Available online at www.sciencedirect.com
ScienceDirect
Metabolism Clinical and Experimental 60 (2011) 669–672
Metabolism
Clinical and Experimental
www.metabolismjournal.com

Serum levels of the adipokine zinc-α2-glycoprotein are increased in chronic hemodialysis

Anne Philipp[a,1], Susan Kralisch[a,1], Anette Bachmann[a], Ulrike Lossner[a], Jürgen Kratzsch[b], Matthias Blüher[a], Michael Stumvoll[a], Mathias Fasshauer[a,*]

[a]*Department of Internal Medicine III, University of Leipzig, 04103 Leipzig, Germany*
[b]*Institute of Laboratory Medicine, University of Leipzig, 04103 Leipzig, Germany*

Received 12 May 2010; accepted 23 June 2010

Abstract

Zinc-α2-glycoprotein (ZAG) has recently been proposed as a new adipokine involved in body weight control. In the current study, we investigated renal elimination of this adipokine by comparing circulating ZAG levels in patients on chronic hemodialysis (CD) with controls. Sixty CD patients and 60 controls with a glomerular filtration rate greater than 50 mL/min were included. Serum concentrations of ZAG were determined by enzyme-linked immunosorbent assay; and its relationship with renal function, glucose and lipid metabolism, as well as inflammation was studied in both groups. Median ZAG serum levels were almost 2-fold higher in CD patients (94.4 ± 29.4 mg/L) as compared with controls (48.3 ± 23.5 mg/L) ($P < .001$). Furthermore, circulating ZAG was negatively correlated with fasting insulin, homeostasis model assessment of insulin resistance, and leptin in controls in univariate analysis. Moreover, CD independently predicted ZAG concentrations in multiple regression analysis. Renal filtration appears to be an important route of ZAG elimination, and markers of renal function should be included in studies on ZAG physiology.

1. Introduction

Obesity is a rapidly growing nutritional disorder characterized by excessive accumulation of adipose tissue [1]. Both hyperplasia and hypertrophy of fat cells are found when weight is gained [1]. Increased body weight is associated with insulin resistance and type 2 diabetes mellitus (T2DM) [2]. In T2DM, 2 main defects are found: insulin resistance of peripheral tissues such as fat, liver, and muscle, as well as secretory failure of pancreatic β-cells [1,3]. The molecular mechanisms that link obesity on one hand and insulin resistance, as well as T2DM, on the other hand have not been completely understood so far and are the subject of intensive research. In recent years, adipose tissue has been recognized as an endocrine organ besides its role in energy storage. Adipocytes secrete various bioactive peptides termed *adipokines* that play a central role in whole-body glucose and energy homeostasis by influencing a variety of biological and physiologic processes, including food intake, energy expenditure, insulin action, and lipid metabolism.

In 2004, zinc-α2-glycoprotein (ZAG) was introduced as a novel adipokine that is secreted from human and murine adipose tissue and induces body weight loss [4]. Homozygous ablation of ZAG in mice led to increased body weight, and adipocytes from these animals showed reduced lipolysis [5]. Furthermore, administration of ZAG to mice [6,7] and ZAG overexpression in mice [8] induced marked body weight loss. Interestingly, the transgenic mice showed decreased fatty acid synthesis and increased hormone-sensitive lipase messenger RNA (mRNA) expression in epididymal fat [8]. Recently, hormonal regulation of ZAG and the influence of the adipokine on adiponectin expression in adipocytes have been studied in vitro in more detail. Thus, ZAG mRNA synthesis was up-regulated by treatment with dexamethasone [4], the peroxisome proliferator–activated receptor γ agonist rosiglitazone [9], and the selective β3-adrenoreceptor agonist BRL37344 [4], whereas insulin resistance–inducing tumor necrosis factor α decreased ZAG mRNA expression in differentiated adipocytes [9].

* Corresponding author. Tel.: +49 341 9713318; fax: +49 341 9713389.
E-mail address: mathias.fasshauer@medizin.uni-leipzig.de (M. Fasshauer).
[1] These authors equally contributed to this work.

0026-0495/$ – see front matter © 2011 Elsevier Inc. All rights reserved.
doi:10.1016/j.metabol.2010.06.019

Furthermore, recombinant ZAG stimulated adiponectin protein secretion from SGBS adipocytes [10]; and overexpression of ZAG in 3T3-L1 adipocytes up-regulated adiponectin mRNA synthesis [11].

