Cardiometabolic Correlates and Heritability of Fetuin-A, Retinol-Binding Protein 4, and Fatty-Acid Binding Protein 4 in the Framingham Heart Study

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Context: Fetuin-A, retinol-binding protein 4 (RBP4), and fatty-acid binding protein 4 (FABP4) are novel biomarkers that may link adiposity to insulin resistance and the metabolic syndrome (MetSyn).

Objective: The aim of this study was to investigate the correlates of these three adiposity biomarkers in a large community-based sample.

Design, Setting, Participants, and Outcomes: Serum concentrations of fetuin-A, RBP4, and FABP4 were assayed in 3658 participants of the Third Generation Framingham Heart Study cohort (mean age 40 yr, 54% women). We used multivariable regression to cross-sectionally relate biomarkers to insulin resistance, cardiovascular risk factors, and the MetSyn. The genetic contribution to inter-individual variation in biomarker levels was assessed using variance-components analysis.

Results: All three biomarkers exhibited sexual dimorphisms (levels higher in women for fetuin-A and FABP4 but greater in men for RBP4) and were associated positively with insulin resistance assessed using the homeostasis model, with high-sensitivity C-reactive protein, and with prevalent MetSyn ($P < 0.01$ for all). The biomarkers showed distinct patterns of association with metabolic risk factors. RBP4 levels were correlated with body mass index only in unadjusted but not in adjusted models. None of the biomarkers were associated with prevalent diabetes in multivariable models. Circulating fetuin-A, RBP4, and FABP4 levels showed modest heritability, ranging from 15–44% (all $P < 0.0001$).

Conclusions: In our large young- to middle-aged community-based sample, we observed that circulating levels of fetuin-A, RBP4, and FABP4 are associated with insulin resistance and with distinct components of MetSyn consistent with the multifactorial pathogenesis of metabolic dysregulation. (***J Clin Endocrinol Metab*** **97: E1943–E1947, 2012)**

Obesity is a fundamental precursor of risk factors for cardiovascular disease and of cardiovascular disease itself. Recently, the discovery of several fat- and liver-secreted bioactive mediators has brought new insights into the complex cross talk between excess body fat and metabolic dysregulation. We investigated clinical and bio-

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Abbreviations: BP, Blood pressure; eGFR, estimated glomerular filtration rate; FABP4, fatty-acid binding protein 4; HDL, high-density lipoprotein; HOMA-IR, homeostasis model assessment for insulin resistance; hsCRP, high-sensitivity C-reactive protein; MetSyn, metabolic syndrome; RBP4, retinol-binding protein 4.

chemical correlates of three select novel biomarkers of the metabolic syndrome (MetSyn), fetuin-A, retinol-binding protein 4 (RBP4), and fatty-acid binding protein 4 (FABP4) (Supplemental Fig. 1).

Fetuin-A is a hepatic secretory glycoprotein that inhibits insulin receptor tyrosine kinase activity (1). Epidemiological studies have demonstrated that higher circulating fetuin-A levels are associated with greater prevalence of insulin resistance and the MetSyn and with higher incidence of myocardial infarction and ischemic stroke (2–5). Conversely, fetuin-A inhibits calcium precipitation and has been favorably associated with survival in dialysis patients (6).

RBP4 has been proposed to play a key role in linking obesity to insulin resistance. RBP4 is secreted by adipose tissue and the liver, it impairs insulin action in muscle and adipocytes, and promotes hepatic gluconeogenesis (7, 8). Elevated blood RBP4 levels have been associated with obesity, diabetes or insulin resistance, fatty liver, and MetSyn in some epidemiological studies (7, 9–11), but evidence is not consistent (12–14).

FABP4 is another endogenous factor that has been identified as a key mediator of insulin resistance in obesity. FABP4 is produced by adipocytes and released into plasma in abundant amounts (15). Circulating levels have been associated with prevalent MetSyn in smaller studies (15–17).

Given the potential important roles of fetuin-A, RBP4, and FABP4 in mediating the metabolic consequences of adiposity, we evaluated the clinical correlates of their circulating levels and relations to insulin resistance and MetSyn in a large community-based sample, the Framingham Study.

