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Serum levels of angiopoietin-related growth factor in diabetes mellitus and chronic hemodialysis

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Abstract

Angiopoietin-related growth factor (AGF) was recently introduced as a novel liver-derived protein that antagonizes obesity and insulin resistance. In the current study, we investigated circulating AGF levels in relation to renal function and type 2 diabetes mellitus (T2DM). Angiopoietin-related growth factor was determined by enzyme-linked immunosorbent assay in subjects with a glomerular filtration rate greater than 50 mL/min (n = 60, 30 diabetic and 30 nondiabetic) and in patients on chronic hemodialysis (CD; n = 60, 32 diabetic and 28 nondiabetic). Furthermore, AGF was correlated to clinical and biochemical measures of renal function, glucose and lipid metabolism, as well as inflammation. Median serum AGF levels were significantly lower in CD patients (125.9 ± 96.3 μg/L) as compared with subjects with a glomerular filtration rate greater than 50 mL/min (164.0 ± 95.4 μg/L) ($P < .05$). Furthermore, AGF serum levels were significantly increased in diabetic patients (161.7 ± 114.2 μg/L) as compared with nondiabetic subjects (123.0 ± 88.2 μg/L) ($P < .01$). Moreover, CD negatively and T2DM positively predicted AGF concentrations in multiple regression analysis. In addition, fasting serum glucose was independently and positively correlated with circulating AGF in all patients and controls. Our results suggest that renal dysfunction is negatively and T2DM is positively associated with AGF serum levels. Further studies are needed to better elucidate the physiologic significance of circulating AGF in human disease.

1. Introduction

Obesity is a rapidly growing disorder in industrialized countries and is associated with insulin resistance, type 2 diabetes mellitus (T2DM), dyslipidemia, and hypertension [1,2]. In recent years, it has been suggested that dysregulated secretion of various factors from adipocytes and hepatocytes contributes to metabolic and cardiovascular disease found in obesity [1,2]. Among those, tumor necrosis factor–α and interleukin-6 have been characterized as insulin resistance–inducing cytokines that increase the risk of cardiovascular disease [1,3]. In contrast, the adipokine adiponectin and hepatocyte-derived fibroblast growth factor 21 have been shown to significantly improve glucose tolerance [1,4].

Recently, angiopoietin-related growth factor (AGF, also known as *Angptl6*) has been introduced as a novel liver-derived protein that antagonizes obesity and insulin resistance. Thus, AGF knockout mice developed marked obesity and insulin resistance, as well as lipid accumulation in skeletal muscle and liver [5]. Furthermore, AGF deficiency leads to reduced energy expenditure [5]. In accordance with these findings, mice with transgenic overexpression of AGF were lean, were insulin sensitive, and showed increased energy expenditure [5]. Furthermore, AGF transgenic mice were resistant to high-fat diet–induced obesity, insulin resistance, and nonadipose tissue steatosis [5]. Similar effects were also found in mice with hepatic overexpression of AGF by adenoviral transduction [5].

Whereas the role of AGF as a novel hepatocyte-derived metabolic regulator has been well established in rodents, no

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study has determined regulation of this factor in human disease in vivo until now. Furthermore, renal elimination has been established as a major route by which physiologic levels of various cytokines implicated in the metabolic syndrome are maintained [6,7]. In contrast, the association between AGF and renal dysfunction has not been studied so far. Therefore, we determined AGF serum levels in 60 patients on chronic hemodialysis (CD; 32 diabetic and 28 nondiabetic subjects) and 60 controls (30 diabetic and 30 nondiabetic subjects) with a glomerular filtration rate (GFR) greater than 50 mL/min using a novel commercial enzyme-linked immunosorbent assay system from Adipogen (Seoul, South Korea). Furthermore, this hepatocyte-secreted factor was correlated to clinical and biochemical measures of renal function, glucose and lipid metabolism, as well as inflammation.

