutaneous fat area; VFA, visceral fat area; WC, waist circumference.

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Conflicts of interest

The authors declare no conflict of interest that pertain to this work.

Please refer to the accompanying ICMJE disclosure forms for further details.

Authors' contributions

YL, PC, and XH made substantial contributions to the conception and design of the study. YL, PC, and XH analysed the data. YL, PC, and XH drafted the manuscript. All the authors assisted in the acquisition and interpretation of data and contributed to the critical revision of the manuscript for important intellectual content and approved the final version.

Data availability statement

De-identified data in our study will not be made available publicly. For further detailed data access policy and procedure, please contact the corresponding author.

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Supplementary data

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Author names in bold designate shared co-first authorship.

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