Lipocalin-2 Is an Inflammatory Marker Closely Associated with Obesity, Insulin Resistance, and Hyperglycemia in Humans

Yu Wang,1,2 Karen S. L. Lam,2,3 Edward W. Kraegen,4 Gary Sweeney,5 Jialiang Zhang,2,3 Annette W.K. Tso,3 Wing-Sun Chow,3 Nelson M.S. Wat,3 Jian Yu Xu,2 Ruby L.C. Hoo,2 and Aimin Xu2,3*

Background: Lipocalin-2, a 25-kDa secreted glycoprotein, is a useful biomarker for early detection of various renal injuries. Because lipocalin-2 is abundantly expressed in adipose tissue and liver, we investigated its relevance to obesity-related pathologies.

Methods: We used real-time PCR and in-house immunoassays to quantify the mRNA and serum concentrations of lipocalin-2 in C57BL/KsJ db/db obese mice and their age- and sex-matched lean littermates. We analyzed the association between serum lipocalin-2 concentrations and various metabolic and inflammatory variables in 229 persons (121 men and 108 women) recruited from a previous cross-sectional study, and we evaluated the effect of the insulin-sensitizing drug rosiglitazone on serum lipocalin-2 concentrations in 32 diabetic patients (21 men and 11 women).

Results: Compared with the lean littermates, lipocalin-2 mRNA expression in adipose tissue and liver and its circulating concentrations were significantly increased in db/db diabetic/obese mice (P < 0.001). These changes were normalized after rosiglitazone treatment. In humans, circulating lipocalin-2 concentrations were positively correlated (P < 0.005) with adiposity, hypertriglyceridemia, hyperglycemia, and the insulin resistance index, but negatively correlated (P = 0.002) with HDL cholesterol. There was also a strong positive association between lipocalin-2 concentrations and high sensitivity C-reactive protein (hs-CRP), independent of age, sex, and adiposity (P = 0.007). Furthermore, rosiglitazone-mediated decreases in lipocalin-2 concentrations correlated significantly with increases in insulin sensitivity (r = 0.527; P = 0.002) and decreases in hs-CRP concentrations (r = 0.509; P = 0.003).

Conclusions: Lipocalin-2 is an inflammatory marker closely related to obesity and its metabolic complications. Measurement of serum lipocalin-2 might be useful for evaluating the outcomes of various clinical interventions for obesity-related metabolic and cardiovascular diseases.

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tissue and liver. Many of these inflammatory factors, such as interleukin (IL) 6, tumor necrosis factor α (TNFα), and resistin, can directly induce glucose intolerance and insulin resistance by antagonizing insulin’s metabolic actions at peripheral tissues, especially in liver and skeletal muscle (7, 8). On the other hand, several other adipokines produced from adipocytes, including adiponectin and visfatin, have demonstrated insulin-sensitizing activity and exertion of beneficial effects on glucose and lipid homeostasis (9–11).

Lipocalin-2, also known as 24p3 (12) and neutrophil gelatinase-associated lipocalin (NGAL) (13), is a 25-kDa secretory glycoprotein that was originally identified in mouse kidney cells and human neutrophil granules. This protein has been implicated in diversified functions such as apoptosis and innate immunity. In addition to neutrophils, lipocalin-2 is expressed in several other tissues, including liver, lung, kidney, adipocytes, and macrophages (14–16). Several inflammatory stimuli, such as lipopolysaccharides and IL 1β, can markedly induce lipocalin-2 expression and secretion in these cells (14, 17). Notably, the proinflammatory transcription factor NF-κB has been shown to transactivate lipocalin-2 expression through binding with a consensus motif in the promoter region of the lipocalin-2 gene (18), suggesting that this secretory protein might be involved in the inflammatory responses. Nevertheless, whether lipocalin-2 plays a role in the pathogenesis of obesity-related diseases has not been investigated to date.