2. Subjects and methods

2.1. Subjects

The design of the study has recently been described [8,9]. In brief, 120 white men (n = 62) and women (n = 58) were recruited, with 60 patients having a GFR greater than 50 mL/min (controls) as assessed by Cockroft-Gault formula and 60 patients being on CD. Fifty-five of the 60 controls had a GFR greater than 60 mL/min. Body mass index (BMI) was calculated as weight divided by squared height. Waist-to-hip ratio (WHR) was determined after waist and hip circumferences were assessed. The age ranged from 32 to 85 years; and BMI, from 18.7 to 46.1 kg/m^2. *Type 2 diabetes mellitus* in control and CD patients was defined as fasting blood glucose of at least 126 mg/dL or use of insulin or oral hypoglycemic medications. When these criteria were applied to the study population, 30 of the 60 controls and 32 of the 60 CD patients presented with T2DM. In the 30 controls who did not meet these criteria for T2DM, the disease was further excluded by performing 75-g oral glucose tolerance tests. Here, these patients showed 2-hour glucose levels less than 200 mg/dL. In contrast, T2DM was not excluded by 75-g oral glucose tolerance tests in the 28 CD patients who did not meet the above-mentioned criteria for T2DM because of the necessary fluid restriction. Homeostasis model assessment of insulin resistance (HOMA-IR) was calculated as previously described [10]. 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

All blood samples were taken after an overnight fast. Serum insulin was determined with a 2-site chemiluminescent enzyme immunometric assay for the Immulite automated analyzer (Diagnostic Products, Los Angeles, CA). Circulating AGF (Adipogen) and adiponectin (Mediagnost, Reutlingen, Germany) were determined with commercially available enzyme-linked immunosorbent assays according to the manufacturers' instructions. Leptin levels were assessed using an in-house assay as described previously [11]. Serum creatinine, parathyroid hormone (PTH), free fatty acids (FFA), cholesterol, triglycerides (TG), and C-reactive protein (CRP) were measured in a certified laboratory by standard laboratory methods.

2.3. Statistical analysis

All statistical analyses were performed using SPSS software version 11.5 (SPSS, Chicago, IL). Differences between groups were assessed by Mann-Whitney *U* test and Kruskal-Wallis test with Bonferroni post hoc analysis as indicated in the figure legends. 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. Here, parameters were included as independent variables that showed a significant correlation with circulating AGF levels in univariate analyses. In addition, age and sex were included in all models. Because fasting glucose depends on T2DM and GFR depends on CD, these variables were not included in the same model. Before performing multivariate analyses, distribution was tested for normality using Shapiro-Wilk *W* test. Nonnormally distributed parameters were logarithmically transformed. A *P* value less than .05 was considered as statistically significant in all analyses.

3. Results

3.1. AGF serum levels in CD and diabetic patients

Clinical characteristics of the subjects studied (control and CD) are summarized in Table 1, and all continuous variables are given as median ± interquartile range. Furthermore, the characteristics of the subgroups further divided into nondiabetic and diabetic subjects are presented in Table 2. Serum AGF was 140.7 ± 104.6 μg/L in the total sample. Median circulating AGF was significantly higher in controls (164.0 ± 95.4 μg/L) as compared with subjects on CD (125.9 ± 96.3 μg/L) ($P < .05$) (Table 1). Furthermore, AGF serum levels were significantly higher in diabetic patients (161.7 ± 114.2 μg/L) as compared with nondiabetic subjects (123.0 ± 88.2 μg/L) ($P < .01$). In contrast, a significant difference in serum AGF levels could not be demonstrated depending on sex (men, 131.6 ± 102.7 μg/L; women, 144.9 ± 98.1 μg/L).

