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Short Communication

GlycA, a biomarker of inflammatory glycoproteins, is more closely related to the leptin/adiponectin ratio than to glucose tolerance status

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ABSTRACT

Objectives: Plasma GlycA is a recently developed biomarker whose nuclear magnetic resonance signal originates from glycosylated acute-phase proteins. The aim of our study was to determine potential relationships between GlycA and adiposity, insulin resistance ($HOMA_{ir}$), high sensitive C-reactive protein (hs-CRP), leptin, adiponectin, and the leptin/adiponectin ratio, and to test whether GlycA is elevated in subjects with impaired fasting glucose (IFG) and type 2 diabetes mellitus (T2DM).

Design and methods: Plasma GlycA, hs-CRP, leptin, adiponectin, the leptin/adiponectin ratio, and insulin resistance ($HOMA_{ir}$) were measured in 103 fasting subjects (30 with normal fasting glucose, 25 with IFG and 48 with T2DM).

Results: In all subjects combined, plasma GlycA was correlated positively with body mass index (BMI), $HOMA_{ir}$, hs-CRP, leptin and the leptin/adiponectin ratio, and inversely with adiponectin ($p < 0.05$ to $p < 0.001$). GlycA did not significantly vary according to glucose tolerance category ($p = 0.060$). GlycA was related positively to the leptin/adiponectin ratio ($p = 0.049$), independent of BMI ($p = 0.056$) and $HOMA_{ir}$ ($p = 0.50$).

Conclusions: High plasma GlycA reflects a pro-inflammatory state. Adipose tissue-associated inflammatory processes could contribute to increased circulating levels of glycosylated acute-phase proteins.

Introduction

Protein glycosylation, i.e. the enzymatic process whereby a glycan (carbohydrate) moiety is linked to a protein, is influenced by many biological processes including inflammation [1]. Proton nuclear magnetic resonance (NMR) spectroscopy has the ability to detect signals from circulating glycoproteins [2]. Interestingly, the NMR-based glycoprotein biomarker, designated GlycA, which captures both the protein levels and enhanced glycosylation states of the most abundantly expressed acute-phase proteins, has been shown to predict the development of cardiovascular disease (CVD), as well as the progression to type 2 diabetes mellitus (T2DM) in women [3,4].

Inflammatory processes are characterized by enhanced acute phase protein secretion and glycosylation [5]. Accordingly, a positive relationship of GlycA with high-sensitive C-reactive protein (hs-CRP) has been observed [2,3], but insights into the relationships between GlycA and other cardiometabolic risk factors are still limited. Among adipokines, leptin and adiponectin play key roles in adipocyte differentiation and inflammation [6,7]. The leptin/adiponectin ratio is a determinant of insulin resistance in non-diabetic individuals, highlighting the contribution of adipose tissue dysfunction to the pathogenesis of diminished insulin action [8]. Furthermore, a high leptin/adiponectin ratio may confer increased intima media thickness and predict incident CVD [9–11]. Given that GlycA may predict CVD and progression to T2DM, we hypothesized that GlycA levels may be related to disturbances in pro- and anti-inflammatory adipokines. Therefore, the aim of this study was to interrogate potential relationships of GlycA between and hs-CRP, adiposity, insulin resistance, leptin, adiponectin and the leptin/adiponectin ratio. Second, we tested whether plasma GlycA is elevated in subjects with varying degrees of glucose intolerance.

Materials and methods

Subjects

The medical ethics committee of the University Medical Center Groningen approved the study protocol. Participants with and without T2DM were aged >18 years and were recruited by advertisement in local newspapers. Written informed consent was obtained. T2DM had been diagnosed previously (fasting plasma glucose ≥7.0 mmol/L; non-fasting plasma glucose ≥11.1 mmol/L). In non-diabetic subjects,

