UNIVERSIDADE FEDERAL DO RIO GRANDE DO SUL

PROGRAMA DE PÓS-GRADUAÇÃO EM CIÊNCIAS MÉDICAS:

ENDOCRINOLOGIA

DIMENSÕES, CONTEÚDO DE GORDURA E PERFUSÃO DO PÂNCREAS EM PACIENTES COM DIABETES: AVALIAÇÃO POR MÉTODOS DE IMAGEM

TESE DE DOUTORADO

TIAGO SEVERO GARCIA

Porto Alegre, dezembro de 2016

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Orientadora: Profa. Dra. Cristiane Bauermann Leitão

Tese de Doutorado apresentada ao Programa de Pós-graduação em Ciências Médicas: Endocrinologia da Universidade Federal do Rio Grande do Sul (UFRGS) como requisito parcial para a obtenção do título de Doutor em Endocrinologia.

Porto Alegre, dezembro de 2016

DEDICATÓRIA

Ao Colégio Barão do Rio Branco,

Ao Colégio Farroupilha,

Ao Hospital de Clínicas de Porto Alegre,

À Universidade Federal do Rio Grande do Sul.

AGRADECIMENTOS

À minha orientadora, Profa. Dra. Cristiane Bauermann Leitão, pela serenidade, pela motivação, pela disponibilidade e, sobretudo, pela qualidade da orientação e pelo seu imenso conhecimento científico. Foi uma experiência muito prazerosa ter sido teu aluno, uma verdadeira honra. Espero poder transmitir aos meus futuros alunos os ensinamentos que recebi de ti. Querida Cris, entre tantas palavras, há três que melhor traduzem meu sentimento em relação a ti, desde o nosso trabalho no tempo da residência médica até o fim dessa tese: admiração, respeito e gratidão.

Aos pacientes do *Institut Jules Bordet* que, mesmo passando por momentos difíceis de suas vidas, aceitaram voluntariamente participar dessa pesquisa.

Aos doutores Jean-Luc Engelholm, Michaël Vouche e Marc Lemort, médicos radiologistas do *Institut Jules Bordet*, por terem aceitado a ideia e por terem colaborado com competência e gentileza nas várias fases da pesquisa.

À doutora Lissandra Dal Lago, médica oncologista do *Institut Jules Bordet*,cujo apoio desde as fases iniciais foi fundamental para o desenvolvimento da pesquisa.

À minha esposa Tatiana, pelo apoio, pela compreensão, pelo suporte, pelo exemplo. Devo muito dessa tese a ti, amor.

Às minhas filhas Marina e Cecília, meu encanto, minha inspiração. Cada abraço e cada beijo que o papai recebe de vocês duas são a coisa mais linda que há.

Aos meus pais Jussara e Vilnei e aos meus irmãos Isadora e Márcio, pelo carinho de sempre, pela companhia. Essa tese é também o resultado da nossa história.

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LISTA DE ABREVIATURAS

ASL	*Arterial spin labeling*
BF	*Blood flow*
BMI	*Body mass index*
BV	*Blood volume*
CCC	*Concordance correlation coefficient*
CI	*Confidence interval*
CT	*Computed tomography*
DM	Diabetes melito ou *diabetes mellitus*
DM1	Diabetes melito tipo 1
DM2	Diabetes melito tipo 2
FIPE	Fundo de Incentivo à Pesquisa e Ensino
HbA1c	*Glycated hemoglobin*
HCPA	Hospital de Clínicas de Porto Alegre
I^2	*I-squared*
kVp	*Kilovolt peak*
MeSH	*Medical Subject Headings*
MRI	*Magnetic resonance imaging*
MTT	*Mean transit time*
RM	Ressonância magnética
ROI	*Region of interest*

SD	*Standard deviation*
TC	Tomografia computadorizada
TTP	*Time to peak*
T1DM	*Type 1 diabetes mellitus*
T2DM	*Type 2 diabetes mellitus*
US	Ultrassonografia ou *ultrasound*
UFRGS	Universidade Federal do Rio Grande do Sul
WMD	*Weighted mean difference*

Esta tese de doutorado será apresentada no formato exigido pelo Programa de Pós-graduação em Ciências Médicas: Endocrinologia. Ela será constituída de uma introdução em português e de três artigos em inglês, estes formatados conforme as exigências das respectivas revistas médicas às quais serão submetidos para avaliação e posterior publicação. Os artigos em inglês desta tese são um artigo do tipo Revisão Sistemática e Meta-Análise e dois do tipo Artigo Original.

RESUMO

A maioria dos estudos com ultrassonografia (US), tomografia computadorizada (TC) e ressonância magnética (RM) mostra que as dimensões do pâncreas são reduzidas em pacientes com diabetes, quando comparados com grupo controle. Dados sobre a perfusão pancreática em pacientes com diabetes são escassos na literatura. Essa tese tem por objetivo avaliar características do pâncreas nos exames de imagem que possam trazer uma melhor compreensão da patogênese e da fisiopatologia do diabetes.

Primeiramente, realizamos uma revisão sistemática com metanálise de estudos que utilizaram métodos de imagem (US, TC ou RM) para a medida das dimensões – diâmetro, área ou volume - e do conteúdo de gordura do pâncreas em pacientes com diabetes tipo 1 (DM1) ou tipo 2 (DM2). Demonstramos que as dimensões pancreáticas são menores nos pacientes com DM1 ou DM2 em comparação com indivíduos sem diabetes. Além disso, o conteúdo de gordura do pâncreas é maior em pacientes com DM2.

Com o intuito de investigar uma possível causa para a redução do volume do pâncreas em pacientes com diabetes, buscamos estudar a vascularização pancreática por meio de TC perfusional. Inicialmente, fizemos um estudo para avaliar a variabilidade intra e interobservador para a medida dos parâmetros de perfusão pancreática por TC (fluxo sanguíneo, volume sanguíneo, tempo de trânsito médio, tempo para o pico de realce), demonstrando que existe uma boa concordância nessas medidas, mesmo entre radiologistas com diferentes níveis de experiência. Em sequência, realizamos um estudo comparando esses parâmetros de perfusão pancreática por TC entre pacientes com DM2 e indivíduos sem diabetes. Mostramos que o volume sanguíneo que perfunde o pâncreas e o seu tempo de trânsito médio pelo órgão são menores em pacientes com DM2 em comparação com indivíduos não diabéticos.

CAPÍTULO 1

Introdução

O diabetes melito é uma doença progressiva caracterizada por hiperglicemia crônica (1) no contexto de resistência à ação da insulina (2) e/ou disfunção e morte das células beta (3). O pâncreas tem um papel central na patogênese e na fisiopatologia do diabetes (4). Entretanto, a avaliação das características desse órgão por métodos de imagem em pacientes com diabetes permanece incompleta.

A maioria dos estudos com ultrassonografia (US), tomografia computadorizada (TC) e ressonância magnética (RM) mostra que as dimensões do pâncreas são reduzidas em pacientes com diabetes, quando comparados com grupo controle (5-7). No que diz respeito ao conteúdo de gordura do pâncreas, seu aumento está associado a uma menor secreção de insulina (8).