3.2. Univariate correlations

When all patients were studied, serum AGF levels positively correlated with BMI, GFR, as well as fasting glucose, and were negatively associated with serum creatinine ($P < .01$) (Table 3). In controls, a positive correlation between AGF on one hand and fasting glucose

Table 1
Baseline characteristics of the study population

	Control	CD
n	60	60
AGF (μg/L)	164.0 ± 95.4	125.9 ± 96.3*
Age (y)	63 ± 17	67 ± 18
Sex (M/F)	27/33	35/25
Diabetic/nondiabetic	30/30	32/28
BMI (kg/m^2)	28.7 ± 5.2	27.0 ± 7.5
WHR	0.91 ± 0.12	0.98 ± 0.13*
SBP (mm Hg)	125 ± 21	120 ± 29
DBP (mm Hg)	75 ± 12	70 ± 20
Creatinine (μmol/L)	74 ± 18	744 ± 300*
GFR (mL/min)	97 ± 41	9 ± 5*
PTH (pmol/L)	4.2 ± 1.7	17.9 ± 20.8*
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 ± 1.2*
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 ± 18.7*

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

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

Table 3
Univariate correlations with serum AGF concentrations in all patients, as well as in control and CD patients

	All patients (N = 120)	Controls (n = 60)	CD (n = 60)
Age (y)	0.146/0.111	0.151/0.249	0.195/0.135
BMI (kg/m^2)	0.238/0.009[a]	0.148/0.260	0.213/0.103
WHR	0.063/0.496	0.123/0.349	0.146/0.267
SBP (mm Hg)	0.092/0.320	0.101/0.444	0.040/0.760
DBP (mm Hg)	−0.069/0.456	−0.158/0.229	−0.025/0.849
Creatinine (μmol/L)	−0.245/0.007[a]	−0.086/0.515	−0.153/0.242
GFR (mL/min)	0.249/0.006[a]	0.076/0.564	0.198/0.129
PTH (pmol/L)	−0.076/0.407	0.098/0.457	0.088/0.505
FG (mmol/L)	0.259/0.004[a]	0.281/0.029[a]	0.186/0.154
FI (pmol/L)	0.045/0.627	0.180/0.169	−0.101/0.444
HOMA-IR	0.145/0.115	0.264/0.042[a]	−0.040/0.761
FFA (mmol/L)	0.010/0.917	0.059/0.655	0.005/0.971
Cholesterol (mmol/L)	−0.007/0.936	−0.110/0.404	−0.048/0.717
HDL (mmol/L)	0.063/0.491	−0.080/0.542	0.007/0.958
LDL (mmol/L)	−0.008/0.928	−0.103/0.434	−0.092/0.490
TG (mmol/L)	−0.048/0.603	−0.024/0.854	0.019/0.884
Adiponectin (mg/L)	−0.113/0.220	−0.233/0.073	0.140/0.285
Leptin (μg/L)	0.035/0.704	0.009/0.946	0.064/0.627
CRP (mg/L)	0.165/0.072	0.166/0.204	0.280/0.030[a]

r/*P* values are given.

[a] Significant correlation.

and HOMA-IR on the other hand was found ($P < .05$) (Table 3). In CD patients, circulating AGF was positively associated with CRP in univariate analyses ($P < .05$) (Table 3). In contrast, AGF did not correlate with age, blood pressure, markers of lipid metabolism (FFA, cholesterol, TG), adiponectin, and leptin (Table 3).

Table 2
Baseline characteristics of the study population further divided into controls without diabetes (control/T2DM−) or with diabetes (control/T2DM+) and CD patients without diabetes (CD/T2DM−) or with diabetes (CD/T2DM+)