Materials and Methods

Study sample

Our study sample was derived from 4095 participants in the Framingham Third Generation cohort. After exclusion of participants with missing data or prevalent cardiovascular disease, n = 3658 individuals were available for the current analyses. Details of the study sample and on coronary calcium measurements are given in Supplemental Text 1 (published on The Endocrine Society's Journals Online web site at http://jcem.endojournals.org).

Biomarker measurements

Serum fetuin-A, FABP4, and RBP4 were measured from fasting samples using commercially available kits: Biovendor (Candler, NC) for fetuin-A and FABP4, R&D Systems Inc. (Minneapolis, MN) for RBP4. The mean interassay coefficients of variation were 2.4, 2.5, and 9.7%, respectively, for fetuin-A, RBP4, and FABP4. Details on insulin and high-sensitivity C-reactive protein (hsCRP) measurements are given in Supplemental Text 2.

Statistical analysis

FABP4 distribution was skewed; therefore, levels were log-transformed for statistical analyses. Clinical and metabolic correlates of fetuin-A, RBP4, and FABP4 were assessed using stepwise regression analyses with biomarkers as the dependent variable. Relations of biomarkers with homeostasis model assessment for insulin resistance (HOMA-IR) and hsCRP were explored by calculating Pearson correlation coefficients, adjusting for age, sex, and BMI. Association of biomarkers with insulin resistance and MetSyn were assessed using logistic regression models, with insulin resistance or MetSyn as the dependent variable. Heritabilities of the biomarkers were assessed using variance-component analysis. Details of statistical analyses are given in Supplemental Text 3. The authors had full access to the data and take responsibility for its integrity. All authors have read and agree to the manuscript as written.

Results

The clinical characteristics of our study sample are shown in Supplemental Table 1. Mean age was 40 yr, and approximately half of the sample was male. Mean BMI was 26.8 kg/m^2, 15.7% had hypertension, and 2.7% had diabetes. The sex-specific distributions of the three biomarkers are shown in Supplemental Fig. 1, and the correlation among them is given in Supplemental Table 2.

Clinical correlates of biomarkers

Table 1 shows the clinical correlates of the three biomarkers, derived from multivariable analyses. Serum fetuin-A concentration was lower in men, correlated inversely with age and alcohol consumption, and had positive associations with BMI and triglycerides. RBP4 levels decreased with age and increasing glomerular filtration rate, were higher in men (compared with women), and were positively related to serum total cholesterol, triglycerides, and high-density lipoprotein (HDL) cholesterol, systolic blood pressure (BP), antihypertensive medication, and alcohol intake. We observed a moderate correlation between RBP4 and BMI (Pearson r = 0.085; $P < 0.0001$) in a secondary, unadjusted analysis. However, this association was abolished by adjustment for age and sex (r = 0.024; $P = 0.14$). Serum FABP4 levels were higher in women compared with men, and levels increased with age, BMI, triglycerides, total cholesterol, diastolic BP, and antihypertensive medication. Inverse associations with FABP4 were observed for HDL and estimated glomerular filtration rate (eGFR).

In a sensitivity analysis, we explored association of RBP4 and FABP4 with blood pressure in 3363 participants without antihypertensive medication. We observed that associations were somewhat attenuated but remained significant (data not shown).

TABLE 1. Clinical correlates of circulating fetuin-A, RBP4, and FABP4 levels (n = 3658)

	β^a	95% confidence interval	*P*
Dependent variable: fetuin-A (mg/liter)			
Age	−23.7	−29.4, −18.0	<0.0001
Male sex	−32.9	−44.5, −21.2	<0.0001
Triglycerides	11.6	5.2, 18.0	0.0004
BMI	15.3	8.8, 21.8	<0.0001
Alcohol intake	−8.4	−13.3, −3.5	0.0008
Dependent variable: RBP4 (ng/ml)			
Age	−0.71	−1.10, −0.32	0.0004
Male sex	4.31	3.38, 5.04	<0.0001
Triglycerides	3.07	1.92, 4.23	<0.0001
HDL	1.75	1.22, 2.27	<0.0001
Total cholesterol	1.07	0.56, 1.58	<0.0001
Systolic BP	0.66	0.30, 1.02	0.0003
Antihypertensive treatment	2.91	1.58, 4.23	<0.0001
eGFR	−2.12	−2.47, −1.77	<0.0001
Alcohol intake	1.75	1.32, 2.18	<0.0001
Dependent variable: log FABP4			
Age	0.074	0.060, 0.088	<0.0001
Male sex	−0.411	−0.438, −0.384	<0.0001
Triglycerides	0.017	0.002, 0.032	0.028
BMI	0.278	0.262, 0.293	<0.0001
HDL	−0.054	−0.069, −0.038	<0.0001
Total cholesterol	0.028	0.013, 0.042	0.0002
Diastolic BP	0.017	0.003, 0.031	0.0173
Antihypertensive treatment	0.085	0.041, 0.128	<0.0001
eGFR	−0.036	−0.049, −0.024	<0.0001