These results indicate that ZAG is a novel adipokine that is regulated by hormones and drugs influencing glucose metabolism and might play a central role in body weight control. To date, few studies have quantified circulating ZAG concentrations in human metabolic disease [8,12-15]; and no data are available about circulating concentrations of this adipokine in end-stage renal disease. Glomerular filtration and renal degradation might influence serum ZAG levels similar to other adipokines including adiponectin [16], leptin [17], retinol-binding protein (RBP)–4 [18], adipocyte fatty acid–binding protein (AFABP) [19], fibroblast growth factor (FGF) 21 [20], and chemerin [21]. If renal elimination was of pathophysiologic significance, we hypothesized that patients on chronic hemodialysis (CD) would accumulate ZAG in serum. We tested our hypothesis by determining circulating ZAG concentrations in 60 CD and 60 control patients. Furthermore, ZAG serum levels were correlated to clinical and biochemical measures of renal function, glucose and lipid metabolism, as well as inflammation in both groups.

2. Research design and methods

2.1. Subjects

As recently described by our group [18-22], a total of 120 white men (n = 62) and women (n = 58) were recruited, with 60 patients having a glomerular filtration rate (GFR) greater than 50 mL/min (controls) as assessed by Cockroft-Gault formula and 60 patients being on CD. Body mass index (BMI) was calculated as weight divided by squared height, and BMI ranged from 18.7 to 46.1 kg/m^2. Waist-to-hip ratio was calculated after waist and hip circumferences were measured. The study population was between 32 and 85 years old. Thirty controls and 32 CD patients had *T2DM*, defined as fasting blood glucose 126 mg/dL or greater or use of insulin or oral hypoglycemic medications. Diabetes mellitus was excluded in the control group by performing 75-g oral glucose tolerance tests. Homeostasis model assessment of insulin resistance (HOMA-IR) was calculated as previously described [23]. Patients with severe conditions including generalized inflammation or end-stage malignant diseases were excluded from the study. The study was approved by the local ethics committee, and all subjects gave written informed consent before taking part in the study.

2.2. Assays

Blood samples were taken after an overnight fast. The ZAG serum concentrations were determined with a commercially available enzyme-linked immunosorbent assay (Biovendor, Modrice, Czech Republic) according to the manufacturer's instructions. Serum insulin was determined with a 2-site chemiluminescent enzyme immunometric assay for the Immulite automated analyzer (Diagnostic Products, Los Angeles, CA). Circulating leptin was assessed using an in-house assay as described previously [24]. Serum creatinine, free fatty acids (FFA), cholesterol, triglycerides (TG), and C-reactive protein (CRP) were measured by standard laboratory methods in a certified laboratory.

2.3. Statistical analysis

SPSS software version 18.0 (SPSS, Chicago, IL) was used for all statistical analyses. Differences between control and CD subjects were assessed by Mann-Whitney *U* test. Univariate analyses were performed using the Spearman rank correlation method. To adjust the effects of covariates and identify independent relationships, multivariate linear regression analyses were performed. Distribution was tested for normality using Shapiro-Wilk *W* test, and nonnormally distributed parameters were logarithmically transformed before multivariate analyses. A P value < .05 was considered as statistically significant in all analyses.

3. Results

3.1. ZAG serum levels are increased in CD patients as compared with controls

In Table 1, clinical characteristics of the subgroups studied (control, CD) are summarized; and all continuous variables are given as median ± interquartile range. Median

Table 1
Baseline characteristics of the study population

	Control	CD
n	60	60
ZAG (mg/L)	48.3 ± 23.5	94.4 ± 29.4*
Age (y)	63 ± 17	67 ± 18
Sex (male/female)	27/33	35/25
Diabetic/nondiabetic	30/30	32/28
BMI (kg/m^2)	28.7 ± 5.2	27.0 ± 7.5*
SBP (mm Hg)	125 ± 21	120 ± 29
DBP (mm Hg)	75 ± 12	70 ± 20
Creatinine (μmol/L)	74 ± 18	744 ± 300*
GFR (mL/min)	87 ± 29	7 ± 4*
FG (mmol/L)	5.8 ± 2.6	4.8 ± 1.7*
FI (pmol/L)	47.7 ± 47.7	38.3 ± 61.8
HOMA-IR	1.8 ± 2.2	1.1 ± 2.5
FFA (mmol/L)	0.5 ± 0.2	0.7 ± 0.5
Cholesterol (mmol/L)	5.1 ± 1.1	4.3 ± 1.3*
HDL (mmol/L)	1.3 ± 0.4	1.0 ± 0.5*
LDL (mmol/L)	3.1 ± 1.1	2.4 ± 1.0*
TG (mmol/L)	1.3 ± 0.8	1.6 ± 1.3*
Adiponectin (mg/L)	6.3 ± 4.8	11.9 ± 15.0*
Leptin (μg/L)	17.5 ± 23.9	20.9 ± 45.2
CRP (mg/L)	2.6 ± 4.2	5.0 ± 18.8*