	Control/T2DM−	Control/T2DM+	CD/T2DM−	CD/T2DM+
n	30	30	28	32
AGF (μg/L)	129.5 ± 84.2	191.6 ± 110.7*	116.5 ± 108.5†	131.0 ± 123.1
Age (y)	63 ± 19	63 ± 16	59 ± 23	68 ± 12
Sex (M/F)	11/19	16/14	15/13	20/12
BMI (kg/m^2)	28.2 ± 5.6	29.1 ± 5.2	25.2 ± 6.5*,†	27.9 ± 6.6
WHR	0.88 ± 0.12	0.94 ± 0.10*	0.96 ± 0.18	1.00 ± 0.14*
SBP (mm Hg)	125 ± 21	126 ± 20	125 ± 38	120 ± 25
DBP (mm Hg)	77 ± 10	73 ± 15	77 ± 20	70 ± 18
Creatinine (μmol/L)	76 ± 17	72 ± 22	829 ± 431*,†	717 ± 221*,†
GFR (mL/min)	85 ± 35	100 ± 42	8 ± 6*,†	9 ± 5*,†
PTH (pmol/L)	4.4 ± 1.5	3.6 ± 1.9	19.1 ± 18.7*,†	16.8 ± 21.5*,†
FG (mmol/L)	5.1 ± 1.3	7.6 ± 3.2*	4.6 ± 1.2†	5.2 ± 3.3†,‡
FI (pmol/L)	45.1 ± 33.3	47.9 ± 62.6	28.2 ± 47.6	50.1 ± 91.6
HOMA-IR	1.4 ± 1.2	2.8 ± 3.0	0.8 ± 1.4†	1.4 ± 3.3
FFA (mmol/L)	0.5 ± 0.2	0.6 ± 0.4	0.6 ± 0.5	0.7 ± 0.5
Cholesterol (mmol/L)	5.3 ± 0.9	4.9 ± 1.5	4.4 ± 1.1*	4.2 ± 1.3*
HDL (mmol/L)	1.4 ± 0.4	1.2 ± 0.5	1.0 ± 0.5*,†	1.0 ± 0.3*,†
LDL (mmol/L)	3.5 ± 1.1	2.9 ± 0.9*	2.7 ± 0.9*	2.1 ± 1.4*,†
TG (mmol/L)	1.1 ± 0.8	1.4 ± 0.9	1.6 ± 0.9*	1.8 ± 1.4*
Adiponectin (mg/L)	6.8 ± 3.8	4.6 ± 4.8	14.3 ± 15.7*,†	11.4 ± 12.6*,†
Leptin (μg/L)	17.8 ± 25.4	16.8 ± 22.3	11.4 ± 35.1	28.0 ± 54.4†,‡
CRP (mg/L)	2.5 ± 4.4	2.8 ± 3.8	3.5 ± 11.9	6.9 ± 24.7*,†

Values for median ± interquartile range are shown. Parameters were analyzed by Kruskal-Wallis test followed by Bonferroni post hoc analysis.
**P* less than .05 as compared with control/T2DM−, †as compared with control/T2DM+, and ‡as compared with CD/T2DM−.

Table 4
Correlation between AGF (dependent variable) and T2DM and CD adjusted for age and sex (model 1), as well as between AGF (dependent variable) and age, sex, FG, GFR, and BMI (model 2)

Dependent variable: AGF			
Model	Independent variable	β	P
1	Age	0.089	.329
	Sex	0.034	.707
	T2DM	0.243	.009[a]
	CD	−0.200	.027[a]
2	Age	0.138	.128
	Sex	0.044	.624
	FG	0.237	.013[a]
	GFR	0.146	.152
	BMI	0.093	.334

β coefficients and P values are given.
[a] Significant correlation.

3.3. Multivariate regression analyses

Multiple linear regression analysis revealed that CD remained negatively and T2DM remained positively associated with circulating AGF levels after adjustment for age and sex ($P < .05$) (Table 4, model 1). In addition, fasting glucose positively predicted circulating AGF independent of age, sex, GFR, and BMI (Table 4, model 2). The positive association between AGF on one hand and fasting glucose and HOMA-IR on the other hand in controls, as well as between AGF and CRP in CD patients, remained significant in multivariate analyses after adjustment for age and sex (data not shown).

