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Circulating IGFBP-2 levels are incrementally linked to correlates of the metabolic syndrome and independently associated with VLDL triglycerides☆

Sophie Carter [a, b, 1], Zhuo Li [a, 1, 2], Isabelle Lemieux [a], Natalie Alméras [a, c], Angelo Tremblay [a, c], Jean Bergeron [d], Paul Poirier [a, b], Yves Deshaies [a, e], Jean-Pierre Després [a, c], Frédéric Picard [a, b, *]

[a] Centre de recherche de l'Institut universitaire de cardiologie et de pneumologie de Québec, Québec, QC, Canada
[b] Faculty of Pharmacy, Université Laval, Québec, QC, Canada
[c] Department of Kinesiology, Faculty of Medicine, Université Laval, Québec, QC, Canada
[d] Lipid Research Center, CHU de Québec Research Center, Québec, QC, Canada
[e] Department of Medicine, Faculty of Medicine, Université Laval, Québec, QC, Canada

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ABSTRACT

Objective: To assess whether plasma IGFBP-2 is independently associated with components of the lipoprotein-lipid profile and to suggest a cutoff value that could identify subjects with the features of the metabolic syndrome. **Methods**: In this cross-sectional study, 379 Caucasian men from the general population and covering a wide range of BMI were recruited through the media. Subjects with type 2 diabetes, BMI values > 40 kg/m^2, or taking medication targeting glucose or lipid metabolism or blood pressure were excluded. Anthropometric data were collected and plasma IGFBP-2 concentrations, glucose tolerance and an extensive plasma lipid profile were determined after an overnight fast. **Results**: Subjects with low IGFBP-2 levels were characterized by increased fat mass ($p < 0.0001$), impaired insulin sensitivity ($p < 0.0001$) and higher plasma triglyceride (TG) levels ($p < 0.0001$). When divided into 6 quantiles, only subjects with the highest IGFBP-2 levels (>221.5 ng/mL) did not meet the NCEP ATP III criteria for the clinical diagnosis of the metabolic syndrome. In addition, circulating IGFBP-2 levels were significantly associated with VLDL-TG ($r = -0.51$, $p < 0.0001$) and HDL-C ($r = -0.27$, $p < 0.0001$) levels. After adjustments, plasma IGFBP-2 was found to be independently associated with VLDL-TG levels but not with HDL-C concentrations. **Conclusions**: In our cohort, IGFBP-2 levels <221.5 ng/mL are incrementally associated with a detrimental plasma lipoprotein-lipid profile. After adjustment for covariates, IGFBP-2 remained independently associated with VLDL-TG but not HDL-C levels. This study supports further investigations in other populations and validation of IGFBP-2 as a biomarker of early dyslipidemia.

1. Introduction

Dyslipidemia often precedes the appearance of type 2 diabetes by several years. This indicates that dysfunctions in lipid metabolism are one of the first elements leading to cardiovascular complications in these patients. It is now recognized that the various components of diabetic dyslipidemia are not isolated abnormalities but are metabolically linked to each other and are partly driven by the hepatic overproduction of large triglyceride (TG)-rich very low-density lipoproteins (VLDL) [1]. This overproduction seems to trigger changes in intravascular lipoprotein metabolism that are associated with an increased cardiovascular

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* Corresponding author. Institut universitaire de cardiologie et de pneumologie de Québec, Y3106 Pavillon Marguerite-d'Youville, 2725 Chemin Ste-Foy, Québec, QC G1V 4G5, Canada.
E-mail address: frederic.picard@criucpq.ulaval.ca (F. Picard).
[1] These authors have contributed equally to the work.
[2] Current address: Endocrinology and Metabolism department, The First Hospital of Jilin University, Jilin, Changchun 130021, China.

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risk [i.e. smaller low-density lipoprotein (LDL) and decrease in size and number of high-density lipoprotein (HDL) particles] [2].

Fat accretion in both hepatic and intra-abdominal (or visceral) adipose tissues has been proposed to be involved in the development of dyslipidemia [3,4]. Studies conducted in humans showed that visceral adipose tissue and liver fat accumulation are both associated with impaired glucose tolerance, insulin resistance and increased VLDL secretion [2,5,6]. These metabolic alterations also influence the production of adipokines and hepatokines [7]. Pro-inflammatory cytokines such as C-reactive protein (CRP), IL-6 and tumor necrosis factor (TNF)-α are upregulated in visceral obesity, which triggers low grade inflammation, a hallmark of obesity.

Circulating insulin-like growth factor-binding protein (IGFBP)-2, can modulate the effects of IGF on metabolism [8] by influencing the binding of IGF to IGF receptors [9] and through IGF-independent effects mediated via integrin signaling [10,11]. In humans, low IGFBP-2 levels have been associated with conditions of obesity and insulin resistance [8,12–15]. In addition, global estimates of the metabolic syndrome [16] and cardiovascular risk markers [17] negatively correlate with circulating IGFBP-2 levels. In line with this concept, IGFBP-2 has been shown to protect against age- and diet-induced insulin resistance when overexpressed in rodents using either transgenic [18,19] or adenoviral techniques [20].

