Association of Glypican-4 With Body Fat Distribution, Insulin Resistance, and Nonalcoholic Fatty Liver Disease

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Context and Objective: Glypican-4 was identified as a novel adipokine capable of enhancing insulin signaling and modulating adipocyte differentiation. We investigated associations between glypican-4 and body composition, insulin resistance, arterial stiffness, and nonalcoholic fatty liver disease (NAFLD) in nondiabetic Asian subjects.

Design and Participants: We analyzed baseline cross-sectional data from the Korean Sarcopenic Obesity Study, an ongoing prospective cohort study. NAFLD was diagnosed by unenhanced computed tomography using the liver attenuation index. We also examined the effects of a 3-month combined aerobic and resistance exercise program on glypican-4 levels and cardiometabolic risk factors.

Results: Circulating glypican-4 levels were higher in men than in women (1.83 [1.19, 2.78] ng/mL vs 1.17 [0.66, 2.00] ng/mL, $P < .001$) and had a significant positive relationship with the waist-to-hip ratio (WHR) ($r = 0.20$, $P = .014$) and the ratio of visceral to sc fat area ($r = 0.30$, $P < .001$). Furthermore, glypican-4 levels in women were correlated with cardiometabolic risk factors, including insulin resistance and arterial stiffness, and were independently associated with NAFLD by multiple logistic regression analysis ($P = .017$, $R^2 = 0.33$). The 3-month combined exercise training program significantly improved several cardiometabolic parameters and reduced retinol binding protein-4 levels. Changes in glypican-4 levels after the exercise program were significantly different between subjects with an increased WHR compared with those with a decreased WHR ($P = .034$).

Conclusion: A gender-based difference in circulating glypican-4 levels was apparent as these were increased in women with NAFLD and related to body fat distribution, insulin resistance, and arterial stiffness. (***J Clin Endocrinol Metab*** **98: 2897–2901, 2013)**

Adipose tissue plays an important role in controlling systemic energy homeostasis through the secretion of various adipokines that interact with the brain, pancreas, skeletal muscle, and liver (1). Because visceral adipose tissue is more pathogenic than sc adipose tissue, the site of fat accumulation plays a pivotal role in metabolic disorders (2). However, little is known about the factors that determine sites of fat accumulation. Recently, Gesta et al found that glypican-4 is differentially expressed in visceral and sc adipose tissue, and that its expression in

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Abbreviations: AST, aspartate aminotransferase; baPWV, brachial ankle pulse wave velocity; BMI, body mass index; HOMA-IR, homeostasis model assessment of insulin resistance; NAFLD, nonalcoholic fatty liver disease; VFA/SFA, ratio of visceral to sc fat area; WHR, waist-to-hip ratio.

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human white adipose tissue is highly correlated with body mass index (BMI) and waist-to-hip ratio (WHR) (3). Very recently, the same group demonstrated for the first time that glypican-4 is released from mouse adipocytes and circulating glypican-4 levels are correlated with insulin sensitivity in humans (4). Furthermore, 3T3-L1 preadipocytes lacking glypican-4 failed to differentiate into mature adipocytes and reduced activation of the insulin signaling pathways (4). In contrast, overexpression of glypican-4 in 3T3-L1 preadipocytes enhanced insulin-stimulated ERK and Akt-Ser473 peak phosphorylation and increased 2-deoxyglucose uptake (4). Taken together, these findings indicate that glypican-4 is a novel adipokine that directly interacts with the insulin receptor to regulate its activation and plays a crucial role in the differentiation and distribution of adipose tissue. However, to the best of our knowledge, there have been no further reports on the metabolic function of glypican-4 as a novel adipokine.

