Serum lipocalin-2 levels are positively associated with not only total body fat but also visceral fat area in Chinese men

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Abstract
Serum lipocalin-2 (LCN2) plays an important role in the regulation of the obesity-associated dysmetabolic state and cardiovascular disease. However, relatively little is known about the relationship between serum LCN2 levels and body fat content and distribution. We examined the associations of total body fat content and abdominal fat distribution with serum LCN2 levels in Chinese men.

The study was based on a cross-sectional analysis of data for 1203 Chinese men aged 22 to 78 years from the Shanghai Obesity Study. Body fat percentage (fat%) was assessed by bioelectrical impedance analysis, and magnetic resonance imaging was adopted to quantify the visceral fat area (VFA) and subcutaneous fat area (SFA). Serum levels of LCN2 were measured with a standard enzyme-linked immunosorbent assay method.

Subjects with a high fat% had higher serum LCN2 levels than those with a normal fat% regardless of their body mass index category (<25 and ≥25 kg/m²). The frequency of isolated high VFA was increased with increasing quintiles of serum LCN2 levels (P < 0.001), but the frequency of isolated high SFA did not differ between quintiles of serum LCN2 levels. A trend of increasing VFA was observed with increasing serum LCN2 levels (P < 0.001). Multiple stepwise regression analysis showed that VFA was positively associated with serum LCN2 levels, independent of overall obesity and other confounding factors (standardized β = 0.082, P = 0.008).

Serum LCN2 levels are positively correlated with body fat content and independently associated with VFA in Chinese men.

Abbreviations: 2hPG = 2-hour postload glucose level, BMI = body mass index, CRP = C-reactive protein, CVD = cardiovascular disease, DBP = diastolic blood pressure, fat% = body fat percentage, FFA = free fatty acid, FINS = fasting insulin, FFM = fat mass, FPG = fasting plasma glucose, HbA1c = glycated hemoglobin, HDL-C = high-density lipoprotein cholesterol, HOMA-IR = homeostasis model assessment of insulin resistance, LCN2 = lipocalin-2, LDL-C = low-density lipoprotein cholesterol, miRNAs = micro ribonucleic acids, MRI = magnetic resonance imaging, mRNA = messenger ribonucleic acid, SBP = systolic blood pressure, SFA = subcutaneous fat area, TC = total cholesterol, TG = triglyceride, VFA = visceral fat area, WC = waist circumference.

Keywords: abdominal fat distribution, adipocytokine, body fat content, Chinese men, lipocalin-2

1. Introduction
Lipocalin-2 (LCN2), also known as neutrophil gelatinase-associated lipocalin and siderocalin, is a 25-kDa glycoprotein originally purified from human neutrophils. It belongs to the lipocalin superfamily, which can bind and transport small lipophilic molecules.[1,2] LCN2 is expressed in many tissues and cell types, including neutrophils, macrophages, kidney, liver, lung, thymus, and small intestine. Moreover, it is abundantly produced by adipocytes. LCN2 was initially discovered to play an important role in the innate immune response to bacterial infection, as well as in the regulation of cell proliferation and apoptosis.[1,3] Recently, LCN2, as an adipose tissue-derived cytokine, has been found to potentially be involved in obesity-related and cardiovascular diseases (CVDs) through the regulation of inflammatory responses.[4–7]

CVDs are the leading health problem worldwide. Therefore, more stringent prognostic and diagnostic biomarkers are needed to lessen the health-related and economic burdens of CVDs.[8] Several genetic polymorphisms (such as CaMK4, PLA2, and GRKs) and micro ribonucleic acids have been suggested to play important roles in regulating metabolic disorders, as well as in the initiation and progression of CVDs.[8–14] Moreover, increasing evidence suggests that the serum LCN2 concentration may be a useful biomarker for evaluating outcomes in various clinical settings of obesity and metabolic or cardiovascular abnormalities. Our previous and other human studies noted elevated LCN2 levels in patients with CVDs.[4,7,15] Another prospective study indicated that the serum LCN2 concentration is a more effective early biomarker for predicting CVD events than the traditional inflammatory marker C-reactive protein (CRP).[15]

