Cytokine 120 (2019) 107–114

Contents lists available at ScienceDirect

Cytokine

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

Associations between intra-pancreatic fat deposition and circulating levels of cytokines

Ruma G. Singh, Ngoc Nhu Nguyen, Aya Cervantes, Gisselle C. Alarcon Ramos, Jaelim Cho, Maxim S. Petrov*

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

ARTICLE INFO

Keywords:
Leptin
Tumor necrosis factor-alpha
Interleukin-6
C-C motif chemokine ligand 2
Intra-pancreatic fat
Abdominal fat distribution

ABSTRACT

While a plethora of studies have been conducted to investigate the associations between pro-inflammatory cytokines and obesity, the inter-relationship between pro-inflammatory cytokines and intra-pancreatic fat deposition (IPFD) has been poorly investigated. In the present study, circulating levels of C-C motif chemokine ligand 2 (CCL2), interleukin-6 (IL-6), leptin, and tumor necrosis factor-alpha (TNFα) were measured in 90 individuals after acute pancreatitis (AP) as well as 21 healthy non-obese individuals. Magnetic resonance imaging was used to quantify IPFD and visceral-to-subcutaneous fat volume ratio by two independent raters. Linear regression analyses were performed to investigate the associations between IPFD and each cytokine, adjusting for demographic, metabolic, and pancreatitis-related factors, as well as abdominal fat distribution. In healthy non-obese individuals, IPFD was not significantly associated with any of the studied cytokines in both the unadjusted and adjusted models. In individuals after AP, IPFD was significantly associated with leptin in the models adjusted for age and sex (β = 0.063 [95% confidence interval: 0.007, 0.119], $P = 0.026$); age, sex, visceral-to-subcutaneous fat volume ratio, glycated hemoglobin, and pancreatitis-related factors (β = 0.056 [95% confidence interval: 0.000, 0.111], $P = 0.049$). Also, IPFD was significantly associated with TNFα in the unadjusted model (β = 0.102 [95% confidence interval: 0.002, 0.202], $P = 0.045$) and the model adjusted for age, sex, visceral-to-subcutaneous fat volume ratio, glycated hemoglobin, and pancreatitis-related factors (β = 0.128 [95% confidence interval: 0.034, 0.223], $P = 0.008$). The associations between IPFD and IL-6, CCL2 were not statistically significant, in both the unadjusted and adjusted models. These findings indicate that leptin and TNFα are associated with IPFD independent of abdominal fat distribution and other covariates in individuals after AP. The role of IPFD in low-grade inflammation warrants further investigations.

1. Introduction

The involvement of cytokines in glucose and lipid metabolism is increasingly recognized [1,2]. Elevated levels of interleukin-6 (IL-6), leptin, C-C motif chemokine ligand 2 (CCL2), and tumor necrosis factor-α (TNFα) have been observed in individuals with diabetes [3–6] and metabolic syndrome [7]. Chronic low-grade inflammation is one of the main underlying pathophysiological features of metabolic disorders, often triggered by excessive fat accumulation in the abdomen. Modern magnetic resonance imaging (MRI) enables accurate quantification of abdominal adiposity and ectopic fat phenotypes, and this has led to a growing attention to intra-pancreatic fat deposition (IPFD). Recent evidence shows that IPFD is associated with at least two-fold increased risk of diabetes mellitus and metabolic syndrome [8]. In obese individuals, IPFD is also significantly associated with elevated circulating levels of leptin [9,10]. However, the associations between IPFD and cytokines other than leptin are largely unknown and no study to date has investigated these associations in healthy non-obese individuals and those after clinical resolution of inflammatory diseases [11–17].

Acute pancreatitis (AP) has traditionally been considered as an acute self-limiting inflammatory disease. However, emerging evidence shows that this disease often has numerous metabolic sequelae [18–21], in part driven by excess abdominal fat and chronic low-grade inflammation [13,21–23]. In individuals after an episode of AP, IPFD and visceral-to-subcutaneous fat volume ratio (V/S fat volume ratio) are significantly higher in comparison with healthy individuals [24].

Abbreviations: AP, acute pancreatitis; CCL2, C-C motif chemokine ligand 2; IL-6, interleukin-6; IPFD, intra-pancreatic fat deposition; MRI, magnetic resonance imaging; TNFα, tumor necrosis factor-alpha; V/S fat volume ratio, visceral-to-subcutaneous fat volume ratio

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

https://doi.org/10.1016/j.cyto.2019.04.011
Received 17 October 2018; Received in revised form 29 March 2019; Accepted 18 April 2019
Available online 01 May 2019
1043-4666/

Further, chronic low-grade inflammation and insulin resistance persist after AP, as evidenced by significantly elevated circulating levels of TNFα, IL-6, and leptin [13]. However, whether these cytokines are associated with IPFD in individuals after AP, independently of abdominal fat distribution, is not known.

The aim of this study was to investigate the associations between a panel of cytokines and IPFD in individuals after AP and healthy non-obese individuals (without history of pancreatic diseases), as well as to determine the effect of abdominal fat distribution and other covariates.