Our laboratory has recently used a genome-wide interrogation strategy to identify differentially expressed genes during the development of obesity and its related pathologies in mice. As a result, we have found that the gene encoding lipocalin-2 was selectively induced in liver and adipose tissue of db/db obese/diabetic mice, and this increase was reversed by the insulin-sensitizing drug rosiglitazone. Accordingly, this study was designed to investigate the relationship between serum lipocalin-2 concentrations and obesity-related pathologies in mice and humans at baseline and after treatment with rosiglitazone (19).

Materials and Methods

ANIMAL STUDIES

The study included 15 C57BL/KsJ db/db diabetic mice and 15 lean littermates (The Jackson Laboratory; age 11–12 weeks). The metabolic characteristics of these mice are shown in Table 1. The mice were housed in a room at controlled temperature [mean (SD) 23 (1) °C], with free access to water and standard mouse chow. Rosiglitazone (a gift from GSK, Middlesex, UK) was administered to db/db mice by daily intragastric gavage (1 mg/kg body weight) for 2 weeks. All of the experiments were conducted in accordance with our institutional guidelines for the humane treatment of laboratory animals.

Table 1. Metabolic parameters of db/db mice and lean mice used in this study.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Lean (n = 15)</th>
<th>db/db (n = 15)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Body weight, g</td>
<td>27.2 (1.3)</td>
<td>46.7 (2.9)*</td>
</tr>
<tr>
<td>Fasting glucose, mmol/L</td>
<td>7.3 (0.6)</td>
<td>23.6 (3.2)*</td>
</tr>
<tr>
<td>Fasting insulin, μg/L</td>
<td>2.8 (1.5)</td>
<td>14.3 (6.7)*</td>
</tr>
<tr>
<td>Serum TG, mmol/L</td>
<td>0.93 (0.06)</td>
<td>2.34 (0.15)*</td>
</tr>
</tbody>
</table>

*P < 0.01 vs lean mice.

HUMAN PARTICIPANTS

We measured lipocalin-2 concentrations in serum samples collected from a total of 229 adults (age range, 33–72 years) who were participants in our previous cross-sectional Hong Kong Cardiovascular Risk Factor Prevalence Study (20, 21). The clinical characteristics of these study participants, as described in our previous reports (20, 21), are shown in Table 2. Participants were selected from our database on the basis of their body mass index (BMI) and included 100 lean (BMI, <25 kg/m²), 80 overweight (BMI, 25–29.9 kg/m²), and 49 obese participants (BMI ≥30 kg/m²), so that a study sample covering a wide range of adiposity was available to evaluate the relationship between circulating adipokine concentrations and various cardio-metabolic parameters. In some analyses, a lower BMI (<23 kg/m²) was used as the cutoff for the lean group, according to guideline for Asian populations (22). Of 229 study participants, 69 had diabetes (fasting plasma glucose, ≥7.0 mmol/L, or 2-h plasma glucose during a 75 g oral glucose tolerance test, ≥11.1 mmol/L, as recommended by the WHO in 1998) and 76 had metabolic syndrome according to National Cholesterol Education Program criteria (23). Serum samples were stored at −70 °C until assayed.

For the rosiglitazone (Avandia) intervention study, 32 T2DM patients (21 men and 11 women; age range, 39–71 years) were treated with rosiglitazone (4 mg twice daily) for 8 weeks. The clinical characteristics of these individuals are listed in Table 2. The study protocol was approved by the Ethics Committee of the Medical Faculty, University of Hong Kong. All study participants provided written informed consent before participation.

REAL TIME QUANTITATIVE PCR

We used the RNasey reagent set (Qiagen) to isolate total RNA from mouse liver, epididymal adipose tissue, and soleus muscle. Subsequently, mRNA was reverse transcribed into cDNA with oligo(dT) primer (Roche). The relative gene abundance of lipocalin-2 was quantified by Taqman real-time PCR with predeveloped reagent sets (Applied BioSystems). The reactions were performed on the ABI 7000 sequence detection system.