In the present study, we explored the associations between circulating glypican-4 levels and body fat distribution, insulin resistance, and nonalcoholic fatty liver disease (NAFLD) in nondiabetic Asian subjects. First, we compared circulating glypican-4 levels between subjects with NAFLD and age- and gender-matched control groups. Second, we evaluated the relationships between serum glypican-4 levels and various cardiometabolic risk factors, including insulin resistance, arterial stiffness (represented by brachial ankle pulse wave velocity; baPWV), and the ratio of visceral to sc fat area (VFA/SFA) measured by abdominal computed tomography. Finally, we investigated the effect of a 3-month combined aerobic and resistance exercise program on changes in circulating glypican-4 levels in obese women.

Subjects and Methods

Study design and participants

We performed a cross-sectional study using database information and blood samples obtained from participants in the Korea Sarcopenic Obesity Study, which is an ongoing prospective, observational, cohort study. Details relating to the study design and objectives were described previously (5, 6). The exclusion criteria and methods used for the diagnosis of NAFLD were the same as those in our previous study (7). Next, we performed a prospective study to examine the effect of a 3-month combined exercise program on circulating glypican-4 levels in 64 obese women. Details of the inclusion criteria for this prospective study and of the combined exercise program were provided in our previous report (8). All participants provided their written informed consent and the Korea University Institutional Review Board, in accordance with the Declaration of Helsinki of the World Medical Association, approved the study protocol.

Anthropometric and laboratory measurements

Plasma glypican-4 levels were assayed using a commercially available ELISA kit (USCNK Life Science, Houston, Texas); the intra- and interassay variations were <9% and <11%, respectively. After each subject had rested in the supine position for 5 minutes, the baPWV was measured using a BP-203RPE II volume-plethymographic apparatus (Colin, Komaki, Japan). The baPWV was calculated as the mean of the left and right baPWV values. Abdominal VFA and total abdominal fat area were measured by computed tomography scan without any iv contrast agent using a Brilliance 64 apparatus (Philips Medical Systems, Cleveland, Ohio). Details of the method used for the analysis of visceral obesity were provided in our previous study (7).

Statistical analyses

A Mann-Whitney *U* test, 2-sample *t* test, or Spearman correlation test was used to determine the relationships between plasma glypican-4 levels and the study variables. All statistical results were based on 2-sided tests. Data were analyzed using SAS 9.2 (SAS Institute, Cary, North Carolina). A P value $<.05$ was considered statistically significant.

Results

The clinical and biochemical characteristics of the study subjects are presented in Table 1. Circulating glypican-4 levels were higher in men than in women (1.83 [1.19, 2.78] ng/mL vs 1.17 [0.66, 2.00] ng/mL, $P < .001$). Women with NAFLD had significantly higher plasma glypican-4 levels than subjects without NAFLD (1.58 [1.01, 2.62] ng/mL vs 0.90 [0.59, 1.28] ng/mL, $P = .001$), whereas men did not show any significant difference in glypican-4 levels based on NAFLD (1.97 [1.17, 3.25] ng/mL vs 1.71 [1.33, 2.71] ng/mL, $P = .496$) (Table 1). Plasma glypican-4 levels showed significant positive correlations with WHR ($r = 0.20$, $P = .014$), VFA/SFA ratio ($r = 0.30$, $P < .001$), and aspartate aminotransferase (AST) levels ($r = 0.27$, $P = .001$) among all subjects. Especially in women, circulating glypican-4 levels were positively correlated with AST ($r = 0.32$, $P = .005$), triglyceride ($r = 0.27$, $P = .020$), glucose ($r = 0.31$, $P = .006$), and homeostasis model assessment of insulin resistance (HOMA-IR) ($r = 0.31$, $P = .006$) levels, baPWV ($r = 0.23$, $P = .048$), and VFA/SFA ratio ($r = 0.30$, $P = .009$) (Figure 1). Furthermore, there was a trend toward positive associations between plasma glypican-4 levels and the number of metabolic syndrome components ($r = 0.23$, $P = .051$) in women. However, there were no significant correlations between plasma glypican-4 levels and various metabolic risk profiles in men (Figure 1). Multiple logistic regression analysis indicated that circulating glypican-4 levels (odds ratio = 2.05, 95% confidence interval: 1.13–3.72. $P = .017$) were independent determining factors for NAFLD along with BMI and insulin resistance in women ($R^2 = 0.33$), whereas BMI and