Several previous in vitro studies and various experimental models of obesity have demonstrated high levels of LCN2 messenger ribonucleic acid (mRNA) expression in adipose...
tissue \cite{16-18} and circulating LCN2 levels were greater in obese individuals than in lean subjects.\cite{17,19} In line with animal studies, human studies have reported a positive association between serum LCN2 levels and obesity determination \cite{14-16,17,20-22} However, these studies used simple methods such as calculation of body mass index (BMI) and measurement of waist circumference (WC) to evaluate the degree of obesity. Notably, BMI does not accurately represent body fat content, whereas biochemical impedance techniques employ the phenomenon that only water (containing electrolytes) can conduct electricity in the human body. Thus, these techniques can be used to accurately distinguish fat mass (FM) and fat-free mass (FFM). In addition, WC does not distinguish between visceral and subcutaneous fat distribution. However, magnetic resonance imaging (MRI) offers the advantage of identifying soft tissue and subcutaneous fat distribution. However, magnetic resonance imaging (MRI) offers the advantage of identifying soft tissue and subcutaneous fat distribution. However, magnetic resonance imaging (MRI) offers the advantage of identifying soft tissue and subcutaneous fat distribution.

A gender-related difference exists in concentrations of circulating LCN2, with studies demonstrating that men have higher serum levels of LCN2 than women. Moreover, in men, but not in women, serum LCN2 levels were found to be associated with obesity and obesity-related metabolic disorders. Therefore, the present study was designed to investigate the associations of serum LCN2 levels with total body fat content and abdominal fat distribution in Chinese men, using biochemical impedance analysis to evaluate total body composition and abdominal MRI to measure visceral fat area (VFA) and subcutaneous fat area (SFA).

2. Materials and methods

2.1. Study population

The present study represents a subgroup analysis of participants from the Shanghai Obesity Study conducted from December 2009 to December 2011, and the population selection and recruitment procedures were described in our previous study.\cite{23} Each participant completed a questionnaire regarding medical history, pharmacological therapy, lifestyle, and exercise habits. Subjects with the following conditions that may influence body weight and/or serum LCN2 levels were excluded from this study: a known history of diabetes; a known history of CVD; hyper- or hypothyroidism; any infection; malignant tumors; hepatic or renal dysfunction; and use of an anti-hypertensive, glucocorticoid, or lipid-lowering drug. After applying these exclusion criteria, a total of 1203 men with completed anthropometric, biochemical, biochemical impedance analysis, and abdominal MRI measurements were included. All participants provided written informed consent at enrollment. Ethical approval was obtained from the Ethics Committee of Shanghai Jiao Tong University Affiliated Sixth People’s Hospital. Data were handled so as to not compromise study participants’ privacy.

2.2. Biomarker analysis

After participants fasted for 10 hours overnight, blood samples were collected for measurement of biochemical parameters, including fasting plasma glucose (FPG), glycated hemoglobin (HbA1c), serum fasting insulin (FINS), serum total cholesterol (TC), serum triglyceride (TG), high-density lipoprotein cholesterol (HDLC), low-density lipoprotein cholesterol (LDLC), CRP, and free fatty acids (FFAs). All participants underwent a standard 75-g oral glucose tolerance test for the determination of the 2-hour postload glucose level (2hPG). The employed laboratory measurement techniques for blood samples have been described previously.\cite{23} The homeostasis model assessment of insulin resistance (HOMA-IR) value was calculated according to the formula: HOMA-IR = FPG (mmol/L) x FINS (mU/L)/22.5. The LCN2 concentration in fasting serum samples was measured using a standard enzyme-linked immunosorbent assay (Antibody and Immunoassay Services, The University of Hong Kong, Hong Kong) following the manufacturer’s instructions. The intra- and interassay coefficients of variation were 1.84% and 6.77%, respectively.