EXPRESSION AND PURIFICATION OF RECOMBINANT MURINE AND HUMAN LIPOCALIN-2

cDNA samples obtained from 3T3-L1 adipocytes were used as templates for amplification of the murine lipoca-
lin-2 gene with 5'-AGT AGG ATC CCA GGA CTC AAC TCA GAA CTT G-3' and 5'-AGT ACT CGA GTC AGT TGT CAA TGC ATT GGT C-3' as upstream and downstream primers, respectively. The amplified PCR products were double-digested by the restriction enzymes BamHI and Xhol and subsequently subcloned into the pPROEX-HTb vector (Invitrogen), leading to an expression vector pPRO-His-mLCN2, which encodes NH2-terminal His6-tagged human lipocalin-2 with 181 amino acid residues starting immediately after secretory signal peptide removed. The inserted human or murine lipocalin-2 cDNA was verified by DNA sequencing.

Fasting glucose, mmol/L 6.3 (2.2) 0.148 0.021 0.142 0.027
2 h postprandial glucose, mmol/L 8.1 (3.9) 0.063 0.451 0.045 0.587
HOMA-IR 3.1 (2.3) 0.305 0.001 0.145 0.025
hs-CRP, mg/L 2.2 (2.0) 0.236 0.002 0.202 0.007
Adiponectin, mg/L 6.4 (3.0) 0.05 0.507 0.115 0.098
A-FABP, ng/mL 24.3 (11.6) 0.245 <0.001 0.143 0.047

* Adjusted for sex and age.

** Adjusted for sex, age, and BMI. Note that the fat percentage was determined by bioelectric impedance analysis (Model TBF-410; Tanita).

*** WC, waist circumference; WHR, waist-hip ratio.

The expression of His-tagged murine or human lipocalin-2 in BL21 cells was induced by the addition of 0.5 mmol/L of isopropyl-β-D-thiogalactopyranoside to the growth medium. The recombinant proteins were purified from the bacterial lysates using the Ni2+–agarose column as we described previously (24). After purification, the NH2-terminal His6 tag was removed by cleavage with recombinant TEV protease, and the endotoxin was removed by use of the Detoxi-gel Endotoxin-Removal Kit (Pierce). The purity of the protein was confirmed by sodium dodecyl sulfate–polyacrylamide gel electrophoresis and fast protein liquid chromatography analysis.

ANTIBODY PRODUCTION AND DEVELOPMENT OF SANDWICH ELISA FOR QUANTIFICATION OF MURINE AND HUMAN LIPOCALIN-2

The polyclonal antibodies against human or murine recombinant lipocalin-2 were generated in New Zealand female rabbits as described previously (25). Antihuman or antimurine lipocalin-2 IgG was purified from the immunized rabbit serum with protein A/G beads, followed by affinity chromatography with their respective antigens as the ligands.

The affinity-purified antihuman or antimurine lipocalin-2 IgG was biotinylated with a reagent set from Pierce and used as the detection antibody. Unlabeled antihuman or antimurine lipocalin-2 IgG was used for coating the immunized rabbit serum with protein A/G beads, followed by affinity chromatography with their respective antigens as the ligands.
(20–40 μg/L) were used to determine the intra- and interassay CVs. These samples were tested 12 times on 1 plate and in 12 separate assays to assess intraassay and interassay variations, respectively.

STATISTICAL ANALYSIS
All the statistical calculations were performed with the SPSS 11.5 statistical software package (SPSS Inc). We used the Kolmogorov–Smirnov test to test variables for normality. The Student unpaired t-test was used for comparison between 2 groups, and a paired t-test for intragroup comparison before and after rosiglitazone treatment. Partial Pearson correlation coefficients were used to establish the relationship between lipocalin-2 concentrations and various clinical characteristics, with adjustment for sex, age, and/or BMI. We also stratified human participants into 5 groups according to number of components of the metabolic syndrome (0, 1, 2, 3, ≥4) and used 1-way ANOVA with Scheffé-type multiple comparison test to compare serum lipocalin-2 concentrations among these groups. In all statistical comparisons, a P value <0.05 was used to indicate a significant difference.