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Table 1. Anthropometric and Metabolic Characteristics of the Study Subjects

	Men (n = 76)			Women (n = 76)		
	Control	NAFLD	*P*	Control	NAFLD	*P*
	(n = 38)	(n = 38)		(n = 38)	(n = 38)	
Age, y	51.0 ± 12.5	50.7 ± 12.8	.921[a]	52.0 ± 13.4	51.7 ± 13.9	.975
BMI, kg/m^2	24.1 (22.3, 25.2)	26.6 (25.0, 27.9)	<.001	22.9 (20.8, 26.5)	26.6 (25.0, 28.9)	<.001
WC, cm	88.4 ± 8.1	92.87 ± 6.82	.005	82.8 ± 10.2	89.8 ± 7.2	.001[a]
WHR	0.89 ± 0.04	0.91 ± 0.04	.129[a]	0.86 ± 0.05	0.88 ± 0.04	.040
SBP, mmHg	122.8 ± 11.5	129.32 ± 12.38	.021[a]	122.4 ± 15.8	122.5 ± 12.0	.987[a]
DBP, mmHg	81.6 ± 8.2	86.21 ± 9.56	.026[a]	80.1 ± 9.8	79.0 ± 9.1	.604[a]
AST, IU/L	19.5 (16.8, 24.0)	26.0 (20.0, 32.3)	<.001	19.0 (16.8, 23.0)	20.0 (16.0, 25.8)	.408
ALT, IU/L	20.0 (16.8, 26.3)	25.0 (17.0, 38.5)	.017	16.0 (13.0, 21.3)	24.0 (17.8, 31.3)	<.001
HDL-C, mmol/L	1.33 (1.09, 1.53)	1.15 (1.01, 1.37)	.051	1.42 (1.24, 1.71)	1.32 (1.14, 1.47)	.014
Triglycerides, mmol/L	1.48 (1.00, 2.26)	1.70 (1.23, 2.52)	.136	1.17 (0.07, 1.92)	1.59 (1.04, 2.62)	.022
LDL-C, mmol/L	2.61 (2.12, 3.23)	2.75 (2.28, 3.18)	.526	2.83 (2.25, 3.28)	2.68 (2.15, 3.67)	1.000
Fasting glucose, mmol/L	5.33 (4.88, 5.72)	5.27 (5.11, 5.83)	.625	5.16 (4.88, 5.50)	5.44 (4.88, 6.11)	.081
HOMA-IR	1.61 (1.36, 1.94)	2.18 (1.64, 2.84)	.002	1.59 (1.20, 2.07)	2.50 (1.79, 3.47)	<.001
hsCRP, mg/dL	0.57 (0.24, 1.40)	0.81 (0.52, 1.86)	.118	0.35 (0.12, 1.04)	0.84 (0.48, 1.36)	.004
baPWV, m/s	14.19 ± 2.96	14.32 ± 2.43	.708	13.21 ± 2.09	13.57 ± 2.25	.472[a]
Adiponectin, μg/mL	9.33 ± 3.60	7.23 ± 2.12	.003[a]	12.35 ± 4.74	8.65 ± 2.84	<.001
Glypican-4, ng/mL	1.97 (1.17, 3.25)	1.71 (1.33, 2.71)	.496	0.90 (0.59, 1.28)	1.58 (1.01, 2.62)	.001
VFA, cm^2	133.7 ± 48.8	171.2 ± 56.4	.003[a]	112.8 ± 55.1	152.7 ± 49.4	.002[a]
SFA, cm^2	131.6 ± 69.3	162.5 ± 81.2	.030	211.5 ± 96.8	241.4 ± 92.0	.171[a]

Abbreviations: ALT, alanine aminotransferase; HDL, high-density lipoprotein; hsCRP, high-sensitivity C-reactive protein; LDL, low-density lipoprotein; WC, waist circumference; SBP, systolic blood pressure; DBP, diastolic blood pressure; HDL-C, HDL cholesterol; LDL-C, LDL cholesterol. Data are expressed as mean ± SD or median (interquartile range).

