Journal of Clinical Lipidology (2016) 10, 512–518

Journal of Clinical Lipidology

# Higher circulating GlycA, a pro-inflammatory glycoprotein biomarker, relates to lipoprotein-associated phospholipase $A_2$ mass in nondiabetic subjects but not in diabetic or metabolic syndrome subjects

**Eke G. Gruppen***, **Margery A. Connelly, PhD, Robin P. F. Dullaart, MD, PhD**

*Department of Endocrinology, University Medical Center Groningen, University of Groningen, Groningen, The Netherlands (Drs Gruppen and Dullaart); and LabCorp, Raleigh, NC, USA (Dr Connelly)*

**KEYWORDS:**
Diabetes mellitus;
Glycoproteins;
GlycA;
High-sensitivity C-reactive protein;
Lipoprotein-associated phospholipase $A_2$;
Metabolic syndrome

**BACKGROUND:** Lipoprotein-associated phospholipase $A_2$ (Lp-$PLA_2$) is a cardiovascular risk marker, which is in part complexed to low-density lipoproteins, where it exerts pro-inflammatory properties. GlycA is a pro-inflammatory proton nuclear magnetic resonance spectroscopy biomarker whose signal originates from a subset of *N*-acetylglucosamine residues on the most abundant glycosylated acute-phase proteins.

**OBJECTIVE:** We compared plasma GlycA and Lp-$PLA_2$ mass between subjects without type 2 diabetes mellitus (T2DM) or the metabolic syndrome (MetS) and subjects with T2DM and/or MetS. We also tested the relationship of GlycA with Lp-$PLA_2$ in each group.

**METHODS:** Plasma GlycA, Lp-$PLA_2$ mass, high-sensitivity C-reactivity protein (hsCRP) and lipids were measured in 40 subjects with neither T2DM nor MetS (group 1) and in 58 subjects with T2DM and/or MetS (group 2).

**RESULTS:** GlycA and hsCRP were higher ($P < .01$ for each), whereas Lp-$PLA_2$ was lower in group 2 vs group 1 ($P < .001$). GlycA was positively related to hsCRP in each group ($P < .001$). In contrast, GlycA was correlated positively with Lp-$PLA_2$ in group 1 ($r = 0.384$, $P = .015$), but not in group 2 ($r = 0.045$; $P = .74$; interaction term for difference: $P = .059$). Although Lp-$PLA_2$ was correlated positively with non–high-density lipoprotein cholesterol and low-density lipoprotein cholesterol in each group ($P \leq .02$), its inverse relationship with high-density lipoprotein cholesterol in group 1 ($r = -0.381$, $P = .013$) was absent in group 2 ($r = -0.101$, $P = .42$).

**CONCLUSIONS:** A pro-inflammatory glycoprotein biomarker, GlycA, is higher in subjects with either T2DM, MetS, or both. The normally present positive relationship of GlycA with Lp-$PLA_2$ is blunted in subjects with T2DM and/or MetS.



* Corresponding author. Department of Endocrinology, University Medical Center Groningen, University of Groningen, P.O. Box 30.001, Groningen 9700 RB, The Netherlands.

E-mail address: e.g.gruppen@umcg.nl

Submitted August 5, 2015. Accepted for publication November 12, 2015.

1933-2874/
http://dx.doi.org/10.1016/j.jacl.2015.11.009

## Introduction

It is well recognized that inflammatory processes are intricately involved in the development of atherosclerosis.[1,2] Lipoprotein-associated phospholipase $A_2$ (Lp-$PLA_2$), also known as platelet-activating factor acetylhydrolase, is secreted by inflammatory cells in the arterial wall.[2] Lp-$PLA_2$ is able to hydrolyse low-density lipoprotein (LDL)-derived oxidized phospholipids, which results in the generation of oxidized free fatty acids, fatty acid hydroperoxides, and lysophospholipids.[2] It is likely that Lp-$PLA_2$ has predominant pro-inflammatory effects and plays a pathogenetic role in coronary artery plaque vulnerability.[2–4] Plasma Lp-$PLA_2$ relates positively to carotid artery intima media thickness, an established marker of subclinical atherosclerosis.[5] Importantly, 2 meta-analyses have demonstrated that plasma Lp-$PLA_2$ mass and activity predict incident cardiovascular disease (CVD), even independent of established risk factors.[6,7] Lp-$PLA_2$ is to a major extent complexed to LDL, as supported by a decrease in plasma Lp-$PLA_2$ in response to pharmacologic and non-pharmacologic maneuvers that lower LDL cholesterol,[8,9] but is to a variable extent also associated with high-density lipoproteins (HDLs).[2]