[a] The β -values indicate mean increase in fetuin-A, RBP4, or log FABP4 per 1 SD increase of continuous traits or presence of a dichotomous trait. For example, men have 32.9 mg/liter lower mean fetuin-A levels and 34% ($e^{-0.411} = 0.66$) lower mean FABP4 levels than women, adjusting for all other covariates in the respective model.

Association of biomarkers with MetSyn, insulin resistance, and systemic inflammation

Adjusted mean serum concentrations of all three biomarkers increased with an increasing number of present MetSyn components (Fig. 1). None of the biomarkers were associated with prevalent diabetes; however, these analyses were limited by the low prevalence of diabetes in our sample. All three biomarkers were associated with insulin resistance (odds ratio per 1 SD increase = 1.18–1.33; all $P \leq 0.003$) and presence of the MetSyn (odds ratio = 1.24–3.43; all $P < 0.0001$; Supplemental Table 3). To assess the relations of fetuin-A, RBP4, and FABP4 with glucose homeostasis, we explored association of these biomarkers with HOMA-IR (Supplemental Table 4). Adjusting for age and sex, we found a strong correlation between HOMA-IR and FABP4 (r = 0.42; $P < 0.0001$). After additional adjustment for BMI, the association was attenuated but remained significant (r = 0.11; $P < 0.0001$).

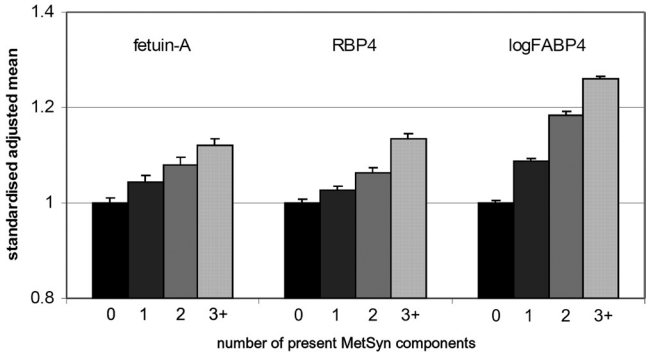

FIG. 1. Mean adjusted levels of fetuin-A, RBP4, and FABP4 in relation to number of present components of the MetSyn. Data are multivariable-adjusted means of fetuin-A, RBP4, and log-transformed FABP4, standardized to the mean values of individuals without any component of the MetSyn. Analyses were adjusted for the correlates identified in Table 1 for each respective biomarker, excluding constituents of the MetSyn (see Supplemental Text 3 for details).

Associations for fetuin-A and RBP4 were markedly weaker but still significant (both $P \leq 0.003$) and were little influenced by adjustment for BMI. A graphical representation of the correlation between HOMA-IR and our biomarkers is provided in Supplemental Fig. 3. Insulin resistance and MetSyn are associated with low-grade inflammation; we therefore assessed relations of fetuin-A, RBP4, and FABP4 with hsCRP. The correlations were very similar to those observed for HOMA-IR (Supplemental Table 4). In sensitivity analyses, we assessed correlations of our biomarkers with fasting glucose and fasting insulin. We found qualitatively similar results, although somewhat weaker (data not shown). As another approach of assessing insulin resistance, we also explored association of fasting insulin with our biomarkers in models adjusting for fasting glucose. Again, results were similar to those observed for HOMA-IR (data not shown).

Fetuin-A and coronary calcium

In age- and sex-adjusted regression models, serum fetuin-A was not associated with coronary calcification ($P = 0.66$ for continous trait; $P = 0.68$ for binary trait).