2.3. Physical examination and body composition and abdominal fat distribution measurements

During physical examinations, participants’ height, weight, WC, and blood pressure were measured as previously described.\cite{23} Height and weight measurements were used to calculate BMI. Body composition (FM, FFM, and body fat percentage [fat%]) was determined using a bioelectrical impedance analyzer (BC-420; Tanita Corp., Tokyo, Japan).\cite{24} VFA and SFA were quantified on the basis of MRI of the abdominal region with a 3.0-T imager (Philips Medical Systems, Amsterdam, The Netherlands) conducted by a single trained technician who was blinded to the participants’ clinical details.\cite{23} The cross-sectional areas corresponding to the VFA and SFA at the umbilical level were determined using image analysis software (Slice-o-matic 4.2 for Windows; Tomovision Inc., Montreal, QC, Canada) as described previously.\cite{25}

2.4. Clinical definitions

According to the definition of obesity of the World Health Organization,\cite{26} participants with a BMI $\geq 25$ kg/m$^2$ were assigned to the overweight/obese group, and those with a BMI $<25$ kg/m$^2$ were classified as the lean group. In addition, a fat% $\geq 25$% was defined as a high fat%,\cite{27} VFA $\geq 80$ cm$^2$ was defined as high,\cite{28} and SFA $\geq 75$th percentile was considered high.\cite{29} Accordingly, participants with a high VFA but normal SFA were classified as isolated high VFA, and those with a high SFA but normal VFA were defined as isolated high SFA. If both the VFA and SFA were high, the participant was classified as high VFA and SFA. Definitions of diabetes and impaired glucose regulation status were based on the 1999 World Health Organization classification criteria.\cite{29} Hypertension was defined according to the 1999 World Health Organization hypertension guidelines as systolic blood pressure (SBP) $\geq 140$ mm Hg and/or diastolic blood pressure (DBP) $\geq 90$ mm Hg.\cite{30} Dyslipidemia was defined according to the guideline of the National Cholesterol and Education Program Adult Treatment Panel III.\cite{31}

2.5. Statistical analyses

All data analyses were performed using SPSS statistical software, version 16.0 (SPSS, Chicago, IL). Results are reported as mean ± standard deviation for normal distribution data, median with interquartile range (25%-75%) for skewed distribution data, and frequencies and proportions for categorical variables. The significance of differences between 2 different groups was determined using the independent sample Student t test for normally distributed data, nonparametric Mann–Whitney U test for skewed data, and $\chi^2$ test for categorical variables. One-way
analysis of variance was used to determine statistically significant differences between serum LCN2 levels and VFA. The partial correlation coefficient was used to determine correlations between serum LCN2 levels and other clinical parameters. Multivariate stepwise regression analysis was performed to assess independent correlations between the clinical parameters and serum LCN2 levels. All statistical tests were two-sided, and \( P < 0.05 \) was considered to indicate statistical significance.

3. Results

3.1. Characteristics of the study population

A total of 1203 men, aged 22 to 78 years (mean, 52.69±8.96 years), were included in this study. Among the study population, 370 (30.8%) men had hyperglycemia, and of these, 102 (8.5%) had newly diagnosed diabetes and 268 (22.3%) had impaired glucose regulation. The prevalence rates of hypertension and dyslipidemia were 19.6% (n = 236) and 40.5% (n = 487), respectively. The median (interquartile range) of serum LCN2 levels among the total population was 39.90ng/mL (range, 29.30–53.50ng/mL).

The study participants were divided into lean (BMI < 25kg/m²) and overweight/obese (BMI ≥ 25 kg/m²) groups. The median LCN2 concentration was significantly higher in the overweight/obese group than in the lean group (\( P = 0.02 \); Table 1). In addition, compared with the lean group, the overweight/obese group had significantly higher levels of obesity determination indices (BMI, WC, FM, FFM, fat%, FFA, and SFA) and metabolism indicators (SBP, DBP, FPG, 2hPG, Hba1c, HOMA-IR, TG, LDL-C, FFA, and CRP), but lower HDL-C levels (Table 1). The frequencies of hyperglycemia, hypertension, and dyslipidemia in the overweight/obese group were also significantly higher than those in the lean group.