P values were calculated by an independent 2-sample *t* test[a] or Mann-Whitney *U* test.

AST were significant risk factors for NAFLD in men (Supplemental Table 1, published on The Endocrine Society's Journals Online web site at http://jcem.endojournals.org).

After the 3-month combined aerobic and resistance exercise program, no significant change in circulating glypican-4 levels was observed (Supplemental Table 2). However, plasma glypican-4 levels in subjects with a decreased WHR showed a tendency to decrease (pre-exercise, 1.55 [0.97, 2.08] ng/mL vs postexercise, 1.24 [0.82, 1.85] ng/mL, $P = .088$), whereas those with an increased WHR showed a tendency to increase (pre-exercise, 0.93 [0.67, 1.35] ng/mL vs postexercise, 1.23 [0.71, 1.87] ng/mL, $P = .192$). This resulted in a significant difference in Δglypican-4 levels between subjects with an increased WHR and those with a decreased WHR (0.06 [−0.31, 0.83] vs −0.17 [−0.78, 0.22], $P = .034$) (Supplemental Figure 1).

Discussion

Glypicans are heparan sulfate proteoglycans that are bound to the outer surface of the plasma membrane by a glycosyl-phosphatidylinositol anchor (9). The main function of glypicans is to regulate the signaling of Wnt, Hedgehog, fibroblast growth factors, and bone morphogenetic proteins (10). However, little is known about the metabolic role of glypicans. Recently, Gesta et al showed for the first time that glypican-4 is differentially expressed in visceral and sc adipose tissue in humans, which suggests that glypican-4 might play an important role in body fat distribution and in functional differences between visceral and sc adipose tissue (3). Because the absolute quantification of any given fat deposit does not reflect its relative distribution, the VFA/SFA ratio has been suggested as an effective measurement of body fat distribution (11). Recently, Kaess et al demonstrated that in the Framingham Heart Study cohort, the VFA/SFA ratio was significantly correlated with metabolic risk factors even after adjusting for BMI and visceral adipose tissue, suggesting that the propensity to store fat viscerally vs sc might be a unique risk factor independent of absolute fat volumes (12). In the present study, circulating glypican-4 levels were significantly positively correlated with the WHR and the VFA/SFA ratio rather than BMI. Furthermore, although the glypican-4 levels showed no significant changes in accordance with weight reduction after the 3-month combined exercise program, the Δglypican-4 level was significantly different between subjects with an increased WHR compared with those with a decreased WHR. These results suggest that the change in glypican-4 levels might be associated with an alteration in body composition, but not with a simple change in body weight.

Recently, Ussar et al found that glypican-4 is an adipokine released from adipose tissue, and that Caucasian

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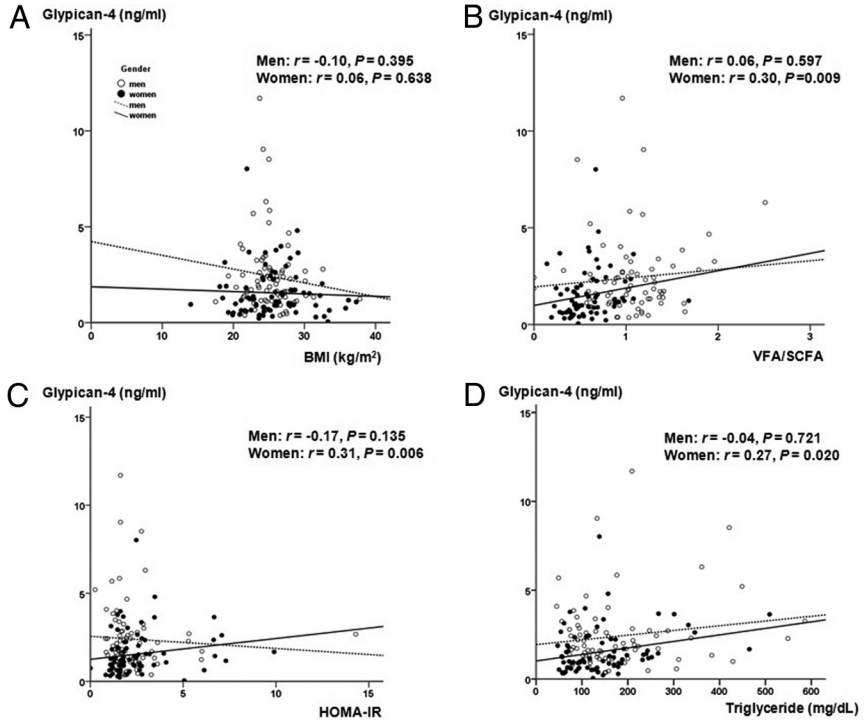