Apesar de representar cerca de 1 a 2% da massa total do pâncreas, as ilhotas de Langerhans recebem de 10 a 23% do fluxo sanguíneo pancreático (9, 10). Muitos estudos avaliaram os valores normais dos parâmetros de perfusão pancreática por TC (11, 12), alterações da perfusão pancreática em doenças do pâncreas (13-18) e modificações da perfusão pancreática pós-terapia oncológica (19). A avaliação dos parâmetros de perfusão pancreática em pacientes com diabetes, no entanto, ainda não foi adequadamente realizada. O único estudo que se dedicou a investigar a perfusão pancreática em pacientes com diabetes melito tipo 2 (DM2) mostrou diferenças de

perfusão de acordo com a duração do diabetes, mas não foi incluído grupo controle (20). Além disso, há poucos dados na literatura a respeito da variabilidade intra e interobservador na interpretação dos parâmetros da perfusão pancreática e os observadores são uma potencial fonte de variabilidade na performance de um teste diagnóstico (21). Portanto, a variabilidade intra e interobservador das medidas da perfusão pancreática devem ser definidas, a fim de garantir a reprodutibilidade das leituras radiológicas.

Diante do exposto, esta tese tem três objetivos:

- Avaliar as dimensões e o conteúdo de gordura do pâncreas em pacientes com diabetes**;**
- Medir a variabilidade intra e interobservador entre radiologistas na leitura dos parâmetros de perfusão pancreática na TC;
- Comparar quantitativamente os parâmetros de perfusão pancreática por TC em pacientes com DM2 e indivíduos não diabéticos.

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CAPÍTULO 2

Pancreatic size and fat content in diabetes: a systematic review and meta-analysis of imaging studies

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ABSTRACT

Background: Imaging studies are expected to produce reliable information regarding the size and fat content of the pancreas, which plays a central role in diabetes. However, the available studies have produced inconclusive results.

Purpose: To perform a systematic review and meta-analysis of imaging studies assessing pancreas size and fat content in patients with type 1 diabetes (T1DM) and type 2 diabetes (T2DM).

Data sources: Medline and Embase databases and hand search of selected reference lists.

Study selection: Studies evaluating pancreatic size (diameter, area or volume) and/or fat content by ultrasound, computed tomography, or magnetic resonance imaging in patients with T1DM and/or T2DM as compared to healthy controls were selected. Sixteen studies including 2,593 subjects (284 T1DM patients, 1,069 T2DM patients, and 1,240 control subjects) were selected for meta-analyses.

Data extraction: Pancreas diameter (two studies), area (two studies), volume (eight studies), density (two studies), and fat percentage (five studies) were evaluated.

Data synthesis: Pancreatic volume was reduced in T1DM and T2DM *vs.* controls (T1DM *vs.* controls: –38.72 cm^3, 95%CI: –52.25 to –25.19, I^2=70.2%, p for heterogeneity=0.018; and T2DM *vs.* controls: –12.18 cm^3, 95%CI: –19.1 to –5.25, I^2=79.3%, p for heterogeneity=0.001). Fat content was higher in T2DM *vs.* controls (+3.53%, 95%CI: 0.85 to 6.21, I^2=83.3%, p for heterogeneity<0.001).

Conclusions: Individuals with T1DM and T2DM have reduced pancreas size in comparison with control subjects. Patients with T2DM have increased pancreatic fat content.

INTRODUCTION

The pancreas plays a key role in diabetes mellitus, a progressive disease characterized by chronic hyperglycemia (1) in the context of insulin resistance (2) and/or beta-cell dysfunction and death (3). Beta-cell loss secondary to apoptosis leads to a reduction in beta-cell mass (4, 5). Although islets of Langerhans represent only 1% of the total pancreas, autopsy studies have demonstrated reduced pancreas size in both type 1 (6) and type 2 diabetic subjects (7). These findings suggest that diabetes may affect exocrine pancreatic function (8, 9).

Insulin deficiency and the lack of a trophic effect of insulin on acinar cells may explain the reduction in pancreas size in type 1 diabetes (T1DM) (6), whereas atherosclerosis might play a role in type 2 diabetes (T2DM) (10, 11). However, the reduction in pancreatic size may also be the cause, and not a consequence, of diabetes; these mechanisms remain to be clarified (12, 13).

Imaging studies are expected to produce reliable information regarding pancreas size. However, while some studies using ultrasound (US), computed tomography (CT), and magnetic resonance imaging (MRI) to assess pancreas size in diabetes have shown reduced pancreatic size in individuals with diabetes as compared to controls (14-16), no differences were observed in others (17, 18). Such inconclusiveness may be related to the small sample size of most studies evaluating this issue.

Interestingly, CT and MRI are widely used to measure liver steatosis (19, 20), which is closely related to obesity and diabetes (20). More recently, imaging protocols have produced accurate non-invasive measurements of pancreatic fat content in humans (21, 22). Excess ectopic fat storage has been linked to

insulin resistance (20), and pancreatic fat content has been negatively associated with insulin secretion (23).

The aim of the present study was to systematically review the literature and synthesize data regarding pancreatic size and fat content in diabetes using meta-analysis.

METHODS

Data sources and searches

To identify observational studies evaluating pancreatic size or fat content by imaging in diabetes, the literature (Medline and Embase) was searched for studies using the three major imaging methods (US, CT, and MRI) for pancreas evaluation from inception until August 2016. No language or date restrictions were applied. Medical subject heading (MeSH) terms and key words included in the search were as follows: pancreas, diabetes, imaging, radiology, ultrasound, tomography, and magnetic resonance. Detailed Medline and Embase search strategies are shown as supplementary material (Supplemental Figure S1). Also, the references of selected articles were manually searched. The titles and abstracts of all retrieved articles were independently reviewed by two physicians, T.S.G (radiologist) and T.H.R. Disagreements were resolved by consensus. The full text of selected articles was examined.

Study selection

We included prospective and retrospective observational studies evaluating pancreatic size and/or fat content by US, CT, or MRI in T1DM and/or T2DM patients as compared to non-diabetic subjects. Exclusion criteria were as

follows: case report design, inclusion of pediatric patients, absence of control group, and no clear description of imaging and post-processing technique. Two studies in Czech language were excluded. If duplicate studies were detected, the most complete report with the longest follow-up was included.

Data extraction

One reviewer (T.S.G) conducted data extraction and a second investigator checked data extraction for accuracy (T.H.R). Data were extracted on year of publication, number of T1DM, T2DM, and non-diabetic subjects, and imaging method used for pancreatic assessment. In addition, the following data were extracted on pancreatic parameters (mean and standard deviation) in the three groups of interest (T1DM, T2DM, and non-diabetic subjects): pancreatic diameter in cm, area in cm^2, volume in cm^3, density in Hounsfield units (HU), and percentage of pancreatic fat.

Quality assessment

Quality assessment of studies included in meta-analyses was performed using the Newcastle-Ottawa scale (24).

Data synthesis and analysis

Absolute changes in size (diameter, area, or volume), density, and fat percentage in patients with diabetes and control groups are presented as means ± standard deviation (SD). Cochran's Q test was used to evaluate heterogeneity between studies. A p value <0.1 was considered statistically significant. The I^2 test was also conducted to evaluate the magnitude of the heterogeneity between studies. Heterogeneity was defined as I^2>50%. A random effects model was used for all analyses.