Results
LIPOCALIN-2 EXPRESSION IS INCREASED IN db/db DIABETIC/OBSESE MICE AND IS SUPPRESSED AFTER TREATMENT WITH THE PPARγ AGONIST ROSIGLITAZONE
We used microarray analysis for systematic identification of genes differentially expressed in db/db obese/diabetic mice and their lean littermates. Our results showed that the expression of a gene encoding lipocalin-2, a secretory glycoprotein, was markedly induced in the liver tissue of db/db obese/diabetic mice. To explore the relevance of lipocalin-2 with obesity, we used real-time quantitative PCR analysis to further compare the expression profiles of lipocalin-2 in male db/db mice and their age- and sex-matched lean littermates in various tissues. In both liver and adipose tissue, the steady-state mRNA concentrations of the lipocalin-2 gene in obese/diabetic db/db mice were substantially higher than those in their age- and sex-matched lean littermates (Fig. 1), and these changes were reversed after 2 weeks of treatment with rosiglitazone. On the other hand, the concentrations of lipocalin-2 mRNA in several other tissues, including lung, kidney, and spleen were comparable between the obese and lean mice.

To investigate whether altered mRNA expression in adipose tissue and liver leads to changes in circulating concentrations of lipocalin-2, we developed a sandwich ELISA method for measuring this protein in serum samples. To this end, we generated a polyclonal antibody against mouse lipocalin-2, using the recombinant protein as the antigen (Fig. 2). The specificity of this antibody was validated by its selective immunoprecipitation of recombinant lipocalin-2 from bacterial lysate and serum samples (data not shown). The sandwich ELISA standard curve generated with recombinant murine lipocalin-2 yielded a consistent r value >0.985. This analysis revealed that circulating concentrations of lipocalin-2 in db/db mice were significantly higher than those in their lean littermates. On the other hand, rosiglitazone treatment decreased circulating lipocalin-2 concentrations in db/db mice.

SERUM CONCENTRATIONS OF LIPOCALIN-2 ARE INCREASED IN OBSESE HUMANS AND ARE CLOSELY ASSOCIATED WITH SEVERAL ANTHROPOMETRIC, BIOCHEMICAL, AND INFLAMMATORY VARIABLES
To investigate the relationship between lipocalin-2 and obesity in humans, we also developed an in-house immunoassay for measurement of human lipocalin-2. Western blot analysis and immunoprecipitation revealed that the affinity-purified antihuman lipocalin-2 antibody selectively recognized an ~25-kDa protein in human serum, which was subsequently confirmed by tandem mass spectrometry analysis to be human lipocalin-2 (data not shown). The immunoassay based on this antibody was highly specific to human lipocalin-2, with no detectable cross-reactivity to several other human adipokines and cytokines, including adiponectin, leptin, resistin, TNFα, C-reactive protein (CRP), IL-6, and other lipocalin family members, including retinol-binding protein 4 (RBP4) and adipocyte fatty acid binding protein (data not shown). The intra- and interassay CVs were 3.8%–6.0% and 3.1%–5.2%, respectively. This analysis showed that the circulating concentrations of lipocalin-2 were 20.9–182.5 μg/L in the 229 study participants and exhibited gaussian distri-
HOMA-IR were still significant even after adjustment for correlations of serum lipocalin-2 with fasting glucose and concentrations of HDL cholesterol. Notably, the positive concentrations were negatively correlated with fasting serum (HOMA-IR) resistance index by homeostasis model assessment of insulin, triglycerides, and glucose; and the insulin age; systolic blood pressure; fasting serum concentrations with waist-to-hip ratio; waist circumference; fat percentage; and BMI (Table 2). After adjustment for sex and age, serum observations between serum lipocalin-2 concentrations and significant for both sexes. A strong positive correlation was than those in lean persons, and this difference was significant in obese persons were significantly higher BMI (Table 3). Notably, the circulating concentrations of lipocalin-2 in obese persons were significantly higher than those in females in both obese and lean groups (Table 3). Notably, the circulating concentrations of lipocalin-2 in obese persons were significantly higher than those in lean persons, and this difference was significant for both sexes. A strong positive correlation was observed between serum lipocalin-2 concentrations and BMI (Table 2). After adjustment for sex and age, serum concentrations of lipocalin-2 were positively correlated with waist-to-hip ratio; waist circumference; fat percentage; systolic blood pressure; fasting serum concentrations of insulin, triglycerides, and glucose; and the insulin resistance index by homeostasis model assessment (HOMA-IR) (26). On the other hand, lipocalin-2 concentrations were negatively correlated with fasting serum concentrations of HDL cholesterol. Notably, the positive correlations of serum lipocalin-2 with fasting glucose and HOMA-IR were still significant even after adjustment for BMI, suggesting that lipocalin-2 might be an independent risk factor for insulin resistance and hyperglycemia in humans. Notably, circulating lipocalin-2 concentrations in current smokers were much higher than those in non-smokers [mean (SD) 93.5 (45.0) vs 66.3 (36.0) µg/L; P = 0.001], and this significance remained even after adjustment for age, sex, and BMI (P = 0.031).