3.2. Correlation of serum LCN2 levels with body fat accumulation and distribution

To investigate the influence of body fat on serum LCN2 levels, each BMI category was further divided into 2 subgroups according to a fat% cutoff value of 25%. In the lean group, participants with a high fat% had significantly higher serum LCN2 levels than those with a normal fat% (42.40 [33.70–52.90] vs 38.30 [27.38–52.70]ng/mL, \( P = 0.02 \)). A similar trend was also observed in the overweight/obese group (42.90 [32.30–55.50] vs 39.50 [28.45–53.00]ng/mL for high and normal fat%, respectively; \( P = 0.04 \)). However, in the same fat% groups, no significant differences in serum LCN2 levels were observed between BMI categories (\( P = 0.58 \) for fat% < 25%, \( P = 0.90 \) for fat% ≥ 25%, respectively; Fig. 1).

To further investigate the relationship between serum LCN2 levels and abdominal fat distribution, we divided participants into 5 groups according to quintiles of serum LCN2 levels. In addition, we separated participants into 3 groups based on their abdominal fat distribution as follows: isolated high VFA, isolated high SFA, and high VFA and SFA. The percentages of participants in each abdominal fat distribution group were calculated for each quintile of serum LCN2, and the frequency of isolated high VFA increased with an increase in the quintile of serum LCN2 level (25.4%, 36.4%, 39.8%, 39.5%, and 44.2%, \( P < 0.001 \)). However, the frequencies of isolated high SFA, as well as high VFA and SFA, did not differ between quintiles of serum LCN2 (Fig. 2).

Next, the participants were divided into 7 groups according to VFA at 20-cm² increments, and an increasing trend in serum LCN2 levels was found to accompany an increase in VFA (median for each group: 32.50, 37.80, 37.70, 39.11, 45.00, 44.20, and 41.75 ng/mL; \( P < 0.001 \); Fig. 3).

3.3. Multivariate analyses for detecting variables that contribute to serum LCN2 levels

Partial correlation analysis adjusted for age was conducted to detect the relationship between serum LCN2 levels and anthropometric indices of obesity and other laboratory parameters. The results showed that serum LCN2 levels were significantly correlated with indices of obesity and other metabolism parameters as shown in Table 2. In contrast, serum LCN2 levels were not significantly correlated with SBP, FPG, Hba1c, TC, or LDL-C (Table 2).

Finally, we conducted a multiple stepwise regression analysis to detect factors that contribute to serum LCN2 level. The serum LCN2 level was designated as dependent variable. Age, all anthropometric indices of obesity (BMI, WC, FM, FFM, VFA, and SFA), and other significant variables identified from partial correlation analysis (DBP, 2hPG, HOMA-IR, TG, LDL-C, FFA, and CRP) were defined as independent variables. The results demonstrated that in addition to HDL-C and CRP, VFA also was independently and positively correlated with serum LCN2 levels (Table 3).

| Table 1 |
|----------|
| Characteristics of the study population divided by body mass index. |
| Variables | BMI < 25kg/m² | BMI ≥ 25kg/m² | \( P \) |
| --- | --- | --- | --- |
| n | 773 | 430 | --- |
| Age, y | 53.88 (47.38–60.39) | 53.25 (46.30–59.02) | 0.06 |
| WC, cm | 80.94 ± 6.75 | 94.00 ± 6.62 | <0.001 |
| FM, kg | 13.30 (10.60–15.70) | 20.75 (18.00–24.80) | <0.001 |
| FFM, kg | 51.25 ± 4.65 | 57.75 ± 5.78 | <0.001 |
| fat%, | 20.50 (17.50–23.00) | 26.60 (23.80–29.75) | <0.001 |
| VFA, cm² | 74.99 ± 34.15 | 127.46 ± 38.44 | <0.001 |
| SFA, cm² | 118.86 (90.52–145.76) | 180.51 (153.40–217.11) | <0.001 |
| SBP, mm Hg | 121.33 (113.33–130.00) | 125.33 (120.00–137.59) | <0.001 |
| DBP, mm Hg | 79.33 (71.33–81.33) | 80.00 (75.33–86.67) | <0.001 |
| FPG, mmol/L | 5.27 (4.93–5.65) | 5.57 (5.19–6.03) | <0.001 |
| 2hPG, mmol/L | 6.11 (4.97–7.40) | 6.76 (5.29–8.72) | <0.001 |
| Hba1c, % | 5.50 (5.30–5.80) | 5.70 (5.40–6.00) | <0.001 |
| FINS, mU/L | 6.35 (4.33–9.09) | 10.11 (7.33–14.16) | <0.001 |
| HOMA-IR | 1.53 (1.01–2.18) | 2.60 (1.82–3.55) | <0.001 |
| TC, mmol/L | 8.46 (3.43–5.52) | 4.98 (3.43–5.56) | 0.22 |
| TG, mmol/L | 1.34 (0.91–1.06) | 1.16 (0.93–2.15) | <0.001 |
| HDL-C, mmol/L | 1.33 (1.14–1.54) | 1.16 (1.04–1.33) | <0.001 |
| LDL-C, mmol/L | 3.15 (2.63–3.71) | 3.35 (2.75–3.89) | 0.001 |
| FFA, μEq/L | 534.00 (405.00–683.00) | 556.00 (444.00–691.00) | 0.003 |
| CRP, mg/L | 56.12 (29.1–10.9) | 1.02 (58.2–2.26) | <0.001 |
| LCN2, ng/mL | 39.20 (28.55–52.70) | 41.35 (30.68–54.30) | 0.02 |