The contribution of individual studies to the overall heterogeneity was explored using meta-regression, subgroup analyses, and sensitivity analyses by removing each study at a time and re-running the meta-analyses. In some cases, these procedures were not feasible due to an insufficient number of studies/patients.

Funnel plot asymmetry was evaluated by Begg and Egger tests. The impact of small-study bias was considered as significant if p value <0.1 (25). Analyses were conducted using Stata software version 11.0 (StataInc, College Station, Texas).

RESULTS

A total of 5,634 potentially relevant studies were initially identified, 1,532 in Medline and 4,102 in Embase. Hand search of reference lists resulted in the inclusion of an additional seven articles (5,641 articles). After removal of 1,047 duplicates, 4,594 citations were screened based on titles and abstracts. Twenty-eight were selected for full-text review, and finally 22 articles and one poster fulfilled the inclusion criteria.

Of the 23 studies selected, seven were not included in the meta-analysis: in six, data were not extractable (22, 26-30), and in one study measuring pancreatic fat divided by splenic fat the parameters could not be combined (29). The remaining 16 studies were included in meta-analyses: two evaluating diameter (17, 31), two evaluating area (14, 32), eight evaluating volume (15, 16, 18, 33-37), two evaluating density (15, 38), and five evaluating fat content (15, 16, 39-41). Studies assessing multiple parameters

were included in more than one meta-analysis. The flowchart of study selection is depicted in Figure 1.

Characteristics of the studies included in the systematic review and meta-analyses are presented in Table 1. The studies were published between 1985 and 2015 and included 2,593 participants: 284 T1DM patients (minimum-maximum: 12-60), 1,069 T2DM patients (11-198), and 1,240 control subjects (9-660). Overall mean age was 59.4 years (33.9 in T1DM, 58.0 in T2DM, and 63.8 in controls), and over all mean BMI was 26.84 kg/m^2 (22.72 in T1DM, 27.12 in T2DM, and 27.05 in controls). Mean duration of disease was 8.9 years in T1DM and 6.5 years in T2DM.

Pancreatic size

Volume

Eight studies were included in meta-analyses focusing on volume (15, 16, 18, 33-37). In four studies (18, 33, 35, 36) with T1DM patients, pancreas volume was reduced as compared to control subjects (–38.72 cm^3; 95%CI: –52.25 to –25.19). However, between-study heterogeneity was high (I^2=70.2%, p for heterogeneity=0.018) (Figure 2A). Heterogeneity was explored by sensitivity analysis and each study was excluded at a time. Heterogeneity was reduced to 47.8% (p for heterogeneity=0.147) after omission of the study by Goda *et al* (18), while no change was observed when other studies were excluded. This may have resulted from patient selection bias, as the mean age of T1DM patients in this study was 48.7 years, while the mean duration of diabetes was 9.4 years – suggesting that T2DM patients may have been misdiagnosed with T1DM. Interestingly, this was the only study using CT for volume

assessment, and thus sensitivity and subgroup analysis of pancreatic volume based on image technique were coincident. Moreover, the present meta-analysis of MRI studies shows a mean reduction of -44.08 cm^3 (95%CI: -57.16 to -30.99) in pancreatic volume in T1DM patients *vs.* controls. Subgroup analyses were performed based on the quality of studies (including only studies with a score of 6-8 in the Newcastle-Ottawa scale or studies where cases and controls were matched by BMI). However, heterogeneity was not affected by these variables (data not shown).

Despite the small number of studies, we performed meta-regression with age, BMI, and duration of diabetes as covariates. Although not statistically significant, a reduction in heterogeneity from 70.2% to 52.76% (p=0.355) was observed in the model considering duration of diabetes. Interestingly, the T1DM patients with longer diabetes duration had the lowest pancreatic volume (Supplemental Figure S2).

Similar results were observed for pancreatic volume in five studies (15, 16, 18, 34, 37) with T2DM patients, whose pancreas was smaller than that of controls (-12.18 cm^3; 95%CI: -19.1 to -5.25, I^2=79.3%, p for heterogeneity=0.001) (Figure 2B). Sensitivity analysis excluding individual studies did not decrease heterogeneity (data not shown). However, subgroup analysis considering imaging methods showed lower heterogeneity for MRI studies (I^2=47.6%, p for heterogeneity=0.167) in comparison with CT studies (I^2=70%, p for heterogeneity=0.035). It should be noted that pancreas volume was smaller in T2DM patients *vs.* controls regardless of imaging technique (MRI: -21.65 cm^3 [95%CI: -31.62 to -11.68] and CT: -7.5 cm^3 [95%CI: -13.65 to -1.36]). As for T1DM, subgroup analyses considering only studies

rated 6-8 in the Newcastle-Ottawa scale or studies with BMI-matched T2DM patients did not change heterogeneity (data not shown). No variable included in meta-regression was associated with heterogeneity.

Only one study (28) compared pancreas volume in T1DM and T2DM patients, precluding meta-analysis. In this CT study, no significant differences in volume were detected between T1DM and T2DM patients.

Diameter

Two studies (17, 31) using US detected a smaller pancreatic diameter (measured at the head and body) in T1DM patients as compared to controls (head diameter: –0.6 cm [95%CI:–0.8 to –0.41], I^2=77.8%, p for heterogeneity=0.034; and body diameter: –0.38 cm [95%CI:–0.73 to –0.03], I^2=97.2%, p for heterogeneity<0.001). However, no differences were found when T2DM patients and controls were compared (head diameter: –0.02 cm [95%CI: –0.63 to 0.6], I^2=97%, p for heterogeneity<0.001; and body diameter: –0.16 cm [95%CI:–0.66 to 0.34], I^2=97.9%, p for heterogeneity<0.001).

A meta-analysis comparing pancreas diameter in T1DM and T2DM revealed that both head diameter (–0.58 cm [95%CI:–1 to –0.16], I^2=94.5%, p for heterogeneity<0.001) and body diameter (–0.22 cm [95%CI:–0.36 to –0.07], I^2=79.3%, p for heterogeneity=0.028) were smaller in T1DM patients. Heterogeneity exploration was not possible regarding diameter, because only two studies were available.

Area

Two US studies (14, 32) analyzed T1DM patients regarding pancreatic area, which was significantly smaller as compared to that of controls (-5.44 cm^2

[95%CI:–6.8 to –4.08], I^2=1.9%, p for heterogeneity=0.313). Another study (26), which was not meta-analyzed due to lack of extractable data, corroborated these findings, showing a reduced pancreas area both in T1DM and T2DM patients, with an even smaller area in T1DM patients.