2. Methods

2.1. Study design and study population

This was a cross-sectional study, as part of the ARIES (Analytic moRphomics In pancrEatic diseaSes) project. Individuals who were at least 18 years old were eligible if they had had primary diagnosis of AP (established prospectively at the time of hospitalization in line with the international guidelines [25]). 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, congenital anomalies of the pancreas, hereditary pancreatitis, autoimmune pancreatitis, cystic fibrosis, pancreatic trauma, interventions involving the pancreas (surgical, endoscopic, or radiologic), received steroid therapy, had malignancy, any metallic foreign body or electronic device implantations, 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 diseases of the exocrine pancreas or diabetes, no upper abdominal pain or nausea, no family history of diseases of the exocrine pancreas, 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. Informed consent was obtained from all study participants.

2.2. MRI acquisition and quantification of variables

Abdominal MRI scans for all participants were performed at the Centre of Advanced MRI (The University of Auckland), using 3.0 Tesla MAGNETOM Skyra MRI scanner (Siemens, Erlangen, Germany). The acquisition protocol was reported elsewhere [23]. Intra-pancreatic fat percentage (%) was measured using the 'MR-opsy' technique [26]. Two candidate slices were selected and three regions of interest were placed in the head, body, and tail of the pancreas. To exclude the non-parenchymal tissue within the selected region of interest, a threshold range of 1–20% was applied [26]. Intra-pancreatic fat % was calculated by taking the average of measurements of two slices. The segmentation of subcutaneous and visceral fat compartments from the second lumbar vertebral level to the fifth lumbar vertebral level was reported elsewhere [27]. The 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. The V/S fat volume ratio was subsequently calculated.

2.3. Inter-rater reliability

Two raters, blinded to characteristics of the study participants, measured intra-pancreatic fat %, visceral fat volume, and subcutaneous fat volume independently. Intra-class correlation coefficients (ICCs) were calculated to assess the inter-rater reliability. The inter-rater reliability was considered excellent if ICC was > 0.90 [28]. The average values of two independent MRI measurements were used for statistical analyses.

2.4. Laboratory analyses

Participants were asked to fast for at least eight hours prior to blood collection. Venous blood was collected from all participants into ethylenediamine-tetra acetic acid tubes. In line with the American Diabetes Association guidelines, glycated hemoglobin was measured at a tertiary referral medical laboratory, LabPlus, using the boronate affinity chromatography assay (Trinity Biotech, Wicklow, Ireland), which is certified by the NGSP (National Glycohemoglobin Standardization Program) and standardized to the Diabetes Control and Complications Trial reference assay.

For analysis of the cytokines – CCL2, IL-6, leptin, TNFα, blood was centrifuged at 4000 g for 7.5 min at 4°Celsius. The separated plasma was further analyzed using the MILLIPLEX® MAP human metabolic hormone magnetic bead panel based on the Luminex xMAP® technology (Luminex Corporation, Austin, USA). Results were quantified based on fluorescent receptor signals recorded by the Luminex xPONENT® software (MILLIPLEX® Analyst 5.1). The intra-assay and inter-assay variation was < 10% and < 15%, respectively. The assay was performed as indicated in the user's manual.

2.5. Pancreatitis-related factors

Etiology of AP was categorized as biliary or non-biliary (including alcohol, idiopathic, other). Acute Physiology and Chronic Health Evaluation II (APACHE II) scores at the time of hospitalization for AP for all participants were calculated. Recurrence of AP was defined as hospitalization 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.

2.6. Statistical analyses

Statistical analyses were performed using the 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 in continuous and categorical characteristics, respectively, between healthy non-obese individuals and individuals after AP. The Mann-Whitney *U* test was performed for continuous variables when the assumption of normal distribution was violated. The inter-relationships between the studied cytokines were evaluated using the Spearman's correlation coefficient.

Linear regression analysis (using generalized linear models) was conducted separately for healthy non-obese individuals and individuals after AP. To investigate the associations between the cytokines (CCL2, IL-6, leptin, and TNFα) and IPFD, each cytokine was analyzed as an independent variable in unadjusted and adjusted models. The analyses of healthy participants 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 glycated hemoglobin. The analyses of individuals after AP, in addition to model 1 and model 2, also included model 3 - adjusted for age, sex, V/S fat volume ratio, glycated hemoglobin, APACHE II score, time since AP, etiology, and recurrence of AP. Outliers in the cytokines data were removed (by visual inspection of boxplots) to obtain the most robust and conservative outputs. Further, a *post-hoc* analysis investigating the inter-relationship between etiology of AP and IPFD was conducted. Etiology (biliary/non-biliary) was analyzed as the independent variable in one unadjusted and three adjusted models (as described above). All results were reported as β

Table 1
Characteristics of study participants.

