Nutrition, Metabolism & Cardiovascular Diseases (2019) **29**, 956–964

Available online at www.sciencedirect.com

Nutrition, Metabolism & Cardiovascular Diseases

journal homepage: www.elsevier.com/locate/nmcd

Serum lipid profile as a biomarker of intra-pancreatic fat deposition: A nested cross-sectional study

Ruma G. Singh, Ngoc N. Nguyen, Aya Cervantes, Jaelim Cho, Maxim S. Petrov*

School of Medicine, University of Auckland, Auckland, New Zealand

Received 28 November 2018; received in revised form 7 June 2019; accepted 8 June 2019
Handling Editor: A. Giaccari
Available online 14 June 2019

KEYWORDS
Lipid profile;
Biomarkers;
Intra-pancreatic fat deposition;
Acute pancreatitis;
Fat distribution

Abstract *Background and aims:* The relationship between intra-pancreatic fat deposition (IPFD) and lipid profile has been investigated in individuals with obesity and/or type 2 diabetes, but not in healthy non-obese individuals and those after acute pancreatitis. The aim of the study was to investigate the association between serum lipid profile and IPFD in the latter individuals and to determine the effect of abdominal fat distribution and other covariates.
Methods and results: A total of 90 individuals with a history of acute pancreatitis as well as 23 healthy non-obese individuals participated in the study. Magnetic resonance imaging was used to quantify IPFD and visceral-to-subcutaneous fat volume ratio, followed by fasting state measurement of high-density lipoprotein cholesterol (HDL-C), low-density lipoprotein cholesterol (LDL-C), total cholesterol (TC), TC/HDL-C ratio, and triglycerides. In healthy non-obese individuals, IPFD was not significantly associated with any of the studied markers. In individuals after acute pancreatitis, IPFD was significantly associated with triglycerides in both unadjusted ($\beta = 0.360$; 95% CI, 0.090–0.629; $p = 0.009$) and adjusted models, with a β-coefficient of 0.280 [(95% CI, 0.016–0.545); $p = 0.038$] in the most adjusted model. Also, IPFD was significantly associated with TC/HDL-C ratio in both unadjusted ($\beta = 0.336$; 95% CI, 0.045–0.626; $p = 0.024$) and adjusted models, with a β-coefficient of 0.375 [(95% CI, 0.090–0.660); $p = 0.010$] in the most adjusted model. Multiple regression yielded triglycerides, but not TC/HDL-C ratio, as a significant marker of IPFD in individuals after acute pancreatitis.
Conclusions: Serum lipid profile is not associated with IPFD in healthy non-obese. Triglycerides, but not other components of lipid profile, is a promising biomarker for IPFD in individuals following acute pancreatitis.

Introduction

Intra-pancreatic fat deposition (IPFD) gained research attention in the past decade and has now emerged as an important ectopic fat phenotype contributing to increased burden of metabolic and pancreatic diseases. In particular, IPFD is associated with at least two-fold increased risk of diabetes mellitus and metabolic syndrome [1]. Furthermore, IPFD contributes to the pathogenesis of diseases of the exocrine pancreas (such as acute pancreatitis (AP) and pancreatic cancer) [2] and their sequelae (e.g., diabetes of the exocrine pancreas, exocrine pancreatic insufficiency) [3,4]. Magnetic resonance imaging (MRI) is the gold standard for measurement of IPFD, considering its non-ionising nature and high soft tissue contrast output [5].

* Corresponding author. Auckland City Hospital, Room 12.085A, Level 12, Auckland 1023, New Zealand. Fax: +64 9 377 9656.
E-mail address: max.petrov@gmail.com (M.S. Petrov).

https://doi.org/10.1016/j.numecd.2019.06.003

However, it is used infrequently in current routine practice as the MRI acquisition is expensive and the post-processing of images is labour-intensive and time-consuming. Therefore, an inexpensive, accurate, and readily available body fluid-based biomarker of IPFD would be desirable for the purpose of identifying high-risk individuals at an early stage.

Serum lipid profile is one of the most commonly acquired blood parameters in routine healthcare practice. Our 2017 systematic review on biomarkers of IPFD has identified all the published studies that investigated the correlations between IPFD and markers of lipid metabolism [6]. Triglycerides and high-density lipoprotein cholesterol (HDL-C) were investigated in 7 and 5 studies, respectively. They had weighted correlation coefficients with IPFD of 0.38 and −0.33, respectively [6]. Other markers of lipid metabolism, such as total cholesterol (TC), low-density lipoprotein cholesterol (LDL-C), and total cholesterol to HDL-C ratio (TC/HDL-C ratio), were investigated in just 3 studies [7–9]. The above correlations between IPFD and markers of lipid metabolism have been mostly investigated in obese individuals or people with abnormal glucose metabolism but rarely in healthy non-obese individuals. Notably, none of the studies in non-obese populations have accounted for abdominal fat distribution [6]. Further, dyslipidaemia (specifically, high levels of triglycerides) and abdominal adiposity were previously reported to contribute to metabolic derangements after AP [10–15]. However, little is known about the inter-relationships between serum lipid profile, abdominal fat distribution, and IPFD in post-pancreatitis setting.

The aim of the present study was to investigate the association between serum lipid profile and IPFD in individuals with a history of AP and healthy non-obese individuals, and to determine the effect of abdominal fat distribution and other covariates.

Methods

Study design and study population

This was a cross-sectional study nested into the prospective longitudinal study as part of the ARIES project [16,17]. The study was approved by the Health and Disability Ethics Committee. All participants gave written informed consent. Individuals were eligible if they were at least 18 years old and had primary diagnosis of AP, established prospectively at the time of hospitalisation. Diagnosis of AP was determined if at least two of the following three criteria were met: [18].

(i) serum amylase and/or lipase (three times the upper limit of normal),
(ii) pain typical of AP, and
(iii) characteristic findings of AP on computed tomography, MRI, or ultrasonography.