Values for median ± interquartile range are shown. DBP indicates diastolic blood pressure; FG, fasting glucose; FI, fasting insulin; FFA, free fatty acids; HDL, high-density lipoprotein; LDL, low-density lipoprotein; SBP, systolic blood pressure.

* P < .05 compared with control as assessed by Mann-Whitney *U* test.

Table 2
Univariate correlations with serum ZAG concentrations

	Control	CD
n	60	60
Age (y)	$r = 0.011/P = .935$	$r = -0.111/P = .400$
BMI (kg/m^2)	$r = -0.096/P = .464$	$r = -0.110/P = .404$
SBP (mm Hg)	$r = -0.003/P = .984$	$r = -0.202/P = .121$
DBP (mm Hg)	$r = 0.005/P = .973$	$r = -0.143/P = .276$
Creatinine (μmol/L)	$r = 0.219/P = .093$	$r = 0.196/P = .133$
GFR (mL/min)	$r = -0.097/P = .460$	$r = -0.170/P = .194$
FG (mmol/L)	$r = 0.007/P = .955$	$r = -0.034/P = .796$
FI (pmol/L)	$r = -0.370/P = .004$*	$r = -0.089/P = .499$
HOMA-IR	$r = -0.328/P = .010$*	$r = -0.092/P = .485$
FFA (mmol/L)	$r = -0.137/P = .297$	$r = -0.081/P = .540$
Cholesterol (mmol/L)	$r = -0.119/P = .367$	$r = 0.002/P = .988$
HDL (mmol/L)	$r = -0.190/P = .147$	$r = -0.044/P = .740$
LDL (mmol/L)	$r = 0.007/P = .958$	$r = 0.049/P = .713$
TG (mmol/L)	$r = -0.006/P = .966$	$r = -0.03/P = .820$
Adiponectin (mg/L)	$r = -0.147/P = .261$	$r = 0.067/P = .612$
Leptin (μg/L)	$r = -0.321/P = .012$*	$r = -0.079/P = .547$
CRP (mg/L)	$r = -0.212/P = .105$	$r = -0.142/P = .280$

r/P values are given.
* Indicates significant correlation.

circulating ZAG was almost 2-fold higher in CD patients (94.4 ± 29.4 mg/L) as compared with controls (48.3 ± 23.5 mg/L) ($P < .001$) (Table 1). In contrast, a significant difference in ZAG concentrations could not be demonstrated depending on sex (female, 59.7 ± 48.1 mg/L; male, 78.6 ± 46.3 mg/L) and T2DM (T2DM, 67.4 ± 44.4 mg/L; non-T2DM, 75.5 ± 54.2 mg/L).

3.2. Univariate correlations

The ZAG serum levels negatively correlated with fasting insulin, HOMA-IR, and leptin in controls ($P < .05$) (Table 2). In CD patients, no significant correlation was detected between circulating ZAG and measures of renal function, glucose and lipid metabolism, as well as inflammation (Table 2).

3.3. Multivariate regression analyses

Multiple linear regression analysis revealed that CD remained independently associated with circulating ZAG levels after adjustment for age, sex, fasting insulin, and leptin ($P < .001$) (Table 3). In contrast, the association between ZAG serum levels on one hand and fasting insulin and leptin on the other hand seen in univariate analysis was lost after adjustment for CD (Table 3). Furthermore, HOMA-IR did not remain a significant predictor of ZAG besides CD in multivariate analyses (data not shown).