Characteristic	Post-pancreatitis (n = 90)	Healthy (n = 21)	*P*
Age (years)	56 (44–66)	44 (29–53)	**0.006** [a]
Sex			0.848
Men	58 (64)	14 (67)	
Women	32 (36)	7 (33)	
Waist circumference (cm)	99 (88–107)	84 (81–89)	< **0.001** [a]
Hip circumference (cm)	102 (92–112)	85 (80–91)	< **0.001**[b]
Height (cm)	172 (165–180)	172 (164–180)	0.781 [a]
Weight (kg)	84 (72–100)	69 (60–84)	< **0.001** [a]
Body mass index (kg/m^2)	27.5 (24.5–33.4)	23.7 (21.4–26.1)	< **0.001**[b]
Visceral fat volume (L)	1.9 (1.2–2.7)	0.7 (0.6–1.2)	< **0.001**[b]
Subcutaneous fat volume (L)	2.9 (2.1–4.1)	1.9 (1.4–2.5)	**0.001**[b]
Glycated hemoglobin (mmol/mol)	37 (34–40)	32 (30–35)	< **0.001**[b]
C-reactive protein (mg/L) [c]	1.1 (0.6–1.8)	0.7 (0.6–3.7)	0.851[b]

Footnote: Data are presented as median (interquartile range) or number (percentage) of participants. Significant ($P < 0.05$) associations are shown in bold.
[a] *P* value obtained from the Student's *t* test.
[b] *P* value obtained from the Mann-Whitney *U* test.
[c] Available for 34 post-pancreatitis and 21 healthy individuals.

coefficients with corresponding 95% confidence intervals (CI). $P < 0.05$ was accepted as statistically significant.

3. Results

3.1. Study population

Ninety individuals after a median (IQR) of 22 (12–35) months since last episode of AP were recruited into the study, comprising of 58 men and 32 women with median (IQR) age of 56 (44–66) years. Of these, 40 individuals had biliary AP; 23 had more than one AP episode; and the median (IQR) APACHE II score of participants at hospital admission was 5 (3–8). Twenty-one healthy non-obese individuals were also recruited into the study, including 14 men and 7 women with a median (IQR) age of 44 (29–53) years. Individuals after AP had a significantly higher IPFD with a median (IQR) intra-pancreatic fat of 9.6 (8.8–10.4)% compared with 7.9 (6.3–8.9)% in healthy non-obese individuals ($P < 0.001$). Other characteristics of study participants are presented in Table 1.

3.2. Inter-rater reliability of MRI measurements

The ICC of intrapancreatic fat % measurements was 0.98 (Fig. 1A). The ICCs of visceral fat volume and subcutaneous fat volume measurements were 0.99 each (Fig. 1B, 1C).

3.3. Associations between intra-pancreatic fat deposition and cytokines in healthy non-obese individuals

The median (IQR) CCL2 level was 74.75 (50.13–114.46) pg/ml. Intra-pancreatic fat deposition was not significantly associated with CCL2, in both unadjusted [0.001 (−0.016, 0.017)] ($P = 0.913$) and adjusted models – model 1 [−0.001 (−0.021, 0.019)] ($P = 0.912$) and model 2 [0.004 (−0.014, 0.021)] ($P = 0.691$). The median (IQR) IL-6 level was 13.51 (11.36–19.43) pg/ml. Intra-pancreatic fat deposition was not significantly associated with IL-6, in both unadjusted [β coefficient (95%CI)], [−0.005 (−0.124, 0.114)] ($P = 0.935$) and adjusted models – model 1 [0.011 (−0.113, 0.135)] ($P = 0.864$) and model 2 [0.027 (−0.084, 0.138)] ($P = 0.632$). The median (IQR) leptin level was 3.57 (1.17–7.70) ng/ml. Intra-pancreatic fat deposition was not significantly associated with leptin, in both unadjusted [0.008 (−0.148, 0.165)] ($P = 0.919$) and adjusted models - model 1 [−0.074 (−0.293, 0.145)] ($P = 0.507$) and model 2 [−0.151 (−0.351, 0.048)] ($P = 0.137$). The median (IQR) TNFα level was 5.22 (3.63–5.78) pg/ml. Intra-pancreatic fat deposition was not significantly associated with TNFα, in both unadjusted [0.067 (−0.293, 0.428)] ($P = 0.714$) and adjusted models - model 1 [0.066 (−0.316, 0.447)] ($P = 0.735$) and model 2 [0.108 (−0.237, 0.454)] ($P = 0.538$). Partial residual plots showing the associations between IPFD and each cytokine in the most adjusted model (model 2) along with adjusted R^2 values are presented in Fig. 2. Correlations between the studied cytokines in healthy non-obese individuals are presented in Table 2.

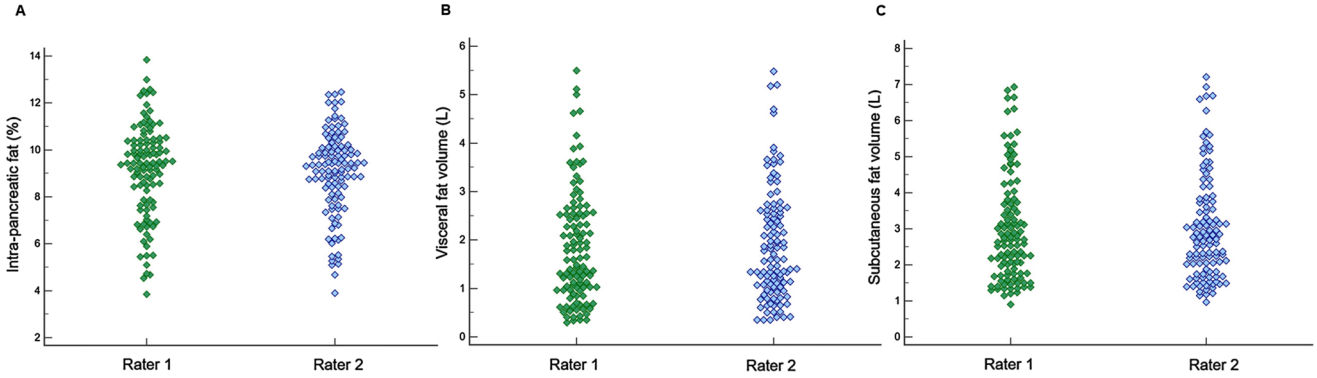

Fig. 1. Measurements of the MRI-derived variables - intra-pancreatic fat% (A), visceral fat volume (B), and subcutaneous fat volume (C) by two independent raters.