Individuals were excluded from the study if they had chronic pancreatitis; post-endoscopic retrograde cholangiopancreatography pancreatitis; intra-operative diagnosis of pancreatitis; pancreatic lipomatosis or lipomatous pseudohypertrophy; ascites; chronic obstructive pulmonary disease severe enough to limit breath-holding; congenital anomalies of the pancreas; hereditary pancreatitis; autoimmune pancreatitis; cystic fibrosis; pancreatic trauma; surgical, endoscopic, or radiologic interventions involving the pancreas; steroid therapy; malignancy; any metallic foreign body or electronic device implants; cognitive disability; or were pregnant (at the time of AP or afterwards).

Healthy non-obese (body mass index < 30 kg/m^2) individuals aged 18 or above were also recruited into the study if they had no history or symptoms of pancreatic disease or diabetes, no upper abdominal pain or nausea, no family history of pancreatic diseases, diabetes, celiac disease, or cystic fibrosis, no history of acute infectious or inflammatory conditions requiring medical treatment or evaluation in 6 months preceding the study.

Magnetic resonance imaging acquisition and quantification of variables

All participants underwent abdominal MRI scans at the Centre of Advanced MRI (The University of Auckland), using 3.0 Tesla MAGNETOM Skyra MRI scanner (Siemens, Erlangen, Germany). Anthropometric measures (i.e., waist circumference, hip circumference, weight, and height) were recorded prior to MRI acquisition. During the scan, participants were asked to lie down in supine position and hold their breath after exhalation. Axial T1-weighted volumetric interpolated breath-hold examination Dixon sequence was applied with the following parameters: true form abdomen shim mode; FOV, 420 mm; base resolution, 320; TE, 1.27 ms, 2.5 ms; TR, 3.85 ms; flip angle, 9°; pixel bandwidth, 920 Hz; slice thickness, 3 mm. For pancreas scan, a higher base resolution (512) was applied with the following parameters: FOV, 440 mm; TE, 2.46 ms, 3.69 ms; TR, 5.82 ms; flip angle, 9°; slice thickness, 5 mm. Four types of images were generated – in-phase, out-of-phase, fat, and water images. These images were retrieved from the scanner and exported as DICOM files for further analyses.

Intra-pancreatic fat percentage (%) was measured using the 'MR-opsy' technique, as described in detail elsewhere [19–21]. In brief, two candidate slices from a series of 5 mm thick slices were selected and three regions of interest (100 mm^2) were placed in the head, body, and tail regions of the pancreas. Further, a thresholding range of 1%–20% was applied to prevent potential inclusion of non-parenchymal tissues (blood vessels, the main pancreatic duct, visceral fat) within the selected region of interest [20]. Intra-pancreatic fat % was obtained by taking the average of measurements of the two slices. For calculation of visceral-to-subcutaneous fat volume ratio (V/S fat volume ratio), the segmentation of subcutaneous and visceral fat compartments was achieved using the ImageJ software

(National Institutes of Health, USA). A series of abdominal fat-phase images from the second lumbar vertebral level to the fifth lumbar vertebral level was selected for measurement of visceral and subcutaneous fat volumes. Using the threshold function, grayscale pixels were converted into binary images. The free-hand tool was then used to delineate the subcutaneous and visceral fat compartments from the abdominal musculature, followed by pixel content measurement for both the compartments. Non-adipose tissue, abdominal organs, blood vessels were excluded from the measurement of visceral fat. For calculation of volumes, the final step involved summation of the pixel contents of all slices in series and multiplying by the pixel area and slice thickness [22].

Intra-class correlation coefficient (ICC) was calculated to assess the inter-rater reliability of MRI-derived measurements done by two raters independently, blinded to characteristics of study participants. The inter-rater reliability was considered excellent if ICC was more than 0.90 [23]. Average value of two independent MRI measurements was used in statistical analyses.

Laboratory analyses

Fasting venous blood samples were collected from all participants. A tertiary referral medical laboratory (LabPlus) located at Auckland City Hospital measured lipid profile, which included HDL-C, LDL-C, TC, TC/HDL-C ratio, and triglycerides. The same laboratory measured glycated haemoglobin (HbA1c), using the boronate affinity chromatography assay (Trinity Biotech, Wicklow, Ireland) that is certified by the NGSP (National Glycohaemoglobin Standardisation Program) and standardised to the Diabetes Control and Complications Trial reference assay.

Pancreatitis-related factors

Aetiology was categorised into biliary, alcohol-related, or other. Biliary AP was diagnosed using modern radiological modalities (i.e., ultrasonography, computed tomography, or MRI). Acute Physiology and Chronic Health Evaluation II (APACHE II) scores at the time of hospitalisation for AP for all participants were calculated. Recurrence of AP was defined as hospitalisation with one or more recurrent episodes (at least 30 days apart) of confirmed AP between first admission for AP and the study date. Time since AP was defined as the number of months since last admission for AP until the study date.

Statistical analyses

Statistical analyses were performed using SPSS for Windows Version 25 (SPSS Inc., Chicago, IL, USA) and MedCalc Statistical Software version 18 (MedCalc Software bvba, Ostend, Belgium). Data were presented as median and interquartile range (IQR) or count frequency. The student's *t* test and Chi square test were used to investigate the differences between the study groups for continuous and categorical characteristics, respectively. Results were reported as β-coefficients with corresponding 95% confidence intervals (CI). $P < 0.05$ was accepted as statistically significant in all analyses.

Having met all statistical assumptions, regression analyses were conducted in two steps, separately for healthy non-obese individuals and individuals after AP. First, the association between each marker of lipid metabolism (HDL-C, LDL-C, TC, TC/HDL-C ratio, and triglycerides) and IPFD was analysed using linear regression analysis, where intra-pancreatic fat% was the dependent variable in unadjusted and adjusted models. The analyses of healthy non-obese cohort included two adjusted models − model 1 was adjusted for age and sex; model 2 was adjusted for age, sex, V/S fat volume ratio, and HbA1c. The analyses of post-AP cohort, in addition to model 1, included model 2 − adjusted for age, sex, use of lipid-lowering drugs, V/S fat volume ratio, and HbA1c; and model 3 − adjusted for age, sex, use of lipid-lowering drugs, V/S fat volume ratio, HbA1c, APACHE II score, time since AP, aetiology, and recurrence of AP.