IGFBP-2 has been negatively associated with circulating TG and cholesterol levels [13,16,17,21]. However, only a limited number of studies have investigated the impact of IGFBP-2 on lipid metabolism [21]. In order to dissociate the interaction of IGFBP-2 with lipids beyond its relation with insulin sensitivity and fat mass, the present study aimed at exploring the associations between circulating levels of IGFBP-2 and several features of the plasma lipoprotein/lipid profile in a cohort of men covering a large range of adiposity. Since features of the metabolic syndrome have been found to be associated with IGFBP-2 levels, this study also aimed at suggesting a cut-off value of circulating IGFBP-2 that could help identify subjects with the features of the metabolic syndrome.

2. Materials and methods

2.1. Study design

This cross-sectional study was performed in a sample of 379 asymptomatic Caucasian adult men, between the ages of 20–65 years from the Québec City metropolitan area. Subjects were recruited by solicitation in the media and selected to cover a wide range of body fatness values. Subjects with type 2 diabetes, body mass index (BMI) values > 40 kg/m^2, or taking medication targeting glucose or lipid metabolism or blood pressure were excluded. Informed written consent was obtained from all participants prior to their inclusion in the study, which was approved by the Medical Ethics Committees of Université Laval and the Institut universitaire de cardiologie et de pneumologie de Québec.

2.2. Anthropometric measurements and computed tomography

Height, weight, waist circumference and fat mass were measured according to standardized procedures [22,23]. Abdominal visceral and subcutaneous fat areas were assessed by computed tomography. Measurements of abdominal adipose tissue depots were obtained at the L4–L5 intervertebral space with subjects lying in the supine position with both arms stretched above the head as previously reported [24]. Visceral adipose tissue area was determined by delineating the middle of the muscle wall surrounding the abdominal cavity. The subcutaneous adipose tissue corresponded to the amount of fat located from the skin to the middle of the muscle wall surrounding the abdominal cavity. Adipose tissue areas (cm^2) were computed using an attenuation range of −190 to −30 Hounsfield units as previously described [24].

2.3. Plasma lipoprotein-lipid profile

Blood samples were collected from the antecubital vein after a 12 h overnight fast for the measurement of plasma lipid and lipoprotein levels. TG and cholesterol levels were determined in plasma and lipoprotein fractions using Technicon RA-500 (Bayer Corporation, Tarrytown, NY, USA); enzymatic reagents were obtained from Randox (Crumlin, UK). TG-rich lipoproteins (VLDL) were first removed by ultracentrifugation [25]. The HDL fraction was obtained after precipitation of the remaining apolipoprotein (apo) B-containing lipoproteins, primarily LDL, in the infranatant (density > 1.006 g/mL) with heparin and $MnCl_2$ [26]. Cholesterol and TG concentrations in the infranatant were measured before and after the precipitation step, allowing the calculation of LDL-C levels [27]. Plasma apo B and A1 concentrations were measured according to standardized procedures [28,29].

2.4. LDL size

The LDL peak particle diameter was obtained from non-denaturing 2–16% polyacrylamide gradient gel electrophoresis as previously described [30]. LDL particle size was extrapolated from the relative migration of four plasma standards of known diameters. The estimate diameter for the major peak in each scan was identified as the LDL peak particle size. The relative proportion of LDL with a diameter smaller than 255 Å was determined by computing the relative area of the densitometric scan corresponding to LDL particles smaller than 255 Å. Sudan black staining was assumed to closely reflect the cholesterol distribution among LDL particles of different sizes [31]. The absolute concentration of cholesterol among particles smaller than 255 Å was calculated by multiplying plasma LDL-C levels by the relative proportion of LDL with a diameter smaller than 255 Å. A similar approach was used to assess the relative and absolute concentrations of cholesterol in LDL particles with a diameter greater than 260 Å.

2.5. HDL size

Nondenaturing 4–30% polyacrylamide gel electrophoresis was performed for the measurement of HDL size as previously described [32]. The mean HDL particle size represents the overall distribution of HDL subspecies and was obtained with the migration of lipid-stainable plasma standards of known diameters [35].

2.6. Oral glucose tolerance test (OGTT)

After a 12 h overnight fast, participants were subjected to a 75 g oral glucose load. Blood samples were taken at −15, 0, 15, 30, 45, 60, 90, 120, 150 and 180 min for the measurement of plasma glucose and insulin concentrations. The total glucose and insulin area under the curve (AUC) during the OGTT were determined by the trapezoid method between 0 and 180 min. The homeostasis model assessment of insulin resistance (HOMA-IR) was calculated from fasting glucose and insulin values, as previously described [33].

2.7. Determination of cardiometabolic risk markers

Fasting plasma adiponectin, IL-6, TNF-α, alanine transaminase (ALT), leptin and hs-CRP levels were assessed on deeply frozen samples (−80 °C). Adiponectin and leptin concentrations were quantified by ELISA (B-Bridge International, Inc. Sunnyvale, CA).