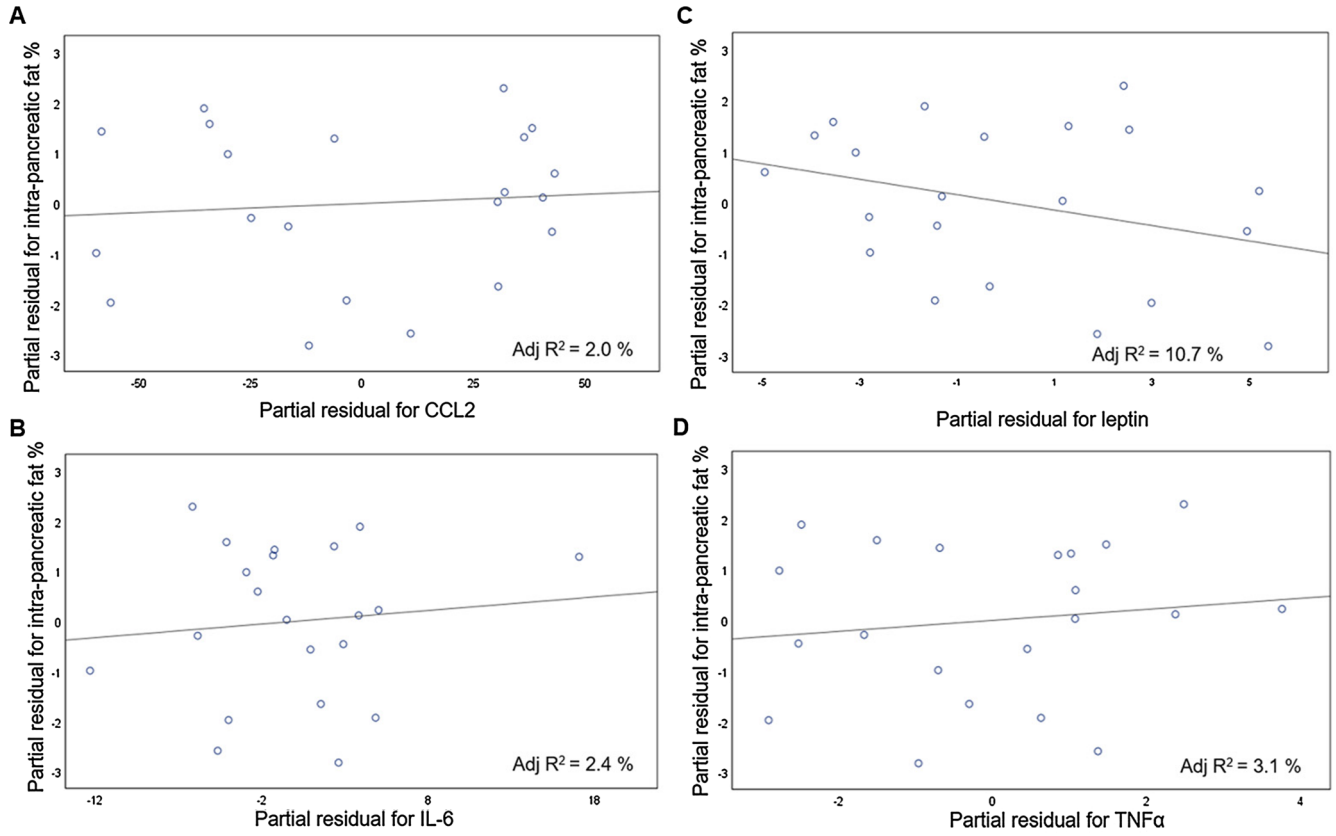

Fig. 2. Associations between intra-pancreatic fat deposition and C-C motif chemokine ligand 2 (CCL2) (A), interleukin-6 (IL-6) (B), leptin (C), tumor necrosis factor-α (TNFα) (D) in healthy non-obese individuals. *Footnote:* All partial residual plots are adjusted for age, sex, V/S fat volume ratio, and glycated hemoglobin.

Table 2
Inter-relationships between the studied cytokines.

	CCL2 (pg/ml)	IL-6 (pg/ml)	Leptin (ng/ml)
(A) Healthy			
CCL2 (pg/ml)	1.00		
IL-6 (pg/ml)	0.434 ($P = 0.05$)	1.00	
Leptin (ng/ml)	0.058 ($P = 0.80$)	−0.154 ($P = 0.51$)	1.00
TNFα (pg/ml)	**0.766 ($P < 0.01$)**	0.216 ($P = 0.35$)	−0.036 ($P = 0.88$)
(B) Post-pancreatitis			
CCL2 (pg/ml)	1.00		
IL-6 (pg/ml)	**0.334 ($P < 0.01$)**	1.00	
Leptin (ng/ml)	**0.237 ($P = 0.03$)**	**0.228 ($P = 0.04$)**	1.00
TNFα (pg/ml)	**0.641 ($P < 0.01$)**	**0.517 ($P < 0.01$)**	**0.369 ($P < 0.01$)**

Abbreviations: CCL2, C-C motif chemokine ligand 2; IL-6, interleukin-6; TNFα, tumor necrosis factor-α.
Footnote: Data are presented as correlation coefficient and *P* value. Significant ($P < 0.05$) associations are shown in bold.