Pancreatic fat content

Five studies (15, 16, 39-41) including only T2DM patients evaluated pancreatic fat content in terms of fat percentage, which was higher in T2DM patients as compared to control subjects (+3.53% [95%CI: 0.85 to 6.21], I^2=83.3%, p for heterogeneity<0.001) (Figure 2C). Heterogeneity was not explained by either sensitivity analysis/meta-regression (data not shown) or subgroup analysis based on imaging methods; only one study (15) measured pancreatic fat content by CT. Meta-analysis of the additional four studies (16, 39-41), all of which using MRI, did not change heterogeneity (I^2=80.2%, p for heterogeneity=0.002). Similarly, heterogeneity was unchanged in subgroup analyses of studies with Newcastle-Ottawa scores of 6-8 or of studies with BMI-matched groups (data not shown).

Pancreatic density is an indirect form of evaluating fat content, as fat-enriched tissues have lower densities. Pancreatic density assessed by CT in two studies (15, 38) was lower in T2DM patients *vs.* control subjects (–4.98HU [95%CI: –6.76 to –3.21], I^2=0%, p for heterogeneity=0.395).

Interestingly, Yokota *et al* demonstrated a decrease in pancreatic density with increasingly impaired glucose homeostasis. Healthy individuals had higher pancreatic densities, which decreased progressively from impaired glucose tolerance to diabetes (27). However, Begovatz *et al* did not find differences in

pancreatic fat content between subjects with normal glucose, impaired fasting glucose, or T2DM patients when pancreatic fat was evaluated by MRI (30).

Quality of studies and small-study bias

The studies included in meta-analyses were assessed for quality using the Newcastle-Ottawa scale (Table 2). Overall, studies had low/moderate quality; most had a score of 6 or 7 points from a maximum of 9.

The funnel plot asymmetry test revealed no major small-study bias regarding volume or fat content in T2DM patients (p=0.458 and 0.621, respectively). However, a possible small-study bias was detected for volume in T1DM patients (p=0.041).

DISCUSSION

In this systematic review with meta-analysis of imaging studies, a reduction in pancreatic size was observed in both T1DM and T2DM patients. In addition, an increase in pancreatic fat content was seen in T2DM subjects.

Pancreatic size was evaluated in terms of diameter, area, and volume. In T1DM patients, the results show decreased pancreatic size in comparison to non-diabetic controls for all three parameters. In turn, volume, but not diameter, was reduced in T2DM patients; area was not meta-analyzed because only one study focusing on this aspect included T2DM subjects. Interestingly, a comparison between T1DM and T2DM revealed smaller pancreatic diameter in T1DM individuals. This analysis was not possible for area and volume because the number of studies was insufficient. A single study assessing pancreatic area showed smaller dimensions in T1DM

individuals *vs*. T2DM individuals, and the only study assessing volume observed no differences between T1DM and T2DM patients.

Volume, which provides three-dimensional data, is the best parameter to assess organ size. The present findings show smaller pancreatic volume in both T1DM and T2DM patients in relation to controls, but data are insufficient to establish a conclusion regarding the comparison between these two types of diabetes. However, our meta-analyses focusing on volume suggest that T1DM subjects may in fact have smaller pancreatic volume in relation to T2DM individuals: a difference of –38.72 cm^3 (95%CI: –52.25 to –25.19) was observed for T1DM *vs*. controls, and a difference of –12.18 cm^3 (95%CI: –19.1 to –5.25) was observed for T2DM *vs*. controls. Although a formal statistical test was not performed, it is fair to assume that pancreatic volume was smaller in T1DM than T2DM patients, since the 95%CIs do not overlap.

An intriguing finding of this systematic review is the low heterogeneity of MRI studies, as opposed to the high heterogeneity of CT studies. Moreover, the magnitude of volume reduction detected by each imaging method was remarkably different (T1DM: –44.08 cm^3 for MRI *vs*. –26.3 cm^3 for CT; T2DM: –21.65 cm^3 for MRI *vs.* –7.5cm^3 for CT), with MRI showing consistently smaller volumes than the results obtained by CT. These differences were unexpected, since the tool used to measure pancreatic volume is similar in both imaging methods and no plausible technical reason can justify lower volumes measured by MRI. Furthermore, a recent study evaluating T1DM patients with MRI or CT did not report differences in pancreas size measured by the two methods (42).

There is a large inter-individual variation in pancreas morphology and volume related to body size and age in healthy populations (43, 44). This may be a relevant source of confusion in studies with T1DM and T2DM individuals – T1DM patients are usually younger, and, as shown in the present study, possibly have a smaller pancreas; conversely, T2DM patients might be older than controls, and pancreas size may decrease with age (34). However, most studies in the present review included BMI- and age-matched controls, and neither subgroup analysis nor meta-regression considering these possible confounders showed an impact on heterogeneity. Reduced pancreatic volume and weight are present from early phases of T1DM, as demonstrated by a study comparing the pancreas of T1DM donors and controls (12), even after correction for confounders (13). Recently, Virostko *et al* (42) have suggested progressively smaller pancreatic volume with increased duration of T1DM (decline rate of 0.013 cm^3/kg per year). This is supported by the findings of our meta-regression showing that TD1M patients with longer disease duration had lower pancreatic volumes. Thus, monitoring variations in pancreatic volume might be useful to predict diabetes in high-risk individuals (12).

Pancreatic fat content is evaluated by means of density or fat percentage, with percentage being more precise. Some studies suggest an association between increased pancreatic fat and diabetes. Kim *et al* (21) have shown that two CT indexes – the difference between pancreatic and splenic density and the pancreas to spleen density ratio – are lower in patients with impaired glucose tolerance than in subjects with normal glucose metabolism. In line with this, a study designed to compare pancreatic fat content and beta cell function found

increased lipid deposition in the pancreas of diabetic patients as compared to healthy subjects (22). Furthermore, in T2DM patients, obesity was associated with lower pancreatic density evaluated by CT, indicating higher pancreatic fat content (29). Our data indicate that pancreatic fat content is increased in T2DM patients, which may reflect a paracrine effect of insulin. Insulin resistance causes increased insulin secretion by beta cells, and the higher local insulin concentration may induce fat deposition. A similar phenomenon occurs in the liver when pancreatic islets are transplanted into the portal vein (45-47). Pancreatic islets delivered to the hepatic sinusoids engraft and produce insulin, and focal steatosis is observed in 20% to 60% of islet recipients (45-47). More interestingly, transplanted islets surrounded by fat have reduced function, probably as a result of lipotoxicity (47, 48). Conversely, a low-fat diet and leptin overexpression have been shown to reduce fat content around islets, improving islet function in an animal model (48). Taken together, these findings suggest that pancreatic fat accumulation might be a result of the higher local insulin levels in an insulin-resistant environment, and that pancreatic lipid deposition may further impair islet function.

Our results have some practical implications. First, the finding of a small or fatty pancreas using imaging techniques should prompt a recommendation for proper biochemical investigation of diabetes. Second, as there is some evidence in the literature linking pancreas atrophy in T1DM and T2DM patients with pancreatic exocrine deficiency (28, 49), differential diagnosis of chronic diarrhea in diabetic patients should consider exocrine pancreatopathy, a hypothesis which could be corroborated by diagnostic imaging.

The present review has limitations that must be addressed. First, there are few studies assessing each parameter, precluding adequate exploration of heterogeneity and increasing the risk for small-study bias. Additionally, the overall quality of studies ranged from low to moderate. However, we believe that the findings of increased fat content and decreased pancreas size consistently point in the same direction and should not be dismissed.