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glucose tolerance status was classified as normal fasting glucose (NFG; plasma glucose <5.6 mmol/L) or impaired fasting glucose (IFG; plasma glucose ≥5.6 and ≤6.9 mmol/L), according to NCEP-ATPIII criteria. T2DM subjects using insulin, current smokers and subjects who used lipid lowering drugs were excluded, as were subjects with a history of CVD, chronic kidney disease, liver function abnormalities or thyroid dysfunction. Subjects using metformin, sulfonylurea or antihypertensive medication were allowed to participate. Blood pressure was measured after 15 min rest at the left arm in sitting position using a sphygmomanometer. Mean arterial pressure was calculated as 1 / 3 × systolic blood pressure + 2 / 3 × diastolic blood pressure. Body mass index (BMI in kg/m^2) was calculated as weight divided by height squared. Homeostasis model assessment of insulin resistance ($HOMA_{ir}$) was used to estimate insulin resistance (fasting insulin (mU/L) × fasting glucose (mmol/L) / 22.5).

Laboratory methods

Venous blood was collected in EDTA containing tubes. Plasma was stored at −80 °C until analysis. Plasma glucose was measured shortly after blood collection with an APEC glucose analyzer. Total cholesterol and triglycerides were measured by routine enzymatic methods. HDL cholesterol was assayed by a homogeneous enzymatic colorimetric test. NMR spectra were obtained from plasma samples using the NMR Profiler [12]. NMR signal amplitudes originating from the *N*-acetyl methyl group protons of the *N*-acetylglucosamine moieties located on the bi-, tri-, and tetra-antennary branches of plasma proteins, predominantly α1-acid glycoprotein, haptoglobin, α1-antitrypsin, α1-antichymotrypsin, and transferrin, were used to calculate the GlycA concentration (μmol/L of *N*-acetyl methyl groups) [2,3]. The intra- and inter-assay coefficients of variation (CV) were 1.9% and 2.6%, respectively [2]. Leptin and total adiponectin were assayed as described [13]. Intra-assay and inter-assay CVs were <6% and <8%, respectively. Insulin, hs-CRP and glycated hemoglobin (HbA_{1c}) were determined as described [13].

Statistical analysis

SPSS (version 20.0, SPSS Inc. Chicago, IL, USA) was used for data analysis. Results are expressed as mean ± SD or as median (interquartile range). Because of skewed distributions, logarithmically transformed values of triglycerides, insulin, $HOMA_{ir}$, hs-CRP, leptin, adiponectin, and the leptin/adiponectin ratio were used. Differences in continuous variables according to glucose tolerance status category were assessed by one-way analysis of variance (ANOVA) with Bonferroni correction for multiple comparisons. Differences in proportions in categorical variables between the glucose tolerance groups were assessed by χ^2-analysis. The relationship of GlycA and hs-CRP with T2DM was also determined using receiver operating characteristic (ROC) analysis, presented as the area under the curve (AUC) with 95% confidence intervals (CI). Univariate relationships were assessed with Pearson correlation coefficients. Partial correlation coefficients were calculated after adjustment for glucose tolerance category, age and sex. Multivariable linear regression analyses were carried out to disclose the independent contribution of variables to GlycA and hs-CRP. Two-sided *p*-values <0.05 were considered significant.

Results

One hundred and three subjects participated, of whom 30 were classified with NFG and 25 with IFG (Table 1). Of the 48 subjects with T2DM, 11 used metformin, 9 used sulfonylurea and 15 used both drugs. Antihypertensive medication (mostly angiotensin-converting enzyme inhibitors, angiotensin II receptor blockers, diuretics and β-blockers, alone or in combination) were used by 23 T2DM subjects. Glucose lowering drugs and antihypertensive medication were not used by non-diabetic subjects. One postmenopausal woman with T2DM, 1 postmenopausal woman with IFG 1, and 1 premenopausal woman with NFD used estrogens. Other medications were not taken.