Second, collinearity between the studied markers of lipid metabolism and their contribution to the variance in intra-pancreatic fat% (dependent variable) was investigated in multiple variable regression analysis. Presence of collinearity was assessed by variance inflation factor (VIF). Using enter method, the first model was built including all five markers of lipid metabolism, followed by step-wise elimination of a marker with highest VIF or VIF >5, until all independent variables in the model had VIF <5 [24,25]. Further, predictive equations for IPFD were derived from the independent variable (marker of lipid metabolism) that was significantly associated with IPFD and had no evidence of collinearity (VIF < 5) in multiple regression analysis. All equations were built using the same covariates included in the above adjusted models. The overall R^2 metric represented the variance in IPFD explained by the marker of lipid metabolism and other covariates.

Results

Characteristics of participants

A total of 90 individuals after AP were recruited into the study. The median (IQR) age of individuals was 56 (44–66) years and women constituted 36% of the post-AP cohort. Of these, 23 individuals had more than one AP episode, 40 had biliary AP, and the median (IQR) APACHE II score of individuals at hospital admission was 5 (3–8). They were followed up after a median (IQR) of 22 (12–36) months since last episode of AP. Seventeen individuals met the diagnostic criteria for diabetes, of whom 8 had pre-existing type 2 diabetes and 9 had new-onset diabetes after AP. Of the individuals with pre-existing diabetes, 3 were on both insulin therapy and oral glucose-lowering drugs, 3 on oral glucose-lowering drugs only. All the individuals with new-onset diabetes after AP were treatment-naïve (Supplementary Table 1). Twenty-three healthy non-obese individuals were also recruited into the study. The median (IQR) age of individuals was 44 (29–54)

years and women constituted 30% of the healthy cohort. The ICC of intra-pancreatic fat% measurements was 0.98 (Fig. 1). Individuals after AP had a significantly higher IPFD with a median (IQR) of 9.6 (8.8–10.4) % compared with 7.9 (6.4–8.9) % in healthy non-obese individuals ($p < 0.001$). Other characteristics of the study participants are presented in Table 1.

Associations between markers of lipid metabolism and intra-pancreatic fat deposition in healthy non-obese individuals

The median (IQR) levels of markers of lipid metabolism in healthy non-obese individuals were as follows: HDL-C: 1.0 (0.8–1.3) mmol/L; LDL-C: 2.8 (1.9–3.5) mmol/L; TC: 4.5 (3.2–5.5) mmol/L; TC/HDL-C ratio: 4 (3.5–4.8); and triglycerides: 1.0 (0.7–1.9) mmol/L. Intra-pancreatic fat deposition was not significantly associated with the studied markers of lipid metabolism in both adjusted and unadjusted models (Table 2).

Associations between markers of lipid metabolism and intra-pancreatic fat deposition in individuals after acute pancreatitis

The median (IQR) level of triglycerides was 1.6 (1.0–2.5) mmol/L. Intra-pancreatic fat deposition was significantly associated with triglycerides in unadjusted model ($\beta = 0.360$; 95% CI, 0.090–0.629; $p = 0.009$), model 1 ($\beta = 0.347$; 95% CI, 0.081–0.612; $p = 0.010$), model 2 ($\beta = 0.312$; 95% CI, 0.043–0.582; $p = 0.023$), and model 3 ($\beta = 0.280$; 95% CI, 0.016–0.545; $p = 0.038$) (Table 3).

The median (IQR) TC/HDL-C ratio was 3.8 (3.0–4.6). Intra-pancreatic fat deposition was significantly associated with TC/HDL-C ratio in unadjusted model ($\beta = 0.336$; 95% CI, 0.045–0.626; $p = 0.024$), model 1 ($\beta = 0.403$; 95% CI, 0.103–0.702; $p = 0.008$), model 2 ($\beta = 0.392$; 95% CI, 0.095–0.689; $p = 0.010$), and model 3 ($\beta = 0.375$; 95% CI, 0.090–0.660; $p = 0.010$) (Table 3).

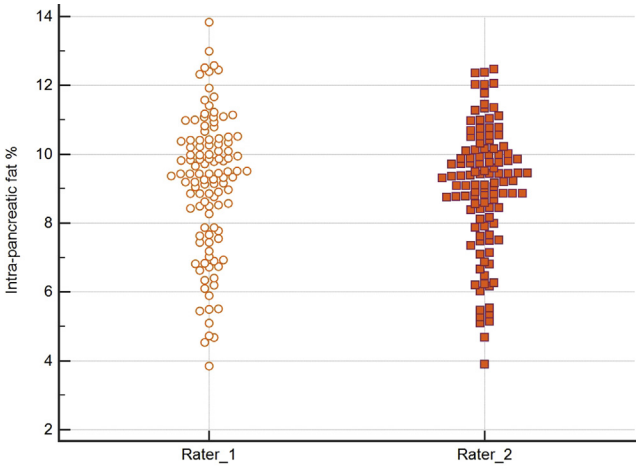

Figure 1 Results of measurements of intra-pancreatic fat % by two independent raters.

Table 1 Characteristics of study participants.

Characteristics	After pancreatitis (n = 90)	Healthy non-obese (n = 23)	*P* value
Age (years)	56 (44–66)	44 (29–54)	**0.007**
Sex			0.645
Men	58 (64)	16 (70)	
Women	32 (36)	7 (30)	
Waist circumference (cm)	99 (88–107)	86 (82–89)	<**0.001**
Hip circumference (cm)	102 (92–112)	85 (80–92)	<**0.001**
Height (cm)	172 (165–180)	173 (165–180)	0.967
Weight (kg)	84 (72–100)	72 (60–84)	**0.001**
Visceral fat volume (L)	1.94 (1.24–2.66)	0.74 (0.61–1.28)	<**0.001**
Subcutaneous fat volume (L)	2.88 (2.09–4.09)	1.94 (1.44–2.65)	<**0.001**
Use of lipid-lowering drugs			<**0.001**
Yes	34 (61)	0 (0)	
No	56 (39)	23 (100)	
Glycated haemoglobin (mmol/mol)	37 (34–40)	33 (30–34)	**0.001**

Data are median (IQR) or number (percentage) of participants. Significant ($p < 0.05$) associations are shown in bold.

The median (IQR) level of TC was 5.0 (4.0–5.8) mmol/L. Intra-pancreatic fat deposition was significantly associated with TC in adjusted model 3 ($\beta = 0.477$; 95% CI, 0.173–0.781; $p = 0.002$), but not in unadjusted or other adjusted models (Table 3).