In summary, the present data indicate that reduced pancreas size and increased fat content are features of diabetes. Further longitudinal studies are required to elucidate the cause and effect relationship between pancreatic size and diabetes, as well as the possible causes of pancreas shrinkage and fat deposition. A better understanding of the mechanisms of altered pancreas morphology and fat deposition in diabetes may lead to new insights in preventing, predicting, and treating patients with diabetes.

Author contributions: T.S.G participated in study conception and design, data acquisition, analysis and interpretation of data, drafting and revision of the manuscript, and statistical analysis. T.H.R participated in data acquisition, analysis and interpretation, and drafting and revision of the manuscript. C.B.L participated in the study conception and design, analysis and interpretation of data, revision of the manuscript, and statistical analysis. C.B.L is the guarantor of this work and, as such, had full access to all data and takes responsibility for the integrity of the data and the accuracy of data analysis.

ACKNOWLEDGMENTS

We thank Caroline K. Kramer, University of Toronto, for expert statistical counseling, and Fundo de Incentivo à Pesquisa e Ensino do Hospital de Clínicas de Porto Alegre (FIPE) for funding.

Funding: Fundo de Incentivo à Pesquisa e Ensino do Hospital de Clínicas de Porto Alegre. CBL receives a scholarship from Conselho de Nacional de Desenvolvimento Científico e Tecnológico (CNPq).

The authors declare that they have no conflict of interest.

LEGENDS TO THE FIGURES

Figure 1. Flowchart of study selection.

Figure 2. Meta-analyses of studies evaluating pancreas by imaging in diabetes. (A) Forest plot comparing pancreas volume (cm^3) in type 1 diabetic patients with a control group. (B) Forest plot comparing pancreas volume (cm^3) in type 2 diabetic patients with a control group. (C) Forest plot comparing fat content (%) in type 2 diabetic patients with a control group. WMD=weighted mean difference.

LEGENDS TO THE SUPPLEMENTARY MATERIAL

Supplemental Figure S1. Search strategy used for study selection.

Supplemental Figure S2. Bubble plot of the relation between diabetes duration (years) and pancreatic volume (cm^3) in type 1 diabetic patients. WMD=weighted mean difference.

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Table 1. Summary of studies evaluating pancreas size and fat content by imaging methods in diabetes

Authors, year	No. of subjects			Method	Parameter
	Type 1 diabetes	Type 2 diabetes	Controls		
Fonseca et al, 1985 (26)	32	22	19	US	Area
Silva et al, 1993* (17)	36	40	60	US	Diameter
Alzaid et al, 1993* (14)	43	14	19	US	Area
Rajput et al, 2001* (32)	0	35	15	US	Area
Basiratnia et al, 2007* (31)	60	60	60	US	Diameter
Gilbeau et al, 1992* (38)	37	20	57	CT	Density
Goda et al, 2001* (18)	29	26	22	CT	Volume
Phillipe et al, 2001 (28)	28	24	0	CT	Volume
Saisho et al, 2007* (34)	165	0	660	CT	Volume
Yokota et al, 2012 (27)	62	0	53	CT	Density
Lim et al, 2014* (15)	156	0	50	CT	Volume, density, fat%
Kim et al, 2014 (22)	18	0	33	CT	p-s, p/s
Kim et al, 2014 (29)	198	0	0	CT	Density
Tushuizen et al, 2007 (23)	12	0	24	MRI	Fat%
Williams et al, 2007* (33)	0	12	12	MRI	Volume
Sequeiros et al, 2010* (35)	0	12	12	MRI	Volume
Lim et al, 2011* (39)	11	0	9	MRI	Fat%
Williams et al, 2012* (36)	0	19	24	MRI	Fat%
Burute et al, 2014* (37)	32	0	50	MRI	Volume
Ma et al, 2014* (40)	24	0	10	MRI	Fat%
Percival et al, 2014* (41)	71	0	9	MRI	Fat%
Macauley et al, 2015* (16)	41	0	14	MRI	Volume, fat%
Begovatz et al, 2015 (30)	14	0	28	MRI	Fat%

US: ultrasound; CT: computed tomography; MRI: magnetic resonance image; fat%: pancreatic fat percentage; P-S: difference between pancreatic and splenic density; P/S: pancreas-to-spleen density ratio. *: studies included in meta-analyses.

Table2. Newcastle-Ottawa quality assessment of studies included in meta-analyses.

	Selection				Comparability	Outcome			
Authors, year	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**Score**
Gilbeau et al, 1992 (38)				*	** (age, diabetes duration)	*	*	*	6
Silva et al, 1993 (17)				*		*	*	*	4
Alzaid et al, 1993 (14)	*	*		*		*	*	*	6
Rajput et al, 2001 (32)	*			*	** (age, sex, BMI)	*	*	*	7
Goda et al, 2001 (18)		*		*	** (age, sex)	*	*	*	7
Basiratnia et al, 2007 (31)	*		*	*	** (age, sex)	*	*	*	8
Saisho et al, 2007 (34)	*	*		*	** (age, BMI)	*	*	*	8
Williams et al, 2007 (33)	*			*		*	*	*	5
Sequeiros et al, 2010 (35)	*			*	** (age, sex)	*	*	*	7
Lim et al, 2011 (39)	*			*	** (age, sex, weight)	*	*	*	7
Williams et al, 2012 (36)	*	*		*	** (age, weight)	*	*	*	8
Lim et al, 2014 (15)	*			*	** (age, BMI)		*	*	6
Burute et al, 2014 (37)	*	*		*	** (age, sex, weight)	*	*	*	8
Ma et al, 2014 (40)	*	*		*	* (age)		*	*	6
Percival et al, 2014 (41)	*			*		*		*	4
Macauley et al, 2015 (16)	*			*	** (age, sex, weight)		*	*	6

The number of stars indicates the quality of each item evaluated: minimum 0, maximum 1 star for selection and outcome; and minimum 0, maximum 2 stars for comparability. The maximum possible overall score is 9.

Figure 1. Flowchart of study selection.