3.4. Associations between intra-pancreatic fat deposition and cytokines in individuals after acute pancreatitis

Intra-pancreatic fat deposition was significantly associated with leptin in model 1 [0.063 (0.007, 0.119)] ($P = 0.026$) and model 3 [0.056 (0.000, 0.111)] ($P = 0.049$) (Table 3). Intra-pancreatic fat deposition was significantly associated with TNFα in the unadjusted model [0.102 (0.002, 0.202)] ($P = 0.045$) and model 3 [0.128 (0.034, 0.223)] ($P = 0.008$) (Table 3). Intra-pancreatic fat deposition was not significantly associated with CCL2 and IL-6 in both unadjusted and adjusted models (Table 3). Partial residual plots showing the

Table 3
Associations between cytokines and intra-pancreatic fat deposition in individuals after acute pancreatitis.

Cytokine	Model	β coefficient	95% CI		*P*
			Lower	Upper	
CCL2 (pg/ml)	Unadjusted	0.003	−0.005	0.010	0.473
	Model 1	0.002	−0.005	0.009	0.534
	Model 2	0.002	−0.005	0.009	0.534
	Model 3	0.003	−0.003	0.009	0.342
IL-6 (pg/ml)	Unadjusted	0.022	−0.018	0.063	0.279
	Model 1	0.018	−0.020	0.057	0.352
	Model 2	0.009	−0.030	0.047	0.651
	Model 3	0.022	−0.016	0.061	0.251
Leptin (ng/ml)	Unadjusted	0.035	−0.022	0.092	0.231
	Model 1	0.063	0.007	0.119	**0.026**
	Model 2	0.056	0.000	0.112	0.051
	Model 3	0.056	0.000	0.111	**0.049**
TNFα (pg/ml)	Unadjusted	0.102	0.002	0.202	**0.045**
	Model 1	0.089	−0.006	0.184	0.066
	Model 2	0.073	−0.024	0.171	0.139
	Model 3	0.128	0.034	0.223	**0.008**

Abbreviations: CI, confidence interval; CCL2, C-C motif chemokine ligand 2; IL-6, interleukin-6; TNFα, tumor necrosis factor-α.
Footnote: Model 1 was adjusted for age and sex; Model 2 was adjusted for age, sex, visceral-to-subcutaneous fat volume ratio, glycated hemoglobin; Model 3 was adjusted for age, sex, visceral-to-subcutaneous fat volume ratio, glycated hemoglobin, APACHE II score, etiology, recurrence, and time since acute pancreatitis. Significant ($P < 0.05$) associations are shown in bold.