The median (IQR) levels of HDL-C and LDL-C were 1.3 (1.1–1.6) mmol/L and 2.8 (1.9–3.3) mmol/L, respectively. Intra-pancreatic fat deposition was not significantly

Table 2 Associations between intra-pancreatic fat deposition and markers of lipid metabolism in healthy non-obese individuals.

Markers	Model	β coefficient	95% CI		*P*
			Lower	Upper	
HDL-C (mmol/L)	Unadjusted	−0.272	−2.163	1.620	0.778
	Model 1	−0.524	−2.344	1.295	0.572
	Model 2	−0.067	−1.731	1.597	0.937
LDL-C (mmol/L)	Unadjusted	−0.246	−1.194	0.703	0.612
	Model 1	−0.306	−1.169	0.556	0.486
	Model 2	−0.300	−1.042	0.443	0.429
Total cholesterol (mmol/L)	Unadjusted	−0.184	−0.844	0.475	0.584
	Model 1	−0.215	−0.814	0.385	0.483
	Model 2	−0.192	−0.732	0.347	0.485
Total cholesterol/ HDL-C ratio	Unadjusted	−0.101	−0.760	0.558	0.764
	Model 1	−0.015	−0.627	0.596	0.961
	Model 2	−0.246	−0.752	0.260	0.340
Triglycerides (mmol/L)	Unadjusted	0.008	−0.869	0.885	0.986
	Model 1	0.156	−0.699	1.011	0.721
	Model 2	0.057	−0.732	0.846	0.888

Abbreviations: CI, confidence intervals, HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol.
Model 1 was adjusted for age and sex; Model 2 was adjusted for age, sex, visceral-to-subcutaneous fat volume ratio, glycated haemoglobin.

Table 3 Associations between intra-pancreatic fat deposition and markers of lipid metabolism in individuals after acute pancreatitis.

Markers	Model	β coefficient	95% CI		*P*
			Lower	Upper	
HDL-C (mmol/L)	Unadjusted	−0.724	−1.710	0.261	0.150
	Model 1	−0.581	−1.644	0.481	0.284
	Model 2	−0.485	−1.626	0.656	0.405
	Model 3	−0.068	−1.210	1.073	0.906
LDL-C (mmol/L)	Unadjusted	−0.008	−0.397	0.380	0.967
	Model 1	0.131	−0.244	0.505	0.493
	Model 2	0.230	−0.153	0.614	0.239
	Model 3	0.368	−0.011	0.748	0.057
Total cholesterol (mmol/L)	Unadjusted	0.210	−0.113	0.533	0.203
	Model 1	0.276	−0.030	0.582	0.077
	Model 2	0.306	−0.007	0.620	0.055
	Model 3	0.477	0.173	0.781	**0.002**
Total cholesterol/ HDL-C ratio	Unadjusted	0.336	0.045	0.626	**0.024**
	Model 1	0.403	0.103	0.702	**0.008**
	Model 2	0.392	0.095	0.689	**0.010**
	Model 3	0.375	0.090	0.660	**0.010**
Triglycerides (mmol/L)	Unadjusted	0.360	0.090	0.629	**0.009**
	Model 1	0.347	0.081	0.612	**0.010**
	Model 2	0.312	0.043	0.582	**0.023**
	Model 3	0.280	0.016	0.545	**0.038**

Abbreviations: CI, confidence intervals, HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol.
Model 1 was adjusted for age and sex; Model 2 was adjusted for age, sex, use of lipid-lowering drugs, visceral-to-subcutaneous fat volume ratio, glycated haemoglobin; Model 3 was adjusted for age, sex, use of lipid-lowering drugs, visceral-to-subcutaneous fat volume, glycated haemoglobin, APACHE II score, aetiology, recurrence, and time since last episode of acute pancreatitis. Significant ($p < 0.05$) associations are shown in bold.

associated with HDL-C and LDL-C in both unadjusted and adjusted models (Table 3).

Partial residual plots showing the associations between IPFD and each marker of lipid metabolism in the most adjusted model (model 3) along with adjusted R^2 values are presented in Fig. 2.

Prediction of intra-pancreatic fat deposition from markers of lipid metabolism

Of the five studied markers of lipid metabolism, none of the markers were significantly associated with IPFD in healthy non-obese individuals (Table 4). In individuals after AP, level of triglycerides was significantly associated with IPFD (in multiple regression analysis after eliminating markers with collinearity) (Table 4) and was further used for building three equations for prediction of IPFD.

i) The model including age and sex yielded R^2 of 16.7% and the equation was:

Intra-pancreatic fat % = 7.428 + 0.347 (Triglycerides in mmol/L) + 0.034 (Age in years) − 0.395 (Sex)

where, Sex: women = 0, men = 1.

ii) The model including age, sex, use of lipid-lowering drugs, V/S fat volume ratio, and HbA1c yielded R^2 of 18.2% and the equation was:

Intra-pancreatic fat % = 6.389 + 0.312 (Triglycerides in mmol/L) + 0.029 (Age in years) − 0.086 (Sex) − 0.102 (Use of lipid-lowering drugs) + 0.584 (V/S fat volume ratio) + 0.016 (HbA1c in mmol/mol).

where, Sex: women = 0, men = 1, Use of lipid-lowering drugs: no = 0, yes = 1.

iii) The model including age, sex, use of lipid-lowering drugs, V/S fat volume ratio, HbA1c, APACHE II score, aetiology, recurrence, and time since AP yielded R^2 of 26.7% and the equation was:

Intra-pancreatic fat % = 6.555 + 0.258 (Triglycerides in mmol/L) + 0.021 (Age in years) − 0.135 (Sex) + 0.064 (Use of lipid-lowering drugs) + 0.943 (V/S fat volume ratio) + 0.006 (HbA1c in mmol/mol) + 1.031 (Aetiology) + 0.012 (APACHE II score) + 0.355 (Recurrence) − 0.005 (Time since AP in months)

where, Sex: women = 0, men = 1, Use of lipid-lowering drugs: no = 0, yes = 1; Aetiology: biliary = 1, non-biliary = 0, Recurrence: no = 0, yes = 1.