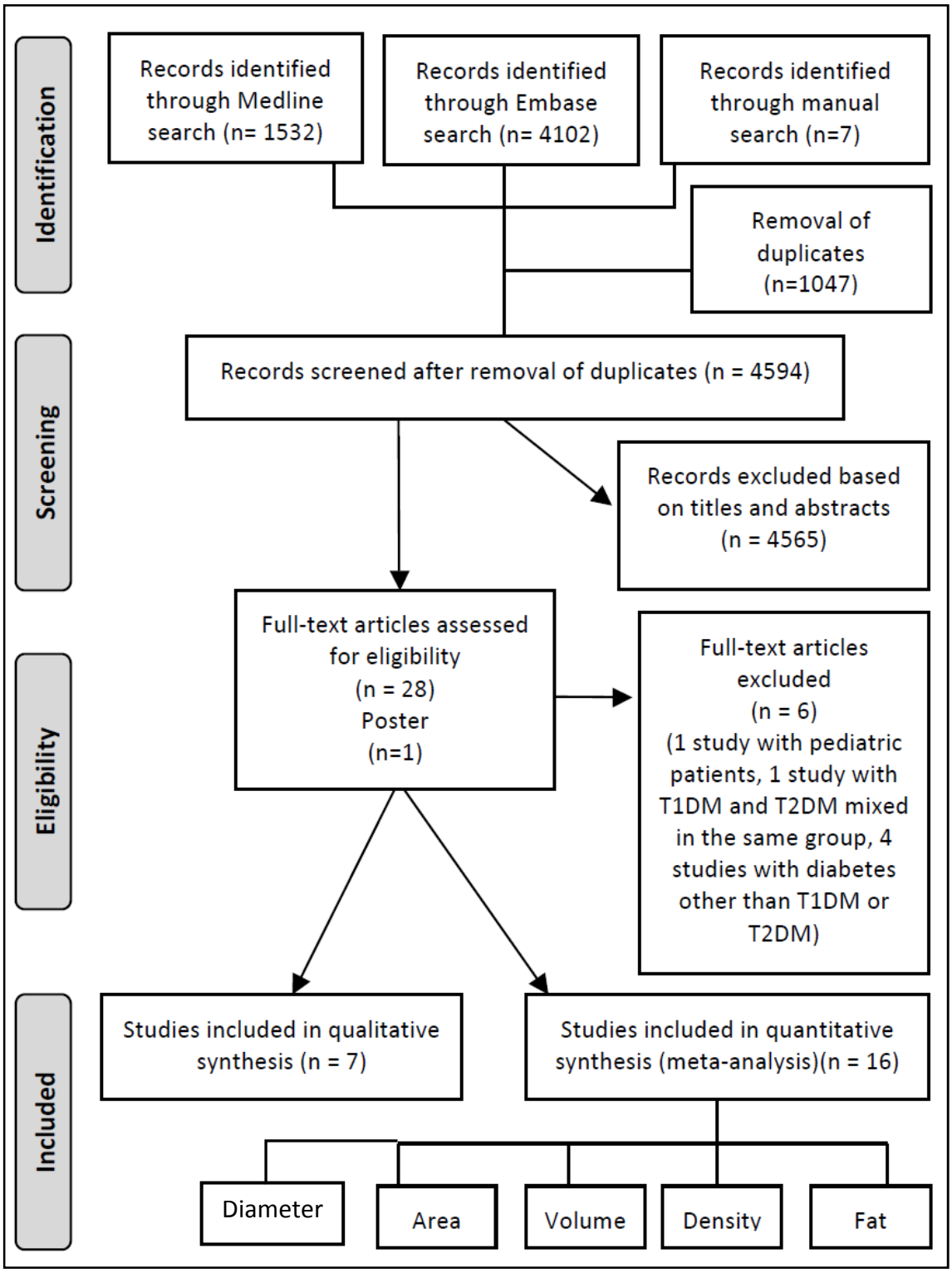


Figure 2. Meta-analyses of studies evaluating pancreas by imaging in diabetes.
2 (A) Forest plot comparing pancreas volume (cm^3) in type 1 diabetic patients with a control group.

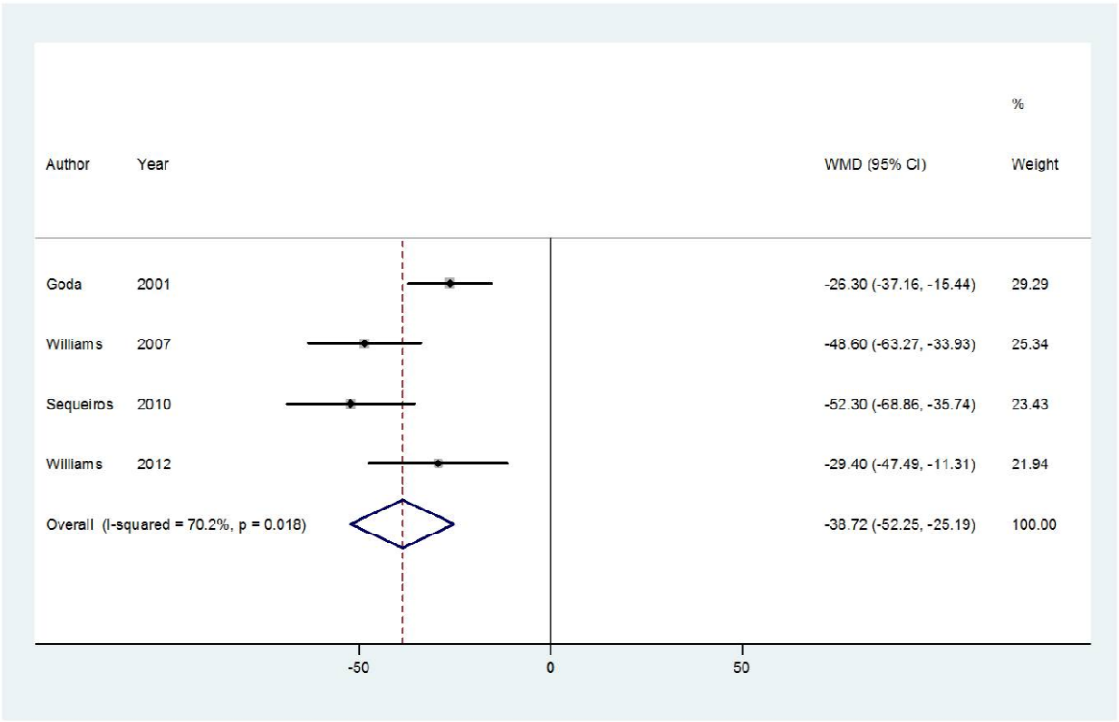


Figure 2. Meta-analyses of studies evaluating pancreas by imaging in diabetes.

2 (B) Forest plot comparing pancreas volume (cm^3) in type 2 diabetic patients with a control group.

