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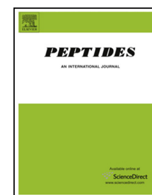

Role of human lipocalin proteins in abdominal obesity after acute pancreatitis

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ABSTRACT

Lipocalin proteins are small regulatory peptides implicated in metabolism, inflammation, and immunity. Although lipocalin proteins have been linked to various clinical conditions, their role in the acute inflammatory setting, such as acute pancreatitis (AP), has only been sparsely investigated. Two members of the lipocalin family, lipocalin-2 (LCN-2) and retinol binding protein −4 (RBP-4), play an important role in obesity and insulin resistance. In this study, we analysed circulating levels of LCN-2 and RBP-4 in 92 individuals after AP, of whom 41 individuals had abdominal obesity and 51 did not. Binary logistic regression analyses were performed to determine whether abdominal obesity was associated with the two lipocalin proteins. Lipocalin-2 was significantly associated with abdominal obesity in the unadjusted model (Odds ratio (OR) = 1.014 [95% confidence interval (CI): 1.000, 1.028], P = 0.05) and after adjusting for patient related (age, ethnicity, and diabetes mellitus) and pancreatitis related (aetiology, severity, recurrence, and duration of AP) characteristics (OR = 1.018 [95% CI: 1.001, 1.036], p = 0.04). Further, the association of LCN-2 with waist circumference was significant in individuals with alcohol aetiology of AP (β=1.082 [95% CI: 1.011, 1.158], p = 0.02]. The association between RBP-4 and abdominal obesity was not significant in both unadjusted and adjusted models. These findings indicate that circulating levels of LCN-2 in patients after AP may play a role in chronic low grade inflammation associated with abdominal adiposity and that alcohol consumption may further exacerbate adipose tissue dysfunction.

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1. Introduction

Visceral fat is more metabolically active than other fat depots and a body of evidence has shown association of abdominal obesity with increased risk of cardiovascular and metabolic disorders [1]. The negative impact of abdominal obesity is also commonly observed in acute inflammatory conditions, such as acute pancreatitis (AP) [2–7], where waist circumference is a better predictor of severity of the disease than body mass index [8]. The increase in incidence of AP over the past decades has coincided with an increasing prevalence of excessive adiposity [9,10]. It is established that chronic low grade inflammation exists in obese individuals [11,12]; however, the underlying pathophysiological mechanisms affecting the adipose tissue in AP have not been fully elucidated [7,13,14].

Among various metabolically active molecules, small molecular lipid chaperones called lipocalin proteins have been implicated in the development of chronic low grade inflammation [15]. Moreover, these proteins, which are strongly linked to cardiovascular and metabolic abnormalities [16,17], are considered as biochemical markers of various clinical conditions, including lipid metabolism disorders, inflammation-related disorders, as well as kidney and liver dysfunctions [18]. Although in general the lipocalin family of proteins, which currently includes 37 proteins [19], are regarded as extracellular carriers for hydrophobic molecules, their widespread expression patterns across different tissues and their specialised structural conformity form the basis for their diverse functions and physiological outcomes.

Lipocalin-2 (LCN-2), a member of the lipocalin superfamily, is released from the adipocytes, neutrophils, macrophages, hepatocytes, and epithelial cells [16,20]. LCN-2 expression in adipose tissue is elevated in obesity [15,16,20,21]. Experimental studies have demonstrated the role of LCN-2 in glucose metabolism, lipid metabolism, insulin resistance, and inflammation [22]. However, in human studies, there is inconsistency in the literature with regards to what drives changes in LCN-2 concentration: while some studies have shown association of LCN-2 with hyperglycemia and insulin

Abbreviations: AP, acute pancreatitis; HOMA-IR, homeostasis model assessment-insulin resistance; LCN-2, lipocalin-2; LPS, lipopolysaccharide; RBP-4, retinol binding protein-4; TNFα, tumour necrosis factor-alpha.

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resistance [16,23], other evidence [24] has not. Another plasma lipocalin, retinol binding protein-4 (RBP-4), is also implicated in the pathogenesis of obesity and insulin resistance [25]. Elevated concentrations of LCN-2 and RBP-4 have been found in pancreatic cancer patients [26] and increased LCN-2 levels have been shown to predict adverse outcomes during the course of AP [27]. However, the role of these lipocalin proteins after AP is not known.

The aim of this study was to investigate the associations between LCN-2, RBP-4, and abdominal obesity in patients after AP; to assess whether this association is influenced by pancreatitis related factors; and to investigate whether glucose metabolism markers, lipid metabolism markers, and adipokines related to insulin sensitivity affect circulating concentrations of lipocalin proteins.

2. Methods

2.1. Study design

This was a cross-sectional follow-up study of patients with AP admitted to Auckland City Hospital. The study was approved by the Health and Disability Ethics Committee (13/STH/182) and the Auckland District Health Board (A+ 6139).