Discussion

This is the first study that has investigated serum lipid profile as a possible biomarker of IPFD and adjusted for MRI-derived abdominal fat parameters in healthy non-obese individuals and those after AP. One of the key findings is that, while none of the studied markers of lipid metabolism were significantly associated with IPFD in healthy non-obese individuals, TC/HDL-C ratio and triglycerides were significantly associated with increased IPFD in individuals after AP. These associations were observed in both unadjusted and adjusted models, which included V/S fat volume ratio, level of HbA1c, and other covariates. Further, the collinearity analysis of the studied markers showed that triglycerides, but not TC/HDL-C ratio, was a significant biomarker of IPFD in the developed predictive equations.

In healthy individuals, lipid metabolism is regulated by dietary fat, *de novo* lipogenesis, transport of lipid metabolites, and lipolysis. *De novo* lipogenesis occurs in the liver and adipocytes, where excess carbohydrates are converted into fatty acids and re-esterified as triglycerides for energy storage [26]. A limited energy storage capacity of the subcutaneous adipose tissue is believed to contribute to excess visceral fat accumulation and increased lipid flux into the non-adipose tissues (e.g., the pancreas) [27]. While it is frequently observed that obese individuals have increased IPFD, the development of IPFD may not always be a consequence of weight gain, as evidenced by the fact that conventional anthropometric measures (such as body mass index and waist circumference) are not significantly associated with IPFD [1]. This suggests that individuals with similar general adiposity may have different metabolic risk profiles. A 2011 study by Rossi et al. [28] showed that visceral fat is the main determinant of IPFD in obese individuals;

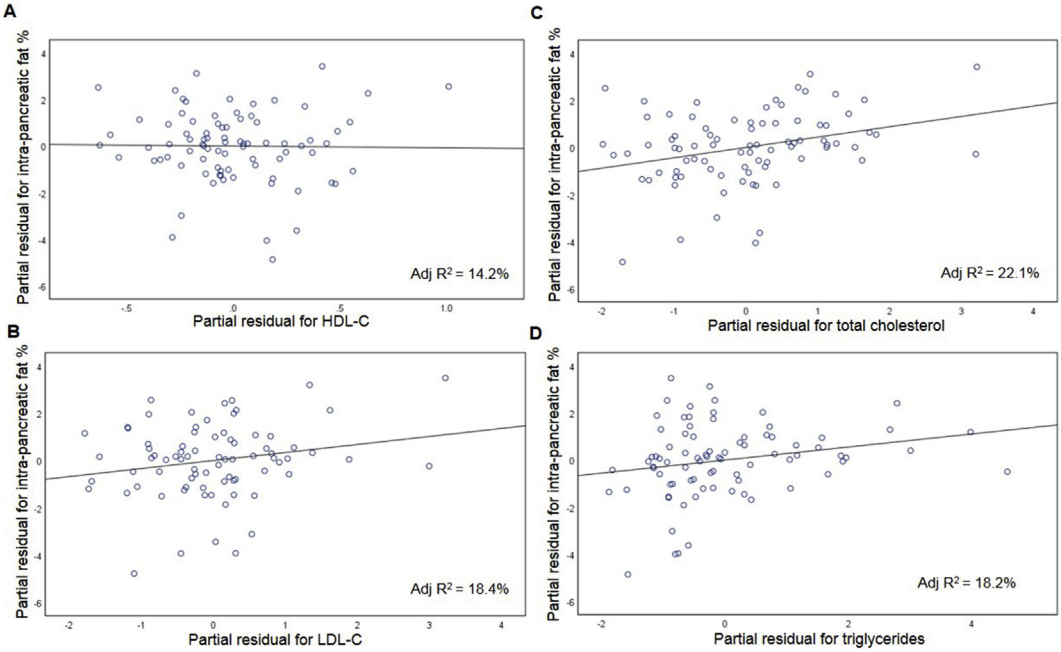

Figure 2 Associations between intra-pancreatic fat deposition and (A) HDL-C, (B) LDL-C, (C) total cholesterol, (D) triglycerides in individuals after acute pancreatitis. *Abbreviations:* HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol. *Footnote:* All partial residual plots are adjusted for age, sex, use of lipid-lowering drugs, visceral-to-subcutaneous fat volume ratio, glycated haemoglobin, APACHEII score, aetiology, recurrence, and time since acute pancreatitis.

however, the contribution of abdominal fat depots to IPFD in non-obese individuals is not completely understood [29]. An earlier cross-sectional study in healthy non-obese individuals reported significant associations between IPFD and triglycerides and HDL-C [30]. However, the study did not account for MRI-derived abdominal fat parameters. In the present study, IPFD in healthy non-obese individuals was not significantly associated with any marker of lipid metabolism, even after adjustment for MRI-derived V/S fat volume ratio. Longitudinal studies are now warranted to confirm this finding and investigate temporal changes in markers of lipid metabolism and their association with IPFD.

The other key finding is that TC/HDL-C ratio and triglycerides were significantly associated with increased IPFD in individuals after AP. During the course of AP, excess visceral fat, IPFD, and dyslipidaemia are well-recognised prognostic factors for adverse in-hospital outcomes [31–33]. However, growing body of evidence also shows that individuals after an episode of AP are at an increased risk of developing metabolic derangements [34–36], and have characteristics of dyslipidaemia, abnormal fat distribution, and chronic low-grade inflammation [12,14,37]. In the present study, the association between MRI-derived IPFD and serum lipid profile was investigated, for the first time, taking into account V/S fat volume ratio and HbA1c.

Table 4 Collinearity between markers of lipid metabolism.