Several previous in vitro studies have suggested lipocalin-2 to be an acute-phase protein that can be induced by a variety of inflammatory stimuli (27, 28). Consistent with these experimental data, we observed a strong positive correlation between circulating lipocalin-2 and high-sensitivity CRP (hs-CRP), an established serum marker for chronic inflammation. This correlation remained significant even after adjusting for age, sex, and adiposity, suggesting that lipocalin-2 is independently associated with inflammation. In addition, serum lipocalin-2 concentrations were positively associated with adipocyte-fatty acid binding protein (A-FABP), a novel serum marker for adiposity and metabolic syndrome (20). On the other hand, there was no significant correlation between serum concentrations of lipocalin-2 and adiponectin (Table 2).

Patients with diabetes had higher mean (SD) serum concentrations of lipocalin-2 than those without this disease [82.4 (43.6) vs 69.6 (32.9) µg/L; P = 0.039 after adjustment for age and sex], but this significance disappeared after adjustment for BMI (P = 0.122). Mean (SD) serum lipocalin-2 concentrations in patients with metabolic syndrome were significantly higher than those without metabolic syndrome [83.2 (39.6) vs 67.5 (32.7) µg/L; P = 0.024 after adjustment for age and sex]. When study patients were further stratified according to the number of

<table>
<thead>
<tr>
<th>Table 3. Comparison of serum lipocalin-2 concentrations (µg/L) in lean (BMI, &lt; 23 kg/m²) and obese (BMI, &gt; 30 kg/m²) humans.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Obese</td>
</tr>
<tr>
<td>-------</td>
</tr>
<tr>
<td>Female</td>
</tr>
<tr>
<td>Male</td>
</tr>
<tr>
<td>Total</td>
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</table>

*a P < 0.05 vs obese group.
*b P < 0.05 vs female group.
*c P < 0.001 vs obese group.
the components of metabolic syndrome, mean (SD) serum concentrations of lipocalin-2 for those with 0, 1, 2, 3 and ≥4 components of metabolic syndrome were 63.7 (29.4) μg/L (n=43), 68.9 (34.6) μg/L (n=61), 69.1 (37.7) μg/L (n=49), 79.3 (36.9) μg/L (n=40), and 87.7 (43.5) μg/L (n=36), respectively. However, the correlation between serum lipocalin concentrations and the number of the metabolic syndrome criteria was not significant (P = 0.097).

CHANGES IN SERUM LIPOCALIN-2 CONCENTRATIONS ARE CORRELATED WITH THE OUTCOMES OF CLINICAL INTERVENTIONS IN T2DM PATIENTS

We next investigated whether serum lipocalin-2 is a useful surrogate marker for monitoring the therapeutic responses to the PPARγ agonist rosiglitazone, a drug with insulin-sensitizing and antiinflammatory activities. To this end, we analyzed serum samples from 32 T2DM patients treated with rosiglitazone (4 mg twice daily) for 8 weeks. The clinical features of these patients are listed in Table 2. During the 8-week intervention, there was no significant change in BMI, waist circumference, or serum triglycerides, whereas fasting glucose, fasting insulin, and HOMA-IR were all significantly decreased (Table 4). Rosiglitazone treatment was also associated with a marked decrease in circulating concentrations of lipocalin-2 and hs-CRP. Furthermore, rosiglitazone-mediated relative changes in serum lipocalin-2 concentrations correlated well with relative changes in insulin sensitivity (r = 0.527; P = 0.002) and CRP (r = 0.509; P = 0.003). Serum concentrations of adiponectin were increased after rosiglitazone treatment (Table 4), but there was no significant correlation between changes in serum adiponectin and lipocalin-2 concentrations.