4. Discussion

In the current study, we show for the first time that serum AGF levels are significantly increased in diabetic patients as compared with nondiabetic subjects and that T2DM is a significant independent positive predictor of circulating AGF in multivariate analysis. Along this line, fasting serum glucose is independently and positively associated with AGF concentrations in multivariate analysis when all patients and controls are studied. Convincing evidence has been presented that AGF is a hepatocyte-derived factor that potently antagonizes obesity and insulin resistance [5]. Furthermore, AGF has been suggested as a candidate gene for obesity in intercross experiments using C57BL/6J and 129S1/SvImJ mice on atherogenic diet [12]. Recently, the mechanisms by which AGF influences glucose metabolism have been elucidated in more detail. Thus, Kitazawa and coworkers [13] present convincing evidence that AGF suppresses glucose production in hepatocytes in a concentration-dependent manner through reduced expression of glucose-6-phosphatase at both the transcriptional and translational level. Furthermore, phosphatidylinositol 3-kinase– and protein kinase B–dependent nuclear export of forkhead box class O1 appears to mediate this effect [13]. Taking these results into consideration, up-regulation of AGF in T2DM might be a compensatory mechanism that partly limits hyperglycemia in humans. Furthermore, it is well possible that AGF antagonizes insulin resistance before the onset of T2DM but is no longer an effective antagonist after the disease is manifest. In addition or alternatively, AGF resistance might be found in T2DM, leading to compensatory up-regulation of this liver-secreted factor. This mechanism would be reminiscent of hyperinsulinemia and hyperleptinemia that are a consequence of increased production in compensation for obesity-associated resistance to insulin and leptin [14]. Here, further studies are needed to investigate whether subjects and animal models with T2DM exhibit decreased AGF sensitivity and impaired receptor or postreceptor signaling in its target tissues. Clearly, more work is needed to better define the physiologic significance of increased AGF levels in T2DM.

In the current study, we show for the first time that circulating AGF is decreased in CD patients as compared with controls with a GFR greater than 50 mL/min. Furthermore, CD remains a significant negative predictor of serum AGF concentrations independent of T2DM in multivariate analysis. These results indicate that renal excretion is not a significant route by which physiologic AGF levels are maintained. Furthermore, our findings suggest that markers of renal function should always be included in studies concerning AGF physiology and regulation. It should be elucidated in future studies by which mechanism impaired renal function might contribute to decreased AGF serum levels. Furthermore, it needs to be tested in additional studies whether inflammatory status contributes to AGF regulation in CD patients.

Some limitations of the present study have to be pointed out: First, cross-sectional data are presented; and therefore, temporality cannot be established. Furthermore, the sample size is relatively small. Moreover, disease may be responsible for the findings via biological mechanisms but also via confounding by other disease-related traits.

Taken together, our results suggest that renal dysfunction is negatively and T2DM is positively associated with AGF serum levels. Further studies are needed to better elucidate the physiologic significance of circulating AGF in human disease.

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References

[1] Fasshauer M, Paschke R. Regulation of adipocytokines and insulin resistance. Diabetologia 2003;46:1594-603.

[2] Trujillo ME, Scherer PE. Adipose tissue–derived factors: impact on health and disease. Endocr Rev 2006;27:762-78.
[3] Hotamisligil GS, Shargill NS, Spiegelman BM. Adipose expression of tumor necrosis factor-alpha: direct role in obesity-linked insulin resistance. Science 1993;259:87-91.
[4] Kharitonenkov A, Shiyanova TL, Koester A, et al. FGF-21 as a novel metabolic regulator. J Clin Invest 2005;115:1627-35.
[5] Oike Y, Akao M, Yasunaga K, et al. Angiopoietin-related growth factor antagonizes obesity and insulin resistance. Nat Med 2005;11:400-8.
[6] 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.
[7] 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.
[8] 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.
[9] 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.
[10] Matthews DR, Hosker JP, Rudenski AS, et al. Homeostasis model assessment: insulin resistance and beta-cell function from fasting plasma glucose and insulin concentrations in man. Diabetologia 1985;28:412-9.
[11] Kratzsch J, Berthold A, Lammert A, et al. A rapid, quantitative immunofunctional assay for measuring human leptin. Horm Res 2002; 57:127-32.
[12] Su Z, Korstanje R, Tsaih SW, Paigen B. Candidate genes for obesity revealed from a C57BL/6J x 129S1/SvImJ intercross. Int J Obes (Lond) 2008;32:1180-9.
[13] Kitazawa M, Ohizumi Y, Oike Y, Hishinuma T, Hashimoto S. Angiopoietin-related growth factor suppresses gluconeogenesis through the Akt/forkhead box class O1-dependent pathway in hepatocytes. J Pharmacol Exp Ther 2007;323:787-93.
[14] Zhang Y, Scarpace PJ. The role of leptin in leptin resistance and obesity. Physiol Behav 2006;88:249-56.