First model	*P*	VIF	Second model	*P*	VIF	Third model	*P*	VIF
(A) Healthy non-obese individuals								
Total cholesterol/HDL-C ratio	0.924	18.1	Triglycerides	0.893	3.4	Triglycerides	0.896	1.5
Triglycerides	0.216	97.4	LDL-C	0.849	11.0	HDL-C	0.964	2.0
HDL-C	0.215	149.3	HDL-C	0.937	16.0	LDL-C	0.756	2.0
LDL-C	0.212	602.8	Total cholesterol/HDL-C ratio	0.945	18.1			
Total cholesterol	0.200	1210.5						
(B) Post-acute pancreatitis individuals								
Triglycerides	0.397	4.4	HDL-C	0.585	2.1	LDL-C	0.884	1.0
HDL-C	0.565	6.5	Triglycerides	0.415	2.9	Triglycerides	**0.026**	1.3
LDL-C	0.648	7.0	LDL-C	0.470	5.2	HDL-C	0.998	1.3
Total cholesterol	0.352	7.6	Total cholesterol	0.379	6.9			
Total cholesterol/HDL-C ratio	0.747	11.7						

Abbreviations: HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol, VIF, variance inflation factor.
The variance inflator factor (VIF) was used to assess collinearity of markers. The independent variable with highest VIF in a model was eliminated at each stage and the remaining independent variables were included in subsequent models, until VIF < 5 was achieved for all independent variables. Significant association ($P < 0.05$) is shown in bold.

For every 1 mmol/L change in triglycerides, IPFD increased by at least 0.28%; and for every unit change in TC/HDL-C ratio, IPFD increased by at least 0.38%. Notably, the significant associations were independent of V/S fat volume ratio and other covariates. This suggests that the phenomenon of IPFD in the post-pancreatitis setting is more complex than IPFD in metabolic diseases. It is likely to be characterised by not only ectopic accumulation of lipids and infiltration of mature adipocytes in the pancreas (as a consequence of excess abdominal adiposity) [38,39] but also fatty replacement of the pancreas (as a consequence of acinar cell dysfunction and/or trans-differentiation of acinar cells into adipocytes and/or β-cell dedifferentiation) [40,41]. Each of the above-mentioned pathways is likely to be triggered by low-grade inflammation – the hallmark of both excess adiposity and post-pancreatitis state. Further, in the collinearity analyses of the five markers of lipid metabolism, triglycerides emerged as the only significant marker of IPFD. The derived predictive equation for IPFD including triglycerides, V/S fat volume ratio, demographics, and other covariates together explained 26.7% variance in IPFD. Therefore, triglyceride levels hold promise as the most useful component of lipid profile in the follow-up assessment of individuals after AP for the purpose of estimation of IPFD and identification of high-risk individuals. The accuracy and usefulness of triglycerides as a biomarker for IPFD now needs to be investigated in a formal diagnostic accuracy study to determine its sensitivity, specificity, positive and negative predictive values, and optimal threshold prior to considering its use in routine practice.

There are several strengths of the study. First, individuals were recruited, on average, after nearly two years since their last AP attack. Therefore, the study cohort represents a homogenous population without the influence of acute inflammatory response. Second, the study was informed by two previous systematic reviews on IPFD by the group that allowed us to benchmark all published evidence on the subject [1,6]. Third, two independent raters measured IPFD and the inter-rater reliability of measurements was excellent, with an ICC of 0.98. Last, robust statistical models were built to investigate the effect of covariates, including but not limited to use of lipid-lowering drugs, abdominal fat distribution, and HbA1c.

Several limitations of the study need to be acknowledged. First, the cross-sectional study design did not allow inference as to whether dyslipidaemia is a consequence of IPFD or one of the factors causing it. However, the aim of the study was to investigate markers of lipid metabolism that can be potentially used as biomarkers of IPFD in post-pancreatitis setting. To date, all studies investigating the relationship between IPFD and markers of lipid metabolism had a cross-sectional study design. A prospective longitudinal study focussing on changes in IPFD over time and its association with markers of lipid metabolism is now warranted to provide novel insights into the interplay between dyslipidaemia and IPFD. Second, some markers of lipid metabolism (e.g., glycerol, free fatty acids, diacylglycerol, and ceramide) were not investigated. However, the five markers of lipid metabolism investigated in this study are the most commonly tested serum lipids. Of these, triglycerides and cholesterol are the major lipids in the body that are more stable in comparison with circulating free fatty acid levels (which are known to have high variability) [42]. Therefore, from the perspective of identifying a practical biomarker of IPFD, the studied blood parameters met the criteria of readily available and stable biomarkers. Third, detailed data on lifestyle factors (e.g., exercise and energy intake) were not available. However, all individuals included in the study were non-athletic and did not report any sudden weight gain or weight loss. Fourth, some individuals with diabetes in the post-AP group received glucose-lowering medications (i.e., metformin, glipizide, sitagliptin) that might have influenced body fat composition. However, their effect is unlikely to be differential and only three (3%) study participants received insulin (which is known to have the most profound effect on body fat among all glucose-lowering medications). Fifth, patterns of alcohol consumption might have affected body fat composition. Investigating whether the amount, frequency, and quality of alcohol consumption influence IPFD needs to be on the research agenda. Last, the present study did not investigate the genetic make-up of participants; therefore, inherent differences in lipid profile cannot be ruled out [43,44]. Future studies are warranted to determine if the studied associations are affected by genetic variations.

In conclusion, the present study investigated markers of lipid metabolism and their associations with IPFD in healthy non-obese individuals and individuals after AP. There was no significant association in healthy non-obese individuals, even after accounting for demographic factors, abdominal fat distribution, and blood glucose homeostasis. Triglycerides and TC/HDL-C ratio were significantly associated with IPFD in individuals after AP, independent of demographic factors, abdominal fat distribution, levels of HbA1c, use of lipid-lowering drugs, and pancreatitis-related factors. Taking into account that triglycerides and TC/HDL-C ratio are interrelated, the collinearity analysis was conducted and revealed that triglycerides is the only significant independent biomarker of IPFD in individuals after AP.

Funding

This study was part of the Clinical and epidemiOlogical inveStigations in Metabolism, nutritiOn, and pancreatic diseaseS (COSMOS) program. COSMOS is supported in part by the Royal Society of New Zealand (Rutherford Discovery Fellowship to Associate Professor Petrov), which played no role in the study design; collection, analysis, or interpretation of data; or writing of the manuscript.

Conflicts of interest

The Authors declare that there is no conflict of interest.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.numecd.2019.06.003.

References

[1] Singh RG, Yoon HD, Wu LM, Lu J, Plank LD, Petrov MS. Ectopic fat accumulation in the pancreas and its clinical relevance: a systematic review, meta-analysis, and meta-regression. Metabolism 2017; 69:1–13. https://doi.org/10.1016/j.metabol.2016.12.012.