Data are expressed as the median (interquartile range) for skewed distribution variables. Twoipped analysis of variance was used to determine statistically significant differences between serum LCN2 levels and VFA. The partial correlation coefficient was used to determine correlations between serum LCN2 levels and other clinical parameters. Multivariate stepwise regression analysis was performed to assess independent correlations between the clinical parameters and serum LCN2 levels. All statistical tests were two-sided, and \( P < 0.05 \) was considered to indicate statistical significance.
4. Discussion

Many bioactive and inflammatory factors secreted from adipose tissue have critical roles in energy metabolism and inflammatory homeostasis. LCN2 belongs to the lipocalin superfamily of proteins and is an adipokine mainly secreted from adipocytes that plays an important role in regulating energy metabolism.\[32\] Several studies have demonstrated associations of serum LCN2 concentration with various metabolic parameters. LCN2-deficient mice showed significantly decreased fasting glucose and decreased insulin levels, and inflammation of adipose tissue and obesity-induced insulin resistance were improved in these mice.\[33–35\] In addition, LCN2 may be involved in the process of lipid metabolism. Yan et al\[18\] found high LCN2 expression in 3T3-L1 adipocytes during adipogenesis. An animal study demonstrated that the expression and activity of 12-lipoxygenase, an enzyme responsible for metabolizing arachidonic acid, was largely inhibited in adipose tissue of LCN2 knockout mice. However, administration of recombinant LCN2 promoted the expression of 12-lipoxygenase.\[31\] On the other hand, a positive association between LCN2 and lipid profiles was observed in several human studies.\[4–6,17\] Consistently, our results also indicated a close association between the serum LCN2 level and TG, HDL-C, and FFA, after exclusion of the effects of lipid-lowering drug usage. Moreover, human studies also reported that patients with hyperglycemia have significantly higher LCN2 levels than individuals with normal glucose tolerance.\[20,36\] However, a recent study revealed that plasma levels of LCN2 are reduced in Mexican individuals with long-term diabetes.\[37\]
Because the effect of hyperglycemia on the serum LCN2 concentration remains uncertain, we excluded patients with diagnosed diabetes from the present study to eliminate the related confounding factors.

Studies in both humans and animals have demonstrated that LCN2 is closely associated with obesity and obesity-related diseases. Wang et al.[17] showed that LCN2 mRNA expression in liver, adipose tissue, and serum is significantly greater in obese mice than in age- and sex-matched lean littersmates. Population-based studies also demonstrated that obese humans have higher serum levels of LCN2 than lean controls, and serum LCN2 levels are significantly and positively correlated with BMI.[4–6,17,20–22] It is known that BMI reflects the degree of total obesity but cannot distinguish body fat from other body compositions. However, a study in 229 subjects that used bioelectrical impedance analysis to assess fat% reported that after adjustment for age, gender, and BMI, serum LCN2 levels remained positively correlated with fat%.[17] We also applied bioelectrical impedance analysis to evaluate total body fat content in a larger number of subjects, and our results revealed that men with an elevated total body fat content had significantly higher serum LCN2 levels, regardless of their BMI values. These findings suggest that increased body fat content may be primarily responsible for the increased circulating concentrations of LCN2.