4. Discussion

In the current study, we show for the first time that median serum ZAG levels are almost 2-fold higher in CD patients as compared with controls. In addition, CD remains a strong independent predictor of ZAG concentrations in multivariate analysis. These findings indicate that renal elimination is a major route by which physiologic ZAG serum levels are maintained. Interestingly, a comparable mechanism of elimination has been described for other adipokines. Thus, plasma levels of insulin-sensitizing adiponectin are more than 2-fold higher in patients on CD as compared with healthy subjects [16]. Furthermore, plasma leptin concentrations are increased about 2-fold in CD patients [17]. Recently, our group has shown that circulating levels of the adipokines RBP-4 [18], AFABP [19], FGF21 [20], and chemerin [21] are also significantly increased in CD patients as compared with controls. Taking these studies into consideration, renal elimination appears to be a major mechanism influencing serum concentrations of various adipokines including adiponectin, leptin, RBP-4, AFABP, FGF21, chemerin, and ZAG. Furthermore, markers of renal function should always be included in studies concerning ZAG physiology and regulation.

The physiologic significance of increased ZAG serum concentrations in renal failure remains to be elucidated. Patients on CD frequently show increased concentrations of very low-density and intermediate-density lipoproteins considered to result from a defect in degradation rather than formation of TG-rich lipoproteins [25]. This impaired degradation of TG-rich lipoproteins in patients with chronic renal impairment is probably due to both a deficient lipolytic system and an inadequate substrate composition [25]. Furthermore, CD patients show a highly catabolic state leading to significant reductions in lean body mass but not fat mass; and decreased muscle mass is a significant predictor of morbidity and mortality in these patients [26]. Taking these data into consideration, increased circulating levels of ZAG in CD appear paradoxical because ZAG potently stimulates lipolysis and decreases fat mass [6-8]. Here, it needs to be determined in future studies whether inhibition of lipogenic enzymes including fatty acid synthase and up-regulation of lipolytic enzymes including hormone-sensitive lipase by ZAG [8] are blunted in CD patients despite increased concentrations of the adipokine.

In the current study, we demonstrate that serum ZAG concentrations are associated with a beneficial metabolic

Table 3
Correlation between ZAG (dependent variable) and CD adjusted for age and sex (model 1), as well as age, sex, fasting insulin, and leptin (model 2)

Dependent variable: ZAG			
Model	Independent variable	β	P
Model 1	CD	0.693	<.001*
	Age	−0.032	.620
	Sex	−0.125	.057
Model 2	CD	0.707	<.001*
	Age	−0.029	.657
	Sex	−0.086	.231
	FI	−0.078	.282
	Leptin	−0.084	.296

β coefficients and P values are given.
* Indicates significant correlation.

profile in control subjects. Thus, the adipokine is negatively correlated with fasting insulin and HOMA-IR in univariate analysis similar to recent findings [13]. Furthermore, a negative correlation exists with serum leptin levels. In contrast to our findings, other groups describe a positive association between ZAG on one hand and parameters of insulin sensitivity [15] or do not find an association [12,14]. Different patient characteristics might well explain these differences observed in the association between ZAG and metabolic parameters. Circulating ZAG does not correlate to markers of insulin resistance and adiposity in CD patients in the present study. These results emphasize that regulation of ZAG in end-stage renal disease might be different compared with that in patients with a GFR greater than 50 mL/min.

Taken together, our results suggest that renal filtration is an important route of elimination of ZAG and that markers of renal function should be included in studies on ZAG physiology. Prospective studies are needed to better elucidate the potential role of ZAG in metabolic and end-stage renal disease and to define predictors of serum levels of the adipokine.

Acknowledgment

This study was supported by a grant from the Deutsche Forschungsgemeinschaft, KFO 152: “Atherobesity,” project FA476/4-1 (TP 4) to MF, as well as by a grant of the European Section of Aldosterone Council Germany and the Deutsche Diabetes Gesellschaft to SK.