Figure 1. Correlation curves of plasma glypican-4 concentration with BMI (A), VFA/SFA (B), HOMA-IR (C), and triglyceride levels (D) in men and women. Spearman correlation coefficients and corresponding *P* values are displayed. SCFA, sc fat area.

subjects with the highest glypican-4 levels had a significantly higher BMI and body fat percentage. However, they did not find any association between glypican-4 levels and fasting plasma glucose, total cholesterol, high-density lipoprotein cholesterol, low-density lipoprotein cholesterol, or serum adiponectin levels (4). In contrast, the present study with Asian subjects showed that plasma glypican-4 levels had no significant correlation with BMI; however, glypican-4 levels showed significant positive associations with VFA/SFA ratio, AST, triglycerides, glucose, and HOMA-IR levels, especially in women. This is in agreement with our observation of increased glypican-4 levels in women with NAFLD, which is now regarded as a hepatic manifestation of metabolic syndrome. Asians tend to have more prominent abdominal obesity than individuals of European origin with similar BMI values (13), which means that Asians have a higher risk of developing metabolic diseases at lower levels of obesity.

Furthermore, the present study showed that glypican-4 levels in women were correlated with cardiometabolic risk factors, including several components of metabolic syndrome, insulin resistance, and arterial stiffness measured using pulse wave velocity, which is a useful marker for the assessment of increased cardiovascular risk (14). However, no such relationship between circulating glypican-4 levels and metabolic risk profile was observed in men. The exact explanation for this gender-based difference is not clear. It is well known that women are more effective at storing fat sc and men are more effective at storing fat intra-abdominally. Subcutaneous adipose tissue from women has higher lipoprotein lipase activity (15) and insulin-stimulated glucose uptake compared with men (16). Therefore, in women, sc fat may have more metabolically protective effects than the sc fat in men. He et al (17) observed that the VFA/SFA ratio, namely the propensity to store surplus energy in visceral vs sc fat, is independently associated with cardiometabolic risk factors, but only in women, and that VFA is more strongly correlated with cardiometabolic risk than the VFA/SFA ratio in men. Generally, associations between the VFA/SFA ratio and cardiovascular risk factors are stronger in women than in men, implying the relatively greater importance of body fat distribution, especially in women (12). Interestingly, in a study including Caucasian subjects, women showed a continuous increase in glypican-4 levels along a continuum from lean to overweight and obese, whereas the highest glypican-4 levels were observed in men who were overweight and had a visceral fat distribution (4). Further research exploring the possible influence of interactions between race, gender, and body fat distribution and their influence on glypican-4 levels is needed.

In conclusion, the present study revealed a gender difference in glypican-4 levels in Asian subjects as well as a significant association between circulating glypican-4 levels and NAFLD and cardiometabolic risk factors including body fat distribution, insulin resistance, and arterial stiffness in women. Further studies exploring the clinical implications and significance of glypican-4 as an adipokine are needed.