2.2. Study population

Individuals were eligible for the study if they were at least 18 years of age, had primary diagnosis of AP, and provided informed consent. Diagnosis of AP was determined if at least two of the following three criteria were met [28]: (1) serum amylase and/or lipase (three times the upper limit of normal), (2) pain typical of AP, and (3) characteristic findings of AP on computed tomography, magnetic resonance, or ultrasonography. Individuals were excluded if they had chronic pancreatitis, post-endoscopic retrograde cholangio-pancreatography pancreatitis, malignancy, intra-operative diagnosis of pancreatitis, or were pregnant at time of AP or afterwards.

2.3. Variables collected

Abdominal obesity was defined according to the National Cholesterol Education Program Adult Treatment Panel III (ATP III) guidelines for diagnosis of metabolic syndrome as waist circumference ≥102 cm in men and ≥88 cm in women [29]. Waist circumference was measured at the umbilical area, taken over light clothing.

Blood samples were analysed for adipokines, including adiponectin, leptin, resistin; lipocalin proteins including, LCN-2 and RBP-4; lipid metabolism markers, including free fatty acids (FFA), glycerol, total cholesterol, triglycerides, and apolipoprotein B-100 (ApoB100); glucose metabolism markers, including glycated haemoglobin (HBA1c), fasting blood glucose (FBG), and fasting insulin.

Duration from first episode of AP was defined as the time (in months) from the first hospital admission with AP to the time of the study.

Recurrence of AP was defined as admission with one or more episodes of confirmed AP between first admission with AP and study date.

Severity was defined based on the 2012 international classification of AP [30,31].

2.4. Laboratory measurements

The median (IQR) duration of follow up (i.e., the time when blood was collected) was 24 (7–46.5) months. Participants were required to fast for at least eight hours prior to blood collection. Venous blood was collected from all patients in the International Accreditation New Zealand (IANZ) accredited tertiary referral medical laboratory, LabPlus located at Auckland City Hospital. HbA1c was analysed using the boronate affinity chromatography assay (Trinity Biotech). Fasting blood glucose was measured using an enzymatic colorimetric assay (© 2015 F. Hoffmann-La Roche Ltd.). Insulin was measured using chemiluminescence sandwich immunoassay (Roche Products and Roche Diagnostics NZ).

For homeostasis model assessment of insulin resistance (HOMA-IR), FBG (in mmol/l), and fasting insulin (pmol/l) measures were entered into the validated HOMA2 calculator (HOMA2 v2.2.3; Diabetes Trials Unit, University of Oxford) [32].

For analysis of LCN-2, RBP-4, and adipokines (resistin, leptin, and adiponectin), blood was collected into ethylene-diamine-tetra-acetic acid (EDTA) tubes, centrifuged at 4000*g* for 7.5 min at 4° Celsius (C). The separated plasma was aliquoted and subsequently stored at −80° C until further use. Lipocalin-2 and leptin were determined using the MILLIPLEX® MAP Human metabolic magnetic bead panel, based on the Luminex xMAP® (Luminex Corporation, Austin, Texas, USA, 1995) technology. Results were quantified (ng/ml) based on fluorescent receptor signals recorded by the Luminex xPONENT® software (MILLIPLEX® Analyst 5.1). The minimum detection concentration for LCN-2 and leptin were 1.7 pg/ml and 41 pg/ml, respectively. The intra-assay and inter-assay variation was <10% and <15%, respectively. The accuracy of the assay was 91% and the coefficient of variation of standard curve replicates at each dilution level was 1.96%. Adiponectin, RBP-4, resistin, FFA, apolipoprotein B-100 levels were measured using commercial enzyme-linked immunosorbent assay (ELISA) kits, which are in-vitro quantitative sandwich assays with pre-coated specific antibodies for detection of peptide concentration. For each assay, a standard curve was plotted with known concentrations of peptide standard against absorbance to determine the unknown peptide concentration in patient samples. The validity of each assay was established by the quality control values that were within the calculated quality control range. The antibody pair used in the assays were specific to the analysed human proteins. A Rayto Microplate Reader (V-2100C, Santa Fe, Granada, Spain), with an absorbance of 405–630 nm, was used to obtain the results. Glycerol, total cholesterol, and triglycerides were measured using the GM7 Micro-Stat (Analox Instruments Inc., Lunenberg, MA), a multi-assay analyser, as per the manufacturer's instructions.

2.5. Statistical analyses

SPSS for Windows Version 23 (SPSS Inc., Chicago, IL, USA) was used to perform all analyses. Data were presented as median and interquartile range (IQR) or count frequencies.

The statistical analyses were conducted in three stages. First, having met all the statistical assumptions, binary logistic regression analysis was conducted to investigate the association between lipocalin proteins and abdominal obesity. Each lipocalin protein (LCN-2 and RBP-4) was analysed as an independent variable in one unadjusted, and four adjusted models. Model 1 was adjusted for age and ethnicity; model 2–for age, ethnicity, diabetes mellitus; model 3–for aetiology, severity, recurrence, and duration of AP; model 4–for age, ethnicity, diabetes mellitus, aetiology, severity, recurrence, and duration of AP. Given the dependent variable of abdominal obesity had well accepted cut-off values for males and females [29], the adjusted models did not include sex as a covariate. The differences in lipocalin proteins between males and females of the study cohort were evaluated using independent sample *t*-test. Results were reported as odds ratios and 95% confidence intervals (CI). Outliers were excluded to obtain the most robust and conservative output.

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