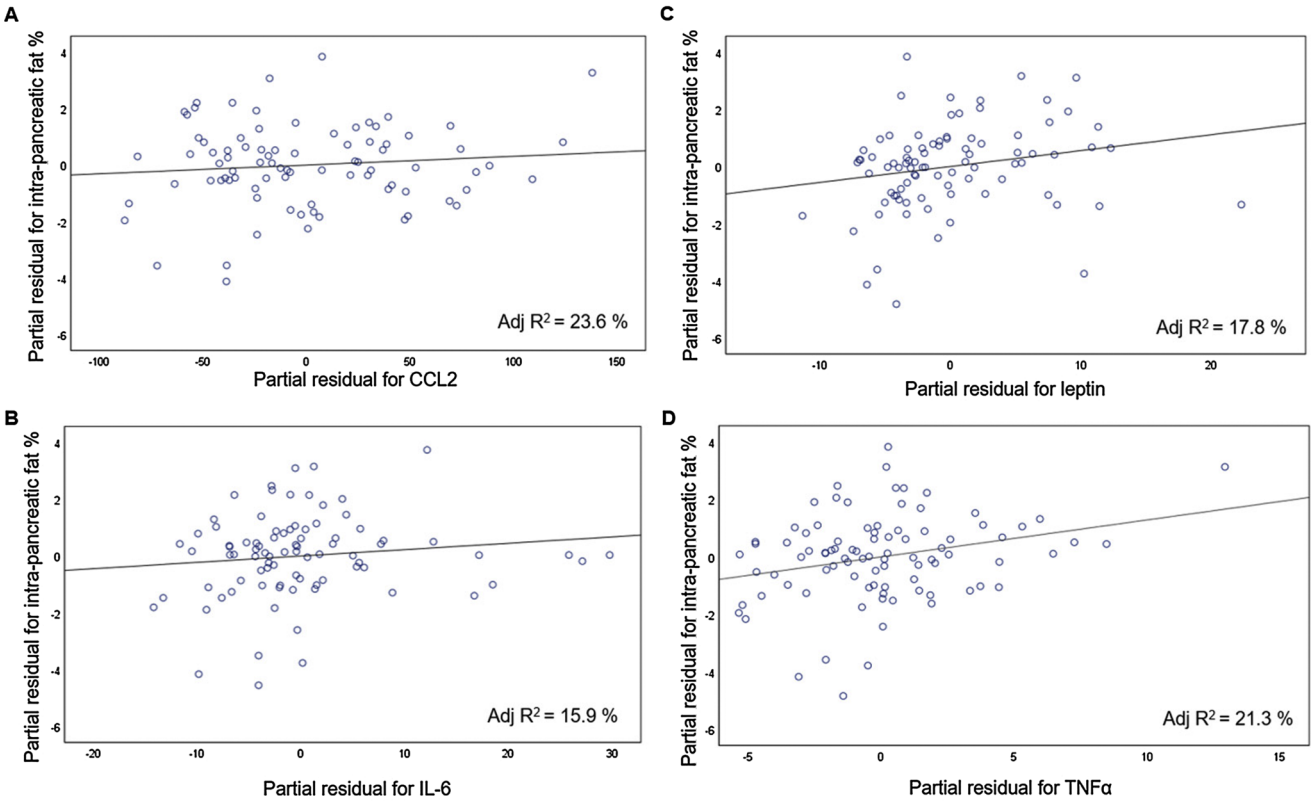

Fig. 3. Associations between intra-pancreatic fat deposition and C-C motif chemokine ligand 2 (CCL2) (A), interleukin-6 (IL-6) (B), leptin (C), tumor necrosis factor-α (TNFα) (D) in individuals after acute pancreatitis. *Footnote:* All partial residual plots are adjusted for age, sex, V/S fat volume ratio, glycated hemoglobin, APACHE II score, etiology, recurrence, and time since acute pancreatitis.

associations between IPFD and each cytokine in the most adjusted model (model 3) along with adjusted R^2 values are presented in Fig. 3. Correlations between the studied cytokines in individuals after AP are presented in Table 2.

3.5. Effect of covariates

The associations between all covariates and IPFD in the analyses of each studied cytokine (the most adjusted model – model 3) in individuals after AP are presented in Table 4. Intra-pancreatic fat deposition was significantly associated with V/S fat volume ratio in the analyses of CCL2, IL-6, and TNFα (Table 4). Of the studied pancreatitis-related factors, biliary etiology of AP was associated with significantly increased IPFD, consistently in the analyses of all the studied cytokines (Table 4).

In a *post-hoc* analysis, biliary etiology of AP (in comparison with non-biliary etiology) was significantly associated with increased IPFD (Fig. 4), consistently in both the unadjusted [0.767 (0.065, 1.469)] ($P = 0.032$) and adjusted models - model 1 [0.865 (0.196, 1.535)] ($P = 0.011$), model 2 [1.038 (0.377, 1.699)] ($P = 0.002$), and model 3 [1.076 (0.412, 1.740)] ($P = 0.001$).

Table 4
Effect of covariates on the studied associations in individuals after acute pancreatitis.

	CCL2		IL-6		Leptin		TNFα	
	β (95%CI)	*P*	β (95%CI)	*P*	β (95%CI)	*P*	β (95%CI)	*P*
Age	0.025 (−0.006, 0.056)	0.114	0.018 (−0.013, 0.049)	0.266	0.020 (−0.013,0.052)	0.230	0.020 (−0.011,0.050)	0.206
Sex								
Men	Reference		Reference		Reference		Reference	
Women	−0.395 (−1.262, 0.472)	0.372	−0.068 (−0.943, 0.806)	0.878	−0.555 (−1.508, 0.398)	0.254	−0.295 (−1.149,0.560)	0.499
V/S fat volume ratio	1.126 (0.033, 2.220)	**0.044**	1.372 (0.253, 2.492)	**0.016**	1.113 (−0.019, 2.244)	0.054	1.290 (0.209, 2.370)	**0.019**
Glycated hemoglobin	−0.005 (−0.042, 0.033)	0.813	0.005 (−0.033, 0.042)	0.807	0.002 (−0.036, 0.041)	0.904	−0.009 (−0.047, 0.029)	0.659
APACHE II score	0.041 (−0.078, 0.159)	0.504	−0.004 (−0.123, 0.115)	0.948	0.010 (−0.111, 0.132)	0.866	−0.008 (−0.126, 0.110)	0.892
Etiology								
Non-biliary	Reference		Reference		Reference		Reference	
Biliary	1.288 (0.639, 1.936)	< **0.001**	1.074 (0.399, 1.750)	**0.002**	0.975 (0.310, 1.641)	**0.004**	1.322 (0.657, 1.986)	< **0.001**
Recurrence								
No	Reference		Reference		Reference		Reference	
Yes	0.109 (−0.645, 0.862)	0.778	0.416 (−0.370, 1.202)	0.300	0.336 (−0.429, 1.100)	0.389	0.130 (−0.607, 0.868)	0.729
Time since AP	−0.011 (−0.028, 0.006)	0.193	0.000 (−0.018, 0.017)	0.959	−0.009 (−0.026, 0.009)	0.326	−0.010 (−0.026, 0.006)	0.226

Abbreviations: AP, acute pancreatitis; CCL2, C-C motif chemokine ligand 2; CI, confidence interval; IL-6, interleukin-6; TNFα, tumor necrosis factor-α, V/S fat volume ratio, visceral-to-subcutaneous fat volume ratio.
Footnote: Significant ($P < 0.05$) associations are shown in bold.