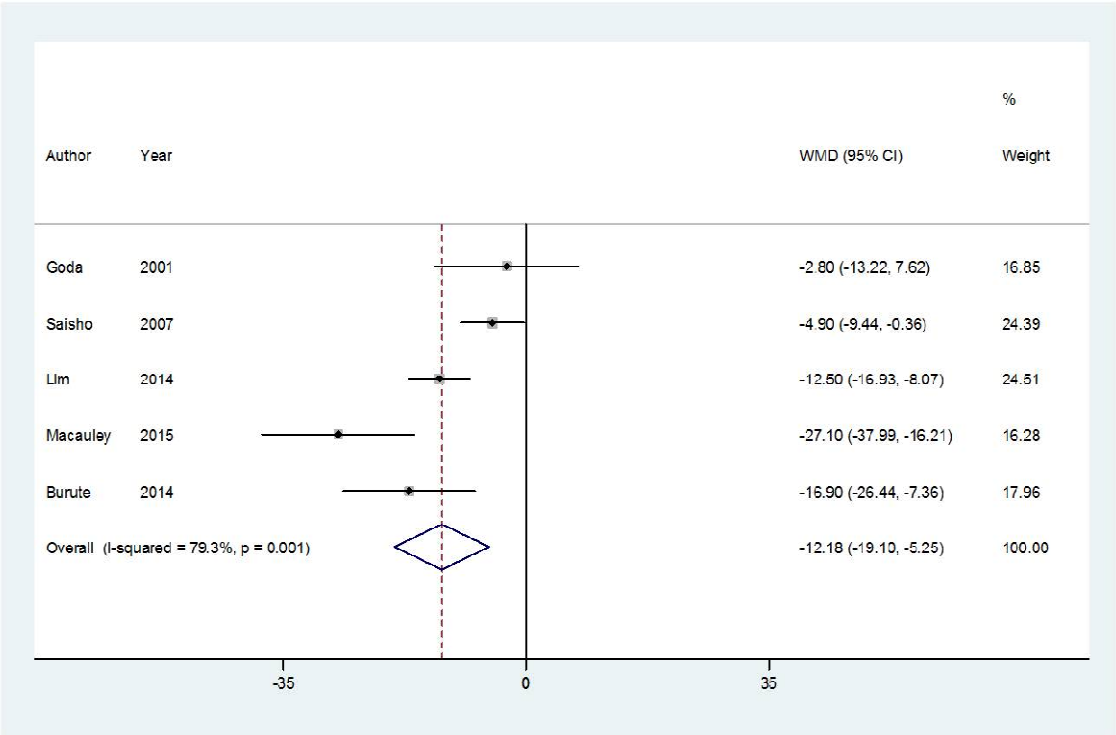
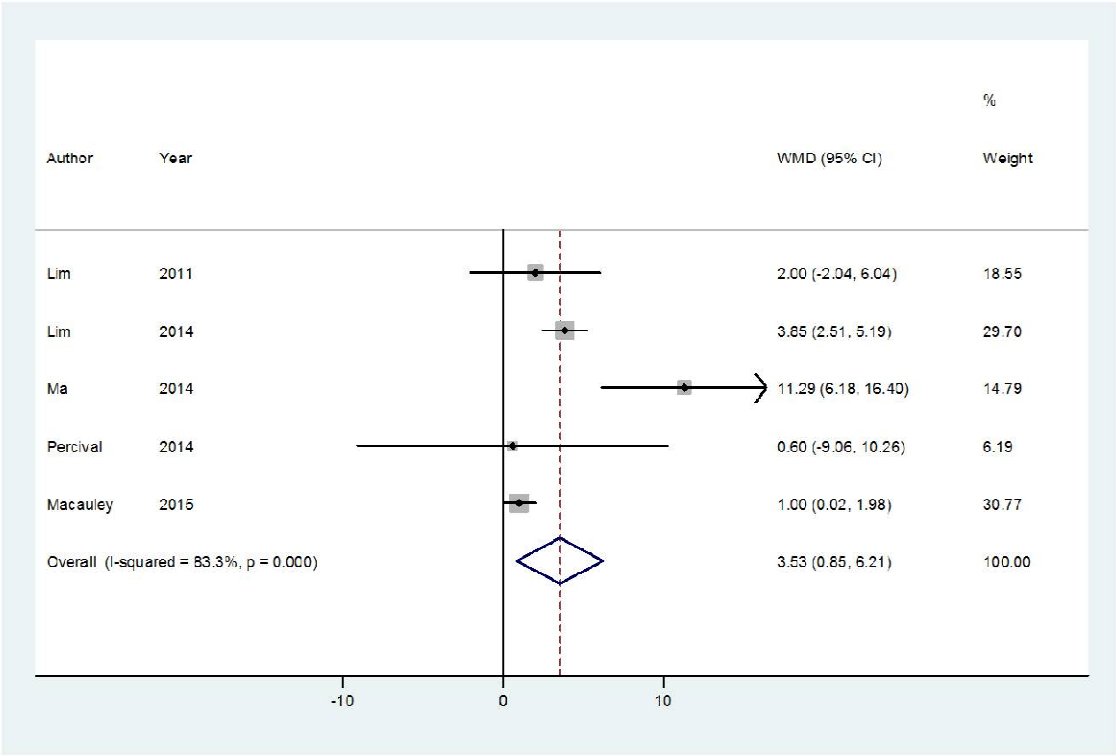


Figure 2. Meta-analyses of studies evaluating pancreas by imaging in diabetes.

2 (C) Forest plot comparing fat content (%) in type 2 diabetic patients with a control group. WMD=weighted mean difference.



Supplemental Figure S1. Search strategy used in the study.

Medline:

((pancreas[MeSH Terms]) AND diabetes[MeSH Terms]) AND tomography[MeSH Terms]

((pancreas[MeSH Terms]) AND diabetes[MeSH Terms]) AND imaging[MeSH Terms]

((pancreas[MeSH Terms]) AND diabetes[MeSH Terms]) AND radiology[MeSH Terms]

((pancreas[MeSH Terms]) AND diabetes[MeSH Terms]) AND magnetic resonance[MeSH Terms]

((pancreas[MeSH Terms]) AND diabetes[MeSH Terms]) AND ultrasound[MeSH Terms]

Embase:

'pancreas'/exp OR pancreas AND ('diabetes'exp OR diabetes) AND ('tomography'/exp OR tomography)

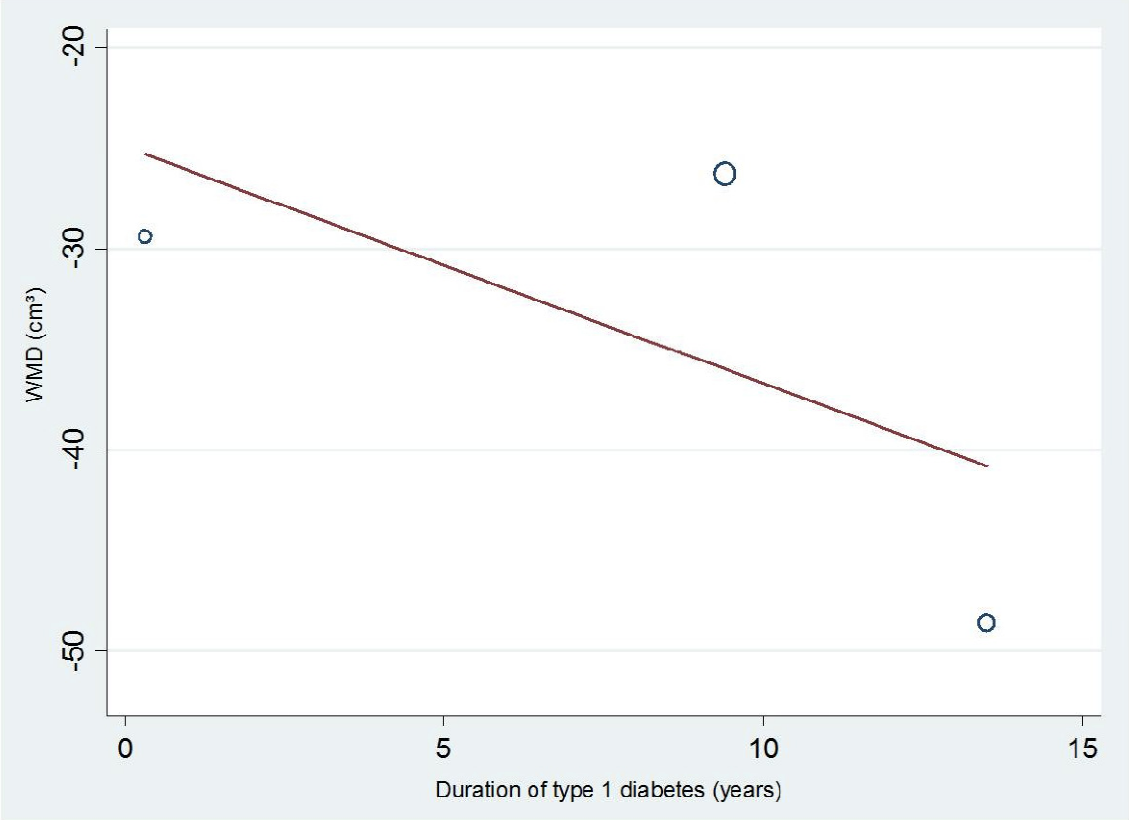
'pancreas'/exp OR pancreas AND ('diabetes'exp OR diabetes) AND imaging