Regional fat distribution, as opposed to total body fat FM, has been recognized as important for understanding the link between obesity and CVD. Evidence has demonstrated that compared with total body fat, excessive visceral fat distribution produces the most profound metabolic abnormalities and is associated with an increased risk for CVD.[12] Compared with Europeans, Asians with similar WC values exhibit relatively greater amounts of visceral adipose tissue accumulation.[38,39] However, to our knowledge, research focusing on the relationship between serum LCN2 levels and body fat distribution has been limited. Most population-based studies used simple measurement of WC to assess abdominal fat,[4–6,20,21] but WC measurement is unable to distinguish VFA and SFA. Only 1 study employed the more accurate method of computed tomography to evaluate visceral fat content in order to examine the relationship between VFA and serum LCN2 levels.[40] In their sample of 90 men with documented coronary artery disease and normal WC, serum LCN2 level was independently correlated with VFA.[40]

However, this result may have been influenced by the subjects’ diagnosed coronary artery disease status and related drug use. Therefore, in our study, we excluded men with a history of diagnosed CVD and applied MRI to distinguish VFA and SFA. We found that compared with a high SFA, high VFA was more closely correlated with increased serum LCN2 concentrations. Furthermore, elevated serum LCN2 levels followed a positive incremental trend according to increases in VFA. After adjustment for other anthropometric indices of obesity and additional relevant confounding factors, VFA was found to be an independent risk factor for elevated serum LCN2 levels, suggesting that abnormal body fat distribution, especially excessive visceral fat deposition, contributed more to the concentrations of serum LCN2 than did excessive total body fat in the present cohort.

Recently, LCN2 was proposed to be an important proinflammatory factor found at elevated levels in obese/inflammatory states. Increasing evidence from human studies indicates significant and positive associations between LCN2 and inflammatory markers (such as CRP, interleukin-6, total leukocyte count, and neutrophil count).[17,19,41] Furthermore, Wang et al.[17] found that the decreased LCN2 concentrations of diabetes patients correlated significantly with decreased CRP concentrations after 8 weeks of rosiglitazone treatment. Consistent with these findings, our present study also revealed a positive association between serum LCN2 and CRP concentrations, and this association remained significant after adjustment for other confounding factors.

However, the mechanisms through which LCN2 regulates inflammation and lipid metabolism remain unclear. The regulation of LCN2 in inflammatory and metabolic gene expression in 3T3-L1 adipocytes demonstrated that the addition of LCN2 induces mRNA expression of peroxisome proliferator-activated receptor-γ and its target genes, such as fatty acid synthase and lipoprotein lipase, indicating an important role for LCN2 in adipogenesis.[16] Moreover, studies have identified that LCN2 mRNA expression in visceral fat of obese subjects is significantly greater than that in lean controls.[19,22,42] However, a study by Auguet et al.[23] showed that the LCN2 protein level in VFA rather than in SFA correlates with circulating LCN2 levels. Visceral adipose accumulation is associated with a proinflammatory state and increased secretion of proinflammatory adipokines[43]; thus, we assumed that LCN2 might be associated with VFA based on its function of regulating inflammation in adipose tissue.[33,34]

The limitations of the present study include the cross-sectional nature of this study, which provides only an instantaneous view of the relationships between LCN2 and VFA. Prospective studies examining the causal relationship between VFA and serum LCN2 levels are needed to determine whether LCN2 should be recommended as a new marker of visceral obesity. In addition, because gender differences exist for both fat distribution and serum LCN2 concentrations, the present study only detected the relationship between serum LCN2 and visceral fat in Chinese men. Thus, our findings should be further verified in women and in populations of other regions.

5. Conclusion

Our study demonstrates that serum LCN2 levels are more closely associated with body fat content than with other body compositions, and for the first time, that VFA is an independent determinant of serum LCN2 levels in Chinese men.

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