Discussion

Although lipocalin-2 was identified more than a decade ago, the physiologic functions of this protein remain poorly understood. Previous studies have focused on the role of this protein in the innate immune response to bacterial infection (29) and in apoptosis (30). Several recent reports suggest that lipocalin-2 might be a sensitive biomarker for early renal injury (31, 32). In cardiopulmonary bypass–induced acute renal injury and cisplatin-induced nephrotic injury, increased de novo synthesis of lipocalin-2 in proximal tubule cells leads to sharply increased concentrations of this protein in both urine and serum.

Our study provides both clinical and experimental evidence demonstrating that circulating lipocalin-2 is a marker for obesity and its associated pathologies. Although many tissues express lipocalin-2, our results suggest that adipose tissue and liver are probably the 2 principal sources that contribute to the increased circulating concentrations of this protein in obesity states. In db/db obese mice, increased serum concentrations of lipocalin-2 are associated with the selective elevation of its mRNA expression in adipose tissue and liver (Fig. 1). In humans, lipocalin-2 concentrations are positively correlated with several adiposity variables, including BMI, waist circumference, and fat percentage (Table 2), suggesting that the increased fat mass might also account for the increased circulating concentrations of this protein in obese humans.

In line with our results, several previous in vitro studies have demonstrated the abundant expression of lipocalin-2 in adipocytes (15) and macrophages (16), the 2 major cell populations in obese adipose tissue. Furthermore, lipocalin-2 expression is sharply increased after conversion of preadipocytes to mature adipocytes or inflammatory stimulation (33). Expression of lipocalin-2 in both adipose tissue and liver can be induced by lipopolysaccharides, suggesting lipocalin-2 to be an acute-phase protein (27). Consistent with our data from db/db diabetic/obese mice, an earlier microarray-based study also found a marked increase in lipocalin-2 expression in white adipose tissue of ob/ob obese mice (34). In contrast, another study of ob/ob mice demonstrated decreased lipocalin-2 expression in adipose tissue but increased expression in liver (27). Although we cannot explain the observed differences between these studies, these data collectively support the role of lipocalin-2 as an adipokine linked to the development of obesity-related metabolic disorders in animal models.

In agreement with the animal data, our clinical study results showed a marked increase of circulating lipocalin-2 in obese humans (Table 3), findings similar to those occurring with many insulin resistance-inducing and inflammatory adipokines and cytokines, such as TNFα, IL6, and resistin (1, 2). Furthermore, we observed a significant positive correlation between lipocalin-2 concentrations and several variables associated with obesity-related metabolic disorders, including adverse lipid profiles (increased serum triglyceride and decreased HDL cholesterol), hyperinsulinemia, fasting glucose concentrations,

### Table 4. Effects of rosiglitazone treatment on serum concentrations of lipocalin-2 and several other metabolic and inflammatory parameters in 32 T2DM patients (21 male and 11 female).