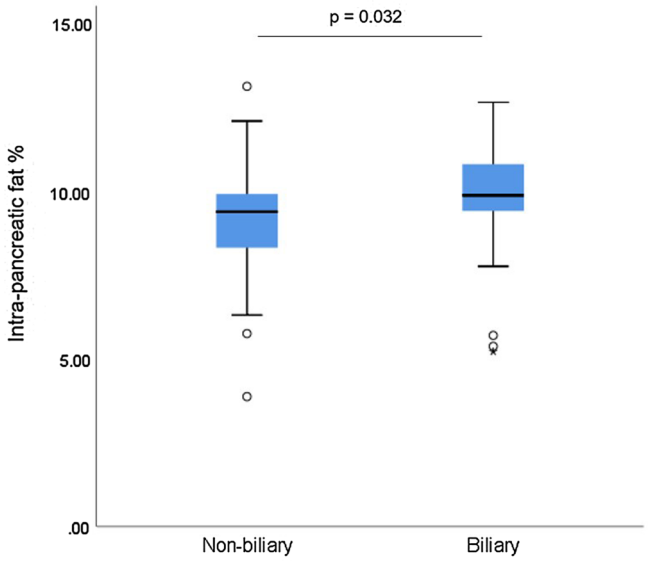

Fig. 4. Intra-pancreatic fat deposition in biliary verus non-biliary acute pancreatitis.

4. Discussion

To date, the role of excess adiposity, IPFD, and elevated pro-inflammatory cytokines has been investigated during the clinical course of AP or in healthy obese individuals. This is the first study to investigate the four pro-inflammatory cytokines and their associations with IPFD in individuals after AP [median (IQR) time since AP was 22 (12–35) months] and in healthy non-obese individuals. The strengths of the study are as follows: it was informed by two complementary systematic literature reviews on IPFD conducted by the group [8,17]; all MRI-derived measurements by two independent raters had excellent inter-rater reliability (ICC > 0.90); and the used statistical models accounted for several relevant covariates (including age, sex, distribution of abdominal fat, level of glycemia). Three findings are worth discussing. First, none of the studied cytokines were significantly associated with IPFD in healthy individuals, both in the unadjusted and adjusted models. Second, two cytokines (leptin and TNFα) were significantly associated with IPFD in individuals after AP. Specifically, leptin explained up to 26% and TNFα explained up to 29% variance in IPFD in individuals after AP. Notably, these associations were independent of V/S fat volume ratio and other covariates. Third, IPFD was significantly higher in individuals with biliary etiology of AP.

More than two decades ago, experimental and animal studies first demonstrated production of IL-6, TNFα, CCL2, and leptin by the adipose tissue [29–32]. It is now established that these cytokines have pro-inflammatory actions and they have been extensively investigated as both inflammatory biomarkers in various disease settings and predictors of cardiovascular events in healthy individuals [33,34]. Expression of these cytokines in the adipose tissue has been shown to be proportional to the amount of fat accumulation in specific abdominal fat depots [35–37], in particular visceral fat. However, there is a paucity of studies investigating the associations between ectopic fat depots (specifically, IPFD) and cytokines in general population [9,10,38–41]. Three studies included children or young adults [39–41] and found that CCL2 [39,41], leptin [39], and TNFα [40] were positively associated with IPFD. Of the studies that included adults, two [9,10] reported a negative association between MRI-derived IPFD and leptin. Notably, all associations in the five studies mentioned above were investigated in obese individuals only and none of the studies adjusted for age, sex, or abdominal fat distribution. The sixth study [38] was an observational study conducted in general population and reported on leptin as an independent risk factor for IPFD; however, ultrasonography (which is known to have inferior accuracy in comparison with MRI) was used to determine IPFD. The present study adds to the literature by reporting no significant association between circulating levels of the four cytokines and MRI-derived IPFD in healthy non-obese individuals, in both the unadjusted analysis and after adjustment for age, sex, glycated hemoglobin, and abdominal fat distribution. The lack of statistical association likely reflects the absence of low-grade inflammation in these individuals.

By contrast, IPFD was significantly associated with TNFα in individuals after AP, independent of V/S fat volume ratio and other covariates. Tumor necrosis factor-α is one of the key regulators of adipocyte metabolism; in particular, it controls the production of adipocyte-derived factors such as lipoprotein lipase, fatty acid transport protein, and acetyl CoA synthase [42], which in turn switches lipid metabolism to favor lipolysis. Further, TNFα downregulates insulin signaling by activating intracellular signaling molecules (c-Jun N-terminal kinase and inhibitor kappa beta kinase) and ultimately leads to insulin resistance [43–45]. The pro-inflammatory state mediated by TNFα is likely to be more pronounced as TNFα also inhibits the production of adiponectin – an anti-inflammatory adipokine [46–48].

In a similar vein, IPFD was significantly associated with leptin in individuals after AP. Leptin, a 16 kDa non-glycosylated peptide mainly produced by adipocytes, is considered one of the key links between metabolic responses and inflammation. During the early course of AP, leptin levels are influenced by the duration of hyperglycemia [49], severity of pancreatitis, and pancreatic necrosis [50]. Furthermore, compared with general adiposity (as measured by body mass index) leptin has higher sensitivity and specificity in predicting pancreatic necrosis [50]. While evidence indicates that leptin may be an important factor increasing the risk of new-onset metabolic derangements after hospital discharge from AP [13], its association with IPFD has been investigated for the first time in this study. The findings show that, for

Table 5
Body composition parameters and cytokines stratified by etiology of acute pancreatitis.