Protein glycosylation, ie, the enzymatic process whereby a glycan (polysaccharide) moiety is added to a protein, is affected by a number of biological processes including inflammation.[10,11] Many circulating proteins are *N*-linked glycoproteins.[12] During an acute-phase response, their glycan moieties are altered consequent to changes in the dynamics between glycosyltransferases and hydrolases.[10] Proton nuclear magnetic resonance (NMR) spectroscopy has the ability to detect circulating glycoproteins by capturing the NMR signal from the *N*-acetyl methyl protons on the carbohydrate side chains.[12,13] A high throughput NMR-based assay has been developed to quantify this NMR signal in plasma samples. This so-called GlycA signal predominantly comprises several major acute-phase proteins, ie, α1-acid glycoprotein, haptoglobin, α1-antitrypsin, α1-antichymotrypsin and transferrin, with specific glycan structures being preferentially detected.[12] Robust positive correlations of GlycA with high-sensitivity C-reactive protein (hsCRP) have been demonstrated recently, indicating that GlycA can be regarded as a pro-inflammatory glycoprotein biomarker.[12–14] Interestingly, GlycA may confer increased CVD risk, even when hsCRP and established risk factors are taken into account.[13] GlycA may also predict the development of type 2 diabetes mellitus (T2DM).[15]

Given the role of Lp-$PLA_2$ in stimulating pro-inflammatory cytokines, such as interleukin-1β (IL-1β) and interleukin-6 (IL-6), and the importance of these cytokines for protein glycosylation,[16–19] it is plausible to hypothesize that higher plasma Lp-$PLA_2$ may coincide with higher GlycA levels. In view of the proposed contribution of GlycA to diabetes prediction[15] and the association of higher GlycA levels with the metabolic syndrome (MetS),[14] it is also relevant to test whether the possible relationship of plasma Lp-$PLA_2$ with GlycA varies according to diabetes and MetS status.

Against this background, we initiated the present study to determine: (1) the extent to which GlycA is related to plasma Lp-$PLA_2$ and (2) whether such a relationship is different in subjects with T2DM and/or MetS compared with nondiabetic subjects without MetS.

## Materials and methods

### Participants

The study was performed in a university hospital setting. Participants (aged >18 years) were of Caucasian descent. Participants were recruited by advertisement in local newspapers and provided written informed consent. The medical ethics committee of the University Medical Center Groningen, the Netherlands, approved the study. Subjects with and without T2DM and with and without MetS participated. T2DM had been previously diagnosed by primary care physicians using guidelines from the Dutch College of General Practitioners (fasting plasma glucose ≥ 7.0 mmol/L and/or non-fasting plasma glucose ≥ 11.1 mmol/L[20]). MetS was defined according to the revised NCEP-ATP III criteria.[21] Three or more of the following criteria were required for categorization of subjects with MetS: waist circumference >102 cm for men and >88 cm for women; hypertension (blood pressure ≥ 130/85 mm Hg or use of antihypertensive drugs); fasting plasma triglycerides ≥ 1.70 mmol/L; HDL cholesterol < 1.0 mmol/L for men and <1.3 mmol/L for women; fasting glucose ≥ 5.6 mmol/L. Based on these criteria, the participants were divided into 2 groups: the first group consisted of subjects without T2DM and without MetS (group 1), whereas the second group consisted of subjects with either T2DM, MetS, or both (group 2). Diabetic subjects who were treated with metformin and/or sulfonylurea were eligible. Subjects using insulin and subjects using lipid-lowering drugs were excluded. The use of antihypertensive medication was allowed. Further exclusion criteria were clinically manifest CVD, renal insufficiency (estimated glomerular filtration rate < 60 mL/min/1.73 $m^2$ and/or proteinuria), thyroid disorders, liver disease, current smoking, and pregnancy.

Physical examination did not reveal cardiac or pulmonary abnormalities. All participants were studied after an overnight fast. Body mass index (BMI) was calculated as weight divided by height squared (in kg/$m^2$). Waist circumference was measured at the midpoint between the 10th rib and the iliac crest.

### Laboratory analyses

Venous blood samples were collected into EDTA-containing tubes (1.5 mg/mL) for the measurement of plasma lipids and apolipoproteins. Plasma was prepared by