References

[1] Kahn BB, Flier JS. Obesity and insulin resistance. J Clin Invest 2000;106:473-81.
[2] Kiberstis PA. A surfeit of suspects. Science 2005;307:369.
[3] Saltiel AR. The molecular and physiological basis of insulin resistance: emerging implications for metabolic and cardiovascular diseases. J Clin Invest 2000;106:163-4.
[4] Bing C, Bao Y, Jenkins J, et al. Zinc-alpha2-glycoprotein, a lipid mobilizing factor, is expressed in adipocytes and is up-regulated in mice with cancer cachexia. Proc Natl Acad Sci U S A 2004;101: 2500-5.
[5] Rolli V, Radosavljevic M, Astier V, et al. Lipolysis is altered in MHC class I zinc-alpha(2)-glycoprotein deficient mice. FEBS Lett 2007;581: 394-400.
[6] Hirai K, Hussey HJ, Barber MD, Price SA, Tisdale MJ. Biological evaluation of a lipid-mobilizing factor isolated from the urine of cancer patients. Cancer Res 1998;58:2359-65.
[7] Russell ST, Zimmerman TP, Domin BA, Tisdale MJ. Induction of lipolysis in vitro and loss of body fat in vivo by zinc-alpha2-glycoprotein. Biochim Biophys Acta 2004;1636:59-68.
[8] Gong FY, Zhang SJ, Deng JY, et al. Zinc-alpha2-glycoprotein is involved in regulation of body weight through inhibition of lipogenic enzymes in adipose tissue. Int J Obes (Lond) 2009;33:1023-30.
[9] Bao Y, Bing C, Hunter L, et al. Zinc-alpha2-glycoprotein, a lipid mobilizing factor, is expressed and secreted by human (SGBS) adipocytes. FEBS Lett 2005;579:41-7.
[10] Mracek T, Ding Q, Tzanavari T, et al. The adipokine zinc-alpha2-glycoprotein is downregulated with fat mass expansion in obesity. Clin Endocrinol (Oxf) 2010;72:334-41.
[11] Gohda T, Makita Y, Shike T, et al. Identification of epistatic interaction involved in obesity using the KK/Ta mouse as a type 2 diabetes model: is Zn-alpha2 glycoprotein-1 a candidate gene for obesity? Diabetes 2003;52:2175-81.
[12] Selva DM, Lecube A, Hernandez C, et al. Lower zinc-alpha2-glycoprotein production by adipose tissue and liver in obese patients unrelated to insulin resistance. J Clin Endocrinol Metab 2009;94: 4499-507.
[13] Ceperuelo-Mallafre V, Naf S, Escote X, et al. Circulating and adipose tissue gene expression of zinc-alpha2-glycoprotein in obesity: its relationship with adipokine and lipolytic gene markers in subcutaneous and visceral fat. J Clin Endocrinol Metab 2009;94:5062-9.
[14] Stejskal D, Karpisek M, Reutova H, et al. Determination of serum zinc-alpha-2-glycoprotein in patients with metabolic syndrome by a new ELISA. Clin Biochem 2008;41:313-6.
[15] Yeung DC, Lam KS, Wang Y, Tso AW, Xu A. Serum zinc-alpha2-glycoprotein correlates with adiposity, triglycerides, and the key components of the metabolic syndrome in Chinese subjects. J Clin Endocrinol Metab 2009;94:2531-6.
[16] Zoccali C, Mallamaci F, Tripepi G, et al. Adiponectin, metabolic risk factors, and cardiovascular events among patients with end-stage renal disease. J Am Soc Nephrol 2002;13:134-41.
[17] Merabet E, Dagogo-Jack S, Coyne DW, et al. Increased plasma leptin concentration in end-stage renal disease. J Clin Endocrinol Metab 1997;82:847-50.
[18] Ziegelmeier M, Bachmann A, Seeger J, et al. Serum levels of the adipokine RBP-4 in relation to renal function. Diabetes Care 2007;30: 2588-92.
[19] Sommer G, Ziegelmeier M, Bachmann A, et al. Serum levels of adipocyte fatty acid binding protein are increased in chronic haemodialysis. Clin Endocrinol (Oxf) 2008;69:901-5.
[20] Stein S, Bachmann A, Lossner U, et al. Serum levels of the adipokine FGF21 depend on renal function. Diabetes Care 2009;32:126-8.
[21] Pfau D, Bachmann A, Lossner U, et al. Serum levels of the adipokine chemerin in relation to renal function. Diabetes Care 2010;33:171-3.
[22] Seeger J, Ziegelmeier M, Bachmann A, et al. Serum levels of the adipokine vaspin in relation to metabolic and renal parameters. J Clin Endocrinol Metab 2008;93:247-51.
[23] Fasshauer M, Bluher M, Stumvoll M, et al. Differential regulation of visfatin and adiponectin in pregnancies with normal and abnormal placental function. Clin Endocrinol (Oxf) 2007;66:434-9.
[24] Kratzsch J, Berthold A, Lammert A, et al. A rapid, quantitative immunofunctional assay for measuring human leptin. Horm Res 2002; 57:127-32.
[25] Lee DM, Knight-Gibson C, Samuelsson O, et al. Lipoprotein particle abnormalities and the impaired lipolysis in renal insufficiency. Kidney Int 2002;61:209-18.
[26] Pupim LB, Flakoll PJ, Ikizler TA. Protein homeostasis in chronic hemodialysis patients. Curr Opin Clin Nutr Metab Care 2004;7:89-95.