[2] Petrov MS. Harnessing analytic morphomics for early detection of pancreatic cancer. Pancreas 2018;47:1051–4. https://doi.org/10.1097/MPA.0000000000001155.

[3] Petrov MS, Yadav D. Global epidemiology and holistic prevention of pancreatitis. Nat Rev Gastroenterol Hepatol 2019;16:175–84. https://doi.org/10.1038/s41575-018-0087-5.

[4] Cho J, Scragg R, Petrov MS. Risk of mortality and hospitalization after post-pancreatitis diabetes mellitus vs type 2 diabetes mellitus: a population-based matched cohort study. Am J Gastroenterol 2019;114:804–12. https://doi.org/10.14309/ajg.0000000000000225.

[5] Idilman IS, Tuzun A, Savas B, Elhan AH, Celik A, Idilman R, et al. Quantification of liver, pancreas, kidney, and vertebral body MRI-PDFF in non-alcoholic fatty liver disease. Abdom Imag 2015;40: 1512–9. https://doi.org/10.1007/s00261-015-0385-0.

[6] Singh RG, Yoon HD, Poppitt SD, Plank LD, Petrov MS. Ectopic fat accumulation in the pancreas and its biomarkers: a systematic review and meta-analysis. Diabetes Metab Res Rev 2017;33. https://doi.org/10.1002/dmrr.2918.

[7] Honka H, Hannukainen JC, Tarkia M, Karlsson H, Saunavaara V, Salminen P, et al. Pancreatic metabolism, blood flow, and β-cell function in obese humans. J Clin Endocrinol Metab 2014;99: E981–90. https://doi.org/10.1210/jc.2013-4369.

[8] Dong Z, Luo Y, Cai H, Zhang Z, Peng Z, Jiang M, et al. Noninvasive fat quantification of the liver and pancreas may provide potential biomarkers of impaired glucose tolerance and type 2 diabetes. Medicine (Baltimore) 2016;95:e3858. https://doi.org/10.1097/MD.0000000000003858.

[9] Targher G, Rossi AP, Zamboni GA, Fantin F, Antonioli A, Corzato F, et al. Pancreatic fat accumulation and its relationship with liver fat content and other fat depots in obese individuals. J Endocrinol Investig 2012;35:748–53. https://doi.org/10.3275/8011.

[10] Gillies NA, Pendharkar SA, Singh RG, Asrani VM, Petrov MS. Lipid metabolism in patients with chronic hyperglycemia after an episode of acute pancreatitis. Diabetes Metab Syndr 2017;11S: S233–41. https://doi.org/10.1016/j.dsx.2016.12.037.

[11] Pendharkar SA, Singh RG, Petrov MS. Pro-inflammatory cytokine-induced lipolysis after an episode of acute pancreatitis. Arch Physiol Biochem 2018;124:401–9. https://doi.org/10.1080/13813455.2017.1415359.

[12] Singh RG, Pendharkar SA, Gillies NA, Miranda-Soberanis V, Plank LD, Petrov MS. Associations between circulating levels of adipocytokines and abdominal adiposity in patients after acute pancreatitis. Clin Exp Med 2017;17:477–87. https://doi.org/10.1007/s10238-017-0453-6.

[13] Singh RG, Pendharkar SA, Plank LD, Petrov MS. Role of human lipocalin proteins in abdominal obesity after acute pancreatitis. Peptides 2017;91:1–7. https://doi.org/10.1016/j.peptides.2017.03.001.

[14] Singh RG, Pendharkar SA, Cervantes A, Cho J, Miranda-Soberanis V, Petrov MS. Abdominal obesity and insulin resistance after an episode of acute pancreatitis. Dig Liver Dis 2018;50:1081–7. https://doi.org/10.1016/j.dld.2018.04.023.

[15] Guzmán S, Nervi F, Llanos O, León P, Valdivieso V. Impaired lipid clearance in patients with previous acute pancreatitis. Gut 1985; 26:888–91. https://doi.org/10.1136/gut.26.9.888.

[16] Singh RG, Cervantes A, Kim JU, Nguyen NN, DeSouza SV, Dokpuang D, et al. Intra-pancreatic fat deposition and visceral fat volume are associated with the presence of diabetes after acute pancreatitis. Am J Physiol Gastrointest Liver Physiol 2019;316: G806–15. https://doi.org/10.1152/ajpgi.00385.2018.

[17] Singh RG, Nguyen NN, Cervantes A, Alarcon Ramos GC, Cho J, Petrov MS. Associations between intra-pancreatic fat deposition and circulating levels of cytokines. Cytokine 2019;120:107–14. https://doi.org/10.1016/j.cyto.2019.04.011.

[18] Maraví-Poma E, Zubia Olascoaga F, Petrov MS, Navarro Soto S, Laplaza Santos C, Morales Alava F, et al. SEMICYUC 2012. Recommendations for intensive care management of acute pancreatitis. Med Intensiva 2013;37:163–79. https://doi.org/10.1016/j.medin.2013.01.007.

[19] Al-Mrabeh A, Hollingsworth KG, Steven S, Taylor R. Morphology of the pancreas in type 2 diabetes: effect of weight loss with or without normalisation of insulin secretory capacity. Diabetologia 2016;59:1753–9. https://doi.org/10.1007/s00125-016-3984-6.

[20] Al-Mrabeh A, Hollingsworth KG, Steven S, Tiniakos D, Taylor R. Quantification of intrapancreatic fat in type 2 diabetes by MRI. PLoS One 2017;12:e0174660. https://doi.org/10.1371/journal.pone.0174660.

[21] Singh RG, Nguyen NN, DeSouza SV, Pendharkar SA, Petrov MS. Comprehensive analysis of body composition and insulin traits associated with intra-pancreatic fat deposition in healthy individuals and people with new-onset prediabetes/diabetes after acute pancreatitis. Diabetes Obes Metab 2019;21:417–23. https://doi.org/10.1111/dom.13523.

[22] Cervantes A, Singh RG, Kim JU, DeSouza SV, Petrov MS. Relationship of anthropometric indices to abdominal body composition: a multi-ethnic New Zealand magnetic resonance imaging study. J Clin Med Res 2019;11:435–46. https://doi.org/10.14740/jocmr3820.