<table>
<thead>
<tr>
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<th>Baseline</th>
<th>8 weeks</th>
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<tbody>
<tr>
<td>Age, years</td>
<td>55.4 (9.8)</td>
<td>25.5 (3.6)</td>
</tr>
<tr>
<td>BMI, kg/m²</td>
<td>25.4 (3.6)</td>
<td>25.8 (3.6)</td>
</tr>
<tr>
<td>Waist circumference, cm</td>
<td>86.8 (10.1)</td>
<td>86.9 (10.3)</td>
</tr>
<tr>
<td>Triglycerides, mmol/L</td>
<td>1.7 (1.0)</td>
<td>1.9 (1.1)</td>
</tr>
<tr>
<td>Fasting glucose, mmol/L</td>
<td>8.7 (1.5)</td>
<td>8.6 (1.2)</td>
</tr>
<tr>
<td>Fasting insulin, mIU/L</td>
<td>10.2 (5.0)</td>
<td>7.9 (3.5)</td>
</tr>
<tr>
<td>HOMA-IR</td>
<td>4.0 (2.2)</td>
<td>2.4 (1.2)</td>
</tr>
<tr>
<td>CRP, mg/L</td>
<td>2.0 (1.9)</td>
<td>1.2 (1.3)</td>
</tr>
<tr>
<td>Serum adiponectin, mg/L</td>
<td>7.7 (5.5)</td>
<td>17.1 (13.0)</td>
</tr>
<tr>
<td>Lipocalin 2, μg/L</td>
<td>84.8 (25.2)</td>
<td>57.1 (22.0)</td>
</tr>
</tbody>
</table>

a P <0.01 vs baseline.  
b P <0.05.
and the insulin resistance index (HOMA-IR). The association of lipocalin-2 with fasting glucose and HOMA-IR remained significant even after adjustment for age, sex, and adiposity, suggesting that this protein might be an independent risk factor for hyperglycemia and insulin resistance in humans.

Chronic low-grade inflammation is now recognized to be a key mediator in the development of obesity-related metabolic and cardiovascular diseases. Numerous epidemiologic studies have demonstrated that circulating hs-CRP, an established marker of chronic inflammation, is an independent risk factor for T2DM as well as atherosclerotic heart diseases (35, 36). In this study, we have demonstrated a close association between lipocalin-2 and hs-CRP, independent of age, sex, and adiposity. Both lipocalin-2 and hs-CRP are increased in obese individuals. Furthermore, the relative changes in serum lipocalin-2 concentrations correlate well with the relative changes in CRP concentrations after treatment with rosiglitazone, a drug with antiinflammatory activities (19). These results suggest that lipocalin-2 can be considered a marker for monitoring obesity-related low-grade inflammation in humans.

The physiologic relevance of lipocalin-2 with respect to obesity and its related pathologies remains to be determined. Notably, 2 other members of the lipocalin family, RBP4 and A-FABP, which are also produced from adipocytes, have recently been reported to play important roles in regulating systemic energy homeostasis, insulin sensitivity, and inflammation in animal models. Transgenic overexpression of RBP4 or injection of recombinant RBP4 in normal mice causes insulin resistance (37). Conversely, genetic deletion of RBP4 enhances insulin sensitivity. Several independent studies have shown that targeted disruption of the A-FABP gene provides significant protection from both dietary and genetic obesity-associated insulin resistance, hyperglycemia, and fatty liver diseases and also leads to marked alleviation of inflammation and atherosclerosis associated with ApoE-deficient mice (38–40). Our preliminary results showed that administration of a neutralizing antibody that blocks the actions of lipocalin-2 can alleviate insulin resistance, hyperglycemia, and inflammation in db/db mice (unpublished observations), suggesting that increased lipocalin-2 might play an etiologic role in the development of obesity-related pathologies, a possibility that is currently under active investigation in our laboratory.

In summary, our study provides the first clinical evidence demonstrating that serum concentrations of lipocalin-2 are closely associated with obesity and its related chronic inflammatory and metabolic complications. Our findings that rosiglitazone-mediated decreases in serum lipocalin-2 concentrations correlate well with decreases in inflammation and insulin sensitivity suggest that serum lipocalin-2 can be a useful marker for evaluating the therapeutic outcomes of clinical interventions for obesity-related metabolic and cardiovascular diseases. The major limitation of this study, however, is the lack of data providing insight into the mechanisms that underlie the increase of serum lipocalin-2 concentrations in obesity and its related pathologies. In addition, we cannot address the question of whether increased lipocalin-2 is a causative factor or simply an irrelevant bystander in the pathogenesis of obesity-associated metabolic abnormalities. Further longitudinal studies should allow us to determine the direction of the observed associations, the regulatory factors that modulate serum lipocalin-2, and the predictive value of this biomarker for obesity-associated metabolic and cardiovascular diseases.

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References