Acknowledgments

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K.M.C. designed the study. H.J.Y. and K.M.C. wrote the manuscript and researched the data. S.Y.H. analyzed the data. G.J.C., H.C.H., H.Y.C., and T.G.H. collected the data. S.M.K. M.B., B.-S.Y., and S.H.B. reviewed and edited the manuscript. K.M.C. is the guarantor of this work and, as such, had full access to all the data used in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis.

Disclosure Summary: B.-S.Y. is employed by AdipoGen, Inc. No other potential conflicts of interest relevant to this article are reported.

References

1. **Blüher M.** Clinical relevance of adipokines. *Diabetes Metab J*. 2012; 36:317–327.
2. **Fox CS, Massaro JM, Hoffmann U, et al.** Abdominal visceral and subcutaneous adipose tissue compartments: association with metabolic risk factors in the Framingham Heart Study. *Circulation*. 2007;116:39–48.
3. **Gesta S, Blüher M, Yamamoto Y, et al.** Evidence for a role of developmental genes in the origin of obesity and body fat distribution. *Proc Natl Acad Sci USA*. 2006;103:6676–6681.
4. **Ussar S, Bezy O, Blüher M, Kahn CR.** Glypican-4 enhances insulin signaling via interaction with the insulin receptor and serves as a novel adipokine. *Diabetes*. 2012;61:2289–2298.
5. **Kim TN, Yang SJ, Yoo HJ, et al.** Prevalence of sarcopenia and sarcopenic obesity in Korean adults: the Korean sarcopenic obesity study. *Int J Obes (Lond)*. 2009;33:885–892.
6. **Kim TN, Park MS, Yang SJ, et al.** Prevalence and determinant factors of sarcopenia in patients with type 2 diabetes: the Korean Sarcopenic Obesity Study (KSOS). *Diabetes Care*. 2010;33:1497–1499.
7. **Yoo HJ, Park MS, Lee CH, et al.** Cutoff points of abdominal obesity indices in screening for non-alcoholic fatty liver disease in Asians. *Liver Int*. 2010;30:1189–1196.
8. **Yang SJ, Hong HC, Choi HY, et al.** Effects of a three-month combined exercise programme on fibroblast growth factor 21 and fetuin-A levels and arterial stiffness in obese women. *Clin Endocrinol (Oxf)*. 2011;75:464–469.
9. **Filmus J, Capurro M, Rast J.** Glypicans. *Genome Biol*. 2008;9:224.
10. **Fico A, Maina F, Dono R.** Fine-tuning of cell signaling by glypicans. *Cell Mol Life Sci*. 2011;68:923–929.
11. **Kim S, Cho B, Lee H, et al.** Distribution of abdominal visceral and subcutaneous adipose tissue and metabolic syndrome in a Korean population. *Diabetes Care*. 2011;34:504–506.
12. **Kaess BM, Pedley A, Massaro JM, Murabito J, Hoffmann U, Fox CS.** The ratio of visceral to subcutaneous fat, a metric of body fat distribution, is a unique correlate of cardiometabolic risk. *Diabetologia*. 2012;55:2622–2630.
13. **He Q, Horlick M, Thornton J, et al.** Sex and race differences in fat distribution among Asian, African-American, and Caucasian prepubertal children. *J Clin Endocrinol Metab*. 2002;87:2164–2170.
14. **Zieman SJ, Melenovsky V, Kass DA.** Mechanisms, pathophysiology, and therapy of arterial stiffness. *Arterioscler Thromb Vasc Biol*. 2005;25:932–943.
15. **Fried SK, Kral JG.** Sex differences in regional distribution of fat cell size and lipoprotein lipase activity in morbidly obese patients. *Int J Obes*. 1987;11:129–140.
16. **Foley JE, Kashiwagi A, Chang H, et al.** Sex difference in insulin-stimulated glucose transport in rat and human adipocytes. *Am J Physiol*. 1984;246:E211–E215.
17. **He H, Ni Y, Chen J, et al.** Sex difference in cardiometabolic risk profile and adiponectin expression in subjects with visceral fat obesity. *Transl Res*. 2010;155:71–77.

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