T2DM subjects were older, had higher blood presssure, were more obese and more insulin resistant compared to NFG and IFG subjects (Table 1). There were no gender differences between the groups. Total cholesterol did not differ between the groups, but triglycerides were higher and HDL cholesterol was lower in T2DM than in IFG and NFG subjects. Leptin and the leptin/adiponectin ratio were higher, whereas adiponectin was lower in T2DM compared to IFG and NFG subjects. hs-CRP was higher in T2DM than in IFG subjects. The difference in GlycA between the 3 glucose tolerance groups was not significant. There was also no difference in GlycA between the glucose tolerance groups after age and sex adjustment ($p > 0.30$). However, the AUC of the ROC curve for GlycA with T2DM vs. no T2DM as the outcome was significant (0.624 (95% CI), 0.515–0.733; $p = 0.030$). For comparison, the AUC for hs-CRP with T2DM was 0.662 (95% CI, 0.558–0.766; $p = 0.005$). For GlycA, equal sensitivity and specificity (0.574) were reached

Table 1
Clinical characteristics, plasma glucose, insulin, insulin resistance, plasma lipoproteins, adipokines, high sensitivity C-reactive protein and GlycA in subjects with normal fasting glucose (n = 30), impaired fasting glucose (n = 25) and type 2 diabetes mellitus (n = 48).

	Normal fasting glucose (n = 30)	Impaired fasting glucose (n = 25)	Type 2 diabetes mellitus (n = 48)	*p*-Value*
Age (years)	52 ± 10[b]	56 ± 9	59 ± 9	0.003
Sex (men/women)	11/19	11/14	28/20	0.15
MAP (mm Hg)	99.3 ± 15.2[b]	96.9 ± 10.9[c]	108.7 ± 10.4	<0.001
BMI (kg/m^2)	25.9 ± 4.6[c]	25.0 ± 3.0[c]	30.7 ± 4.5	<0.001
Waist circumference (cm)	85.2 ± 13.0[c]	87.2 ± 10.5[c]	106.7 ± 12.6	<0.001
Fasting glucose (mmol/L)	5.2 ± 0.3[c]	6.1 ± 0.4[c]	9.4 ± 2.3	<0.001
HbA1c (mmol/mol)	34 ± 3[c]	33 ± 0.3[c]	51 ± 8	<0.001
Fasting insulin (mU/L)	6 (4–9)[c]	6 (5–8)[c]	12 (9–18)	<0.001
$HOMA_{ir}$ (mU × mmol/L^2 × 22.5)	1.4 (1.0–2.0)[c]	1.7 (1.2–2.3)[c]	5.7 (3.5–7.7)	<0.001
Total cholesterol (mmol/L)	5.7 ± 1.1	5.8 ± 0.8	5.5 ± 0.9	0.22
HDL cholesterol (mmol/L)	1.5 ± 0.4[b]	1.6 ± 0.4[c]	1.2 ± 0.3	<0.001
Triglycerides (mmol/L)	1.33 (0.85–2.03)[b]	1.34 (0.92–1.88)[b]	1.94 (1.49–2.54)	0.001
Leptin (μg/L)	6.8 (3.2–22.7)[a]	5.9 (3.3–23.7)[a]	14.7 (6.8–31.9)	0.01
Adiponectin (mg/L)	22.7 (15.0–48.1)[b]	21.3 (16.2–40.7)[b]	14.4 (9.5–26.7)	<0.001
Leptin/adiponectin ratio (μg/mg)	0.34 (0.11–0.74)[c]	0.31 (0.13–0.67)[c]	0.97 (0.39–2.13)	<0.001
GlycA (μmol/L)	373 ± 48	364 ± 60	395 ± 52	0.06
hs-CRP (mg/L)	1.81 (0.59–2.92)	0.85 (0.47–1.90)[b]	2.19 (1.36–4.20)	0.002

Data in mean ± SD or in median (interquartile range). BMI: body mass index; HbA1c: glycated hemoglobin; HDL: high density lipoproteins; $HOMA_{ir}$: homeostasis model assessment of insulin resistance; hs-CRP: high sensitive C-reactive protein; MAP: mean arterial pressure. [a]$p < 0.05$ vs. subjects with type 2 diabetes mellitus; *p*-value*: *p*-value by analysis of variance; [b]$p < 0.01$ vs. subjects with type 2 diabetes mellitus; [c]$p < 0.001$ vs. subjects with type 2 diabetes mellitus.

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