Heritability

We calculated a heritability of $h^2 = 0.15$ for serum fetuin-A in both age- and sex-adjusted and multivariable-adjusted models. Higher heritabilites were observed for FABP4 ($h^2 = 0.44$ and $h^2 = 0.38$ in age- and sex-adjusted and multivariable-adjusted model, respectively) and RBP4 ($h^2 = 0.31$ and $h^2 = 33$ in age- and sex-adjusted and multivariable-adjusted model, respectively).

Discussion

We analyzed clinical and genetic correlates of circulating fetuin-A, RBP4, and FABP4 levels in the Framingham

Heart Study Third Generation cohort. Our principal findings are 3-fold. First, each marker was associated with insulin resistance, prevalent MetSyn, low-grade inflammation (hsCRP), and several cardiovascular risk factors. In particular, levels of all three markers increased with present components of the MetSyn. Second, there were varied patterns of associations for the three biomarkers with metabolic dysregulation in four different domains (BMI, BP, lipids, and glycemia; summarized in Supplemental Table 2). Third, all three biomarkers demonstrated a modest to substantial additive genetic contribution to inter-individual variation in circulating levels.

The findings that serum fetuin-A levels decrease with age and are positively associated with BMI and female sex are in concordance with previous studies (3, 5). The underlying basis for the cross-sectional association with obesity is likely complex. Fetuin-A shows a strong correlation with liver fat, and it has been speculated that fatty liver disease drives fetuin-A expression and by this means contributes to insulin resistance in obesity (2). Conversely, fetuin-A-knockout mice are resistant to weight gain, and hence, fetuin-A seems to be causally involved in the development of obesity (18). We confirm several smaller studies showing association of fetuin-A with insulin resistance and the MetSyn (3–5), consistent with experimental data demonstrating that fetuin-A is a natural inhibitor of the insulin receptor tyrosine kinase activity (1). Independent of its impact on insulin signaling, fetuin-A inhibits vascular calcification and has been reported to correlate inversely with mortality in high-risk patients (6). Our analyses in a community-based sample did not reveal an association of fetuin-A levels with coronary calcification, questioning a relevant protective role of fetuin-A in the general population.

Our data do not support a relevant association of circulating RBP4 levels with adiposity, when accounting for age and sex. In contrast to the commonly held notion that RBP4 is associated with obesity, the evidence in the published literature is inconclusive (12). We observed positive correlations of serum RBP4 levels with insulin resistance and with several components of the MetSyn. Although RBP4 has been discovered as a cause of insulin resistance in rodents (7), epidemiological data are not consistent (10, 12–14). Our study is the largest analysis of this putative association that we are aware of and suggests that RBP4 levels are moderately associated with insulin resistance. We observed positive correlations of RBP4 with triglycerides, total cholesterol, and interestingly, also HDL-cholesterol concentrations. This latter finding is in concordance with the observations of Erikstrup *et al.* (13), whereas other groups found null or inverse associations between HDL and RBP4 (11, 12, 19). Additional studies are warranted to elucidate the functional relation between RBP4 and lipid metabolism.

We confirm previous reports of higher FABP4 levels in women (15, 17) and the association of FABP4 with obesity (15, 17). FABP4 has previously been associated with a dyslipidemic profile (19), which is also reflected in our data. The underlying biological principles are not entirely clear. Adipose tissue of FABP4-knockout mice shows markedly reduced transcription of hormone-sensitive lipase and increased transcription of lipoprotein lipase (20). However, these mechanisms are intracellular, and it is not known whether circulating FABP4 plays a causal role in dyslipidemia.

Strengths of our study are the large sample size, the high quality of data at the Framingham Heart study, and the broad range of clinical and metabolic variables available. However, some limitations of our investigation should be acknowledged. First, its cross-sectional observational design precludes causal inferences. Second, our observations in a middle-aged sample of European ancestry limits generalizability to other ethnicities. Third, we focused on three select markers of insulin resistance, but many mediators in different biological pathways are involved in the pathogenesis of metabolic dysregulation.

Our analysis of a large community-based sample demonstrated consistent associations of circulating fetuin-A, RBP4, and FABP4 concentrations with the presence of insulin resistance and prevalence of the MetSyn. The patterns of association of these biomarkers with metabolic traits were distinctive, suggesting that they may operate along different domains in the evolution of metabolic dysregulation associated with insulin resistance. Additional studies are warranted to confirm our observations and investigate the clinical relevance and potential diagnostic utility of the biomarkers.

Acknowledgments

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