	Overall	Etiology		*P*
		Biliary (n = 40)	Non-biliary (n = 50)	
Waist circumference (cm)	99 (88–107)	101 (88–110)	97 (88–106)	0.332 [a]
Body mass index (kg/m^2)	27.5 (24.5–33.4)	28.5 (24.8–33.7)	27.0 (24.1–32.8)	0.284 [b]
Visceral fat volume (L)	1.9 (1.2–2.7)	1.8 (1.2–2.6)	2.1 (1.3–2.7)	0.553 [b]
Subcutaneous fat volume (L)	2.9 (2.1–4.1)	3.2 (2.4–4.8)	2.7 (1.8–3.7)	**0.016** [b]
CCL2 (pg/ml)	97.4 (65.9–137.8)	85.2 (60.7–135.7)	106.8 (67.7–138.5)	0.381 [b]
IL-6 (pg/ml)	7.4 (3.6–13.5)	5.9 (2.9–10.2)	8.6 (4.3–15.0)	0.086 [b]
Leptin (ng/ml)	4.4 (2.1–10.2)	6.2 (2.4–14.5)	3.9 (1.8–8.3)	0.120 [b]
TNFα (pg/ml)	5.1 (3.4–7.7)	4.3 (2.7–5.2)	6.6 (3.9–8.7)	**0.006** [b]

Abbreviations: CCL2, C-C motif chemokine ligand 2; IL-6, interleukin-6; TNFα, tumor necrosis factor-α.
Footnote: Significant ($P < 0.05$) associations are shown in bold.
[a] *P* value obtained from the Student's *t* test.
[b] *P* value obtained from the Mann-Whitney *U* test.

every 1 ng/ml change in leptin levels, IPFD increased by 0.06%, independent of V/S fat volume ratio and other covariates. Previous studies showed that leptin regulates glucose homeostasis, independent of its effects on food intake and body weight [51]. One of the peripheral sites for leptin signaling is pancreatic β cells that express leptin receptors [52]. Leptin signaling inhibits insulin secretion and β cell mass expansion. It has been proposed that alterations in leptin signaling in β cells or 'leptin desensitization' could result in hyperinsulinemia, which in turn increases adipogenic effect of insulin - promoting fat accumulation and leptin production [53]. Further, leptin mediates pro-inflammatory actions by activating peripheral blood B cells to produce TNFα and IL-6 [54]. In addition, leptin also activates stellate cells, which then produce transforming growth factor β1 (TGFβ-1) and type 1 collagen, contributing to fibrosis [55]. Given the evidence of a central role of pancreatic stellate cells in fibrogenesis associated with progression of pancreatitis [56], it is possible that leptin contributes to increased fibrosis and fatty replacement of pancreatic acini in individuals after AP.

The other notable finding is that individuals with biliary etiology of AP had significantly higher IPFD. In a *post hoc* analysis, individuals with biliary etiology of AP had a significantly higher median subcutaneous fat volume in comparison with those with non-biliary etiology of AP (Table 5), whereas the other studied body composition parameters did not differ significantly. Also, circulating levels of TNFα were significantly lower in individuals with biliary etiology of AP whereas the other studied cytokines did not differ significantly (Table 5). It is possible that biliary etiology is associated with more severe derangements of lipid metabolism in comparison with non-biliary etiology because of over-saturation of bile with cholesterol [57]. Moreover, a perfusion study demonstrated that fatty acid concentrations considerably influence the gallbladder's response to fatty acids [58,59]. However, an effect of unknown confounder cannot be ruled out. Purposely designed studies are now warranted to investigate whether biliary etiology predisposes AP individuals to higher IPFD.

There are several limitations of the study that need to be acknowledged. First, the cross-sectional design did not allow inference of causality. A longitudinal investigation of the associations between cytokines and IPFD should be considered in the future. Second, anti-inflammatory cytokines (e.g., adiponectin) were not investigated in the present study. Adiponectin and leptin, mainly secreted by adipocytes, are known to have an inverse relationship [60]. However, the main impetus behind the present study was to investigate the pro-inflammatory cytokine profile that was previously implicated in chronic low-grade inflammation in post-pancreatitis setting [11,13,14,16]. Last, we did not investigate lifestyle factors such as energy intake, smoking habits, and exercise routine. However, the studied associations between cytokines and IPFD were controlled for V/S fat volume ratio and glycated hemoglobin levels, which are known to be affected by lifestyle factors. Moreover, all individuals included in the study were non-athletic and did not report any sudden weight gain or weight loss.

In conclusion, the present study investigated four main pro-inflammatory cytokines and their associations with IPFD. None of the cytokines were significantly associated with IPFD in healthy non-obese individuals, even after adjustment for covariates. Leptin and TNFα were significantly associated with IPFD in individuals after AP. Notably, these associations were independent of demographic, pancreatitis-related factors, glycemia, and abdominal fat distribution.

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.

Conflict of interest statement

The authors declare no conflict of interest

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