'pancreas'/exp OR pancreas AND ('diabetes'exp OR diabetes) AND magnetic AND resonance

'pancreas'/exp OR pancreas AND ('diabetes'exp OR diabetes) AND radiology

'pancreas'/exp OR pancreas AND ('diabetes'exp OR diabetes) AND ultrasound

Supplemental Figure S2. Bubble plot of the relation between diabetes duration (years) and pancreatic volume (cm3) in type 1 diabetic patients. WMD=weighted mean difference.



CAPÍTULO 3

Intra- and interobserver variability in pancreatic CT perfusion

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Running title: Variability in pancreatic CT perfusion

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Keywords: pancreas; imaging; radiology; tomography and perfusion.

Abstract

Objective: measure intra and interobserver agreement among radiologists with different levels of experience in the assessment of pancreatic perfusion on CT.

Material and methods: thirty-nine perfusion CT scans from subjects who were referred to abdominal CT for reasons not related to pancreatic symptoms or disease at Jules Bordet Institute (Brussels, Belgium) were analyzed. The study was approved by the Ethics Committee of Jules Bordet Institute. Images were analyzed by two radiologists with sixteen (reader 1) and twenty-five years (reader 2) of experience in abdominal imaging. The following parameters were measured: blood flow (BF), blood volume (BV), mean transit time (MTT) and time to peak (TTP).

Results: There was no significant intra-observer variability for reader 1 and reader 2 for BF, BV, MTT or TTP. Regarding interobserver variability, there were significant differences between reader 1 and reader 2 for BV in pancreatic head (mean difference: 0.63mL/100L±1.5; 95%CI: -2.4 to 3.6, p= 0.015), BV in pancreatic body (mean difference: 0.82mL/100L±2.1; 95%CI: -3.3 to 5.0, p= 0.021) and BV in whole pancreas (mean difference: 0.71mL/100L±1.3; 95%CI: 1.9 to 3.3, p= 0.002).

Conclusion: our data support the use of CT perfusion by radiologists of different levels of experience. Caution must be taken for measurement of BV, as interobserver agreement was poor for this parameter.

Introduction

Ultrasound, computed tomography (CT) and magnetic resonance imaging (MRI) may be used in tissue perfusion studies (1-7). CT, however, has a linear relationship between concentration of iodinated contrast media and the recorded density in Hounsfield units (8-10), and may be considered the preferred technique for acquisition of perfusion images (11). Perfusion CT is a relatively recent technique and is able to provide qualitative and quantitative information regarding perfusion parameters of tissues in a non-invasive way.

In 1995, Miles *et al* (12) performed the first study showing the feasibility of pancreatic perfusion CT. Afterwards, several studies have assessed normal values of pancreatic perfusion by CT, pancreatic perfusion impairments in pancreatic and hepatic diseases and the modifications of pancreatic perfusion after oncologic therapy (8, 11, 13-25).

The observers are a substantial source of variability in the performance of a diagnostic test (26). In clinical practice, radiologist-dependent factors may contribute to measurement inconsistencies (e.g., variations in measurement technique or experience) (27-32). Therefore, intra and inter-observer variability has to be assessed in order to guarantee the accuracy of the radiologic readings.

Little information is available about pancreatic perfusion CT regarding intra and interobserver variability (33, 34). Therefore, the purpose of this study is to measure intra and interobserver agreement among radiologists with different levels of experience in the assessment of pancreatic perfusion on CT.

Material and methods

We prospectively analyzed 12 scans from subjects who were referred to abdominal CT perfusion at Jules Bordet Institute (Brussels, Belgium) for reasons not

related to pancreatic symptoms or disease, from October 2015 to September 2016. Twenty-seven scans were additionally included from a CT archive. Informed consent was obtained from all participants that were prospectively included. The study was approved by the Ethics Committee of Jules Bordet Institute. Exclusion criteria were pregnancy, history of allergic reaction to iodinated contrast media, renal insufficiency and history of pancreatic disease.

All patients were scanned in a Siemens Somatom® Force 192-slices scanner (Munich, Germany). The perfusion-CT examination was performed in the interval between unenhanced and portal phases. A test phase, in order to define a correct delay for the perfusion CT, was performed after injection of 10mL of nonionic contrast medium (Iomeron 400) and a bolus of 21mL of saline solution, with a delay of 8s. For this test phase, a region of interest (ROI) was placed on distal thoracic aorta and 15 images were acquired (1 every 2s, rotation time: 0.25s, 40Mas, 100Kvp), so a curve of aortic enhancement could be obtained (software DynEva®, Siemens). The time needed to achieve the peak of aortic enhancement was used to define the delay for the perfusion CT. After that, 50mL of a nonionic contrast medium (Iomeron 400) were injected through a 18-gauge catheter in an antecubital vein using a flow rate of 4.0 mL/s, followed by a chaser bolus of 21mL of saline solution. CT tube voltage was 80 kilovolt peak (kVp). The dynamic imaging sequence consisted of 31 acquisitions of 0.25-second duration (rotation time) with a time interval of 1.5 s (cycle time), resulting in a total examination time of 45.45 s. The perfusion sequence covered a cranio-caudal width of 24 cm (collimation of 48 x 1,2 mm). A portal phase was acquired with a delay of 70s after the beginning of the IV injection of 70mL of contrast media at the end of perfusion CT (Table 1).

Images were analyzed by two radiologists (T.S.G. - reader 1, J.L.E. – reader 2) with sixteen and twenty-five years of experience in abdominal imaging, respectively. Each reader performed two reading sessions, with at least 24 hours of interval. Image data were processed on a workstation (Syngo.via®, Siemens) loaded with commercial perfusion CT software (CT Body Perfusion, Siemens) based on maximum slope model. The following parameters were measured: blood flow (measured in mL/100 mL/min), blood volume (measured in mL/100 mL), time to peak (measured in seconds), and mean transit time (measured in seconds). The arterial input was measured by automatically placed ROI within the abdominal aorta. To obtain perfusion CT parameters, each radiologist manually drew three non-superposable circular ROI of in the head, three in the body and three in the tail of the pancreas to measure these parameters, avoiding visible vessels and ducts