[23] DeSouza SV, Priya S, Cho J, Singh RG, Petrov MS. Pancreas shrinkage following recurrent acute pancreatitis: an MRI study. Eur Radiol 2019;29:3746–56. https://doi.org/10.1007/s00330-019-06126-7.

[24] Alin A. Multicollinearity. Wiley Interdiscip Rev Comput Stat 2010; 2:370–4. https://doi.org/10.1002/wics.84.

[25] Vu DH, Muttaqi KM, Agalgaonkar AP. A variance inflation factor and backward elimination based robust regression model for forecasting monthly electricity demand using climatic variables. Appl Energy 2015;140:385–94. https://doi.org/10.1016/j.apenergy.2014.12.011.

[26] Ameer F, Scandiuzzi L, Hasnain S, Kalbacher H, Zaidi N. De novo lipogenesis in health and disease. Metab Clin Exp 2014;63: 895–902. https://doi.org/10.1016/j.metabol.2014.04.003.

[27] Després J-P. Body fat distribution and risk of cardiovascular disease: an update. Circulation 2012;126:1301–13. https://doi.org/10.1161/CIRCULATIONAHA.111.067264.

[28] Rossi AP, Fantin F, Zamboni GA, Mazzali G, Rinaldi CA, Del Giglio M, et al. Predictors of ectopic fat accumulation in liver and pancreas in obese men and women. Obesity (Silver Spring) 2011; 19:1747–54. https://doi.org/10.1038/oby.2011.114.

[29] Guardiola M, Solà R, Vallvé JC, Girona J, Godàs G, Heras M, et al. Body mass index correlates with atherogenic lipoprotein profile even in nonobese, normoglycemic, and normolipidemic healthy men. J Clin Lipidol 2015;9:824–31. https://doi.org/10.1016/j.jacl.2015.08.001. e1.

[30] Wong VWS, Wong GLH, Yeung DKW, Abrigo JM, Kong APS, Chan RSM, et al. Fatty pancreas, insulin resistance, and β-cell function: a population study using fat-water magnetic resonance imaging. Am J Gastroenterol 2014;109:589–97. https://doi.org/10.1038/ajg.2014.1.

[31] Goodger RL, Asrani VM, Windsor JA, Petrov MS. Impact of metabolic comorbidities on outcomes of patients with acute pancreatitis: a scoping review. Panminerva Med 2016;58:86–93.

[32] Petrov MS. Abdominal fat: a key player in metabolic acute pancreatitis. Am J Gastroenterol 2013;108:140–2. https://doi.org/10.1038/ajg.2012.384.

[33] Noel P, Patel K, Durgampudi C, Trivedi RN, de Oliveira C, Crowell MD, et al. Peripancreatic fat necrosis worsens acute pancreatitis independent of pancreatic necrosis via unsaturated fatty acids increased in human pancreatic necrosis collections. Gut Lond 2016;65:100. https://doi.org/10.1136/gutjnl-2014-308043.

[34] Das SL, Singh PP, Phillips AR, Murphy R, Windsor JA, Petrov MS. Newly diagnosed diabetes mellitus after acute pancreatitis: a systematic review and meta-analysis. Gut 2014;63:818–31. https://doi.org/10.1136/gutjnl-2013-305062.

[35] Pendharkar SA, Mathew J, Petrov MS. Age- and sex-specific prevalence of diabetes associated with diseases of the exocrine pancreas: a population-based study. Dig Liver Dis 2017;49:540–4. https://doi.org/10.1016/j.dld.2016.12.010.
[36] Sankaran SJ, Xiao AY, Wu LM, Windsor JA, Forsmark CE, Petrov MS. Frequency of progression from acute to chronic pancreatitis and risk factors: a meta-analysis. Gastroenterology 2015;149:1490–500. https://doi.org/10.1053/j.gastro.2015.07.066. e1.
[37] Pendharkar SA, Singh RG, Chand SK, Cervantes A, Petrov MS. Pro-inflammatory cytokines after an episode of acute pancreatitis: associations with fasting gut hormone profile. Inflamm Res 2018;67:339–50. https://doi.org/10.1007/s00011-017-1125-4.
[38] Smits MM, van Geenen EJM. The clinical significance of pancreatic steatosis. Nat Rev Gastroenterol Hepatol 2011;8:169–77. https://doi.org/10.1038/nrgastro.2011.4.
[39] Pinnick KE, Collins SC, Londos C, Gauguier D, Clark A, Fielding BA. Pancreatic ectopic fat is characterized by adipocyte infiltration and altered lipid composition. Obesity (Silver Spring) 2008;16:522–30. https://doi.org/10.1038/oby.2007.110.
[40] Bonal C, Thorel F, Ait-Lounis A, Reith W, Trumpp A, Herrera PL. Pancreatic inactivation of c-Myc decreases acinar mass and transdifferentiates acinar cells into adipocytes in mice. Gastroenterology 2009;136:309–19. https://doi.org/10.1053/j.gastro.2008.10.015. e9.
[41] Sun J, Ni Q, Xie J, Xu M, Zhang J, Kuang J, et al. β-Cell dedifferentiation in patients with T2D with adequate glucose control and nondiabetic chronic pancreatitis. J Clin Endocrinol Metab 2019;104:83–94. https://doi.org/10.1210/jc.2018-00968.
[42] Widjaja A, Morris RJ, Levy JC, Frayn KN, Manley SE, Turner RC. Within- and between-subject variation in commonly measured anthropometric and biochemical variables. Clin Chem 1999;45:561–6.
[43] Blackett PR, Sanghera DK. Genetic determinants of cardiometabolic risk: a proposed model for phenotype association and interaction. J Clin Lipidol 2013;7:65–81. https://doi.org/10.1016/j.jacl.2012.04.079.
[44] Ramos-Lopez O, Riezu-Boj JI, Milagro FI, Goni L, Cuervo M, Martinez JA. Differential lipid metabolism outcomes associated with ADRB2 gene polymorphisms in response to two dietary interventions in overweight/obese subjects. Nutr Metab Cardiovasc Dis 2018;28:165–72. https://doi.org/10.1016/j.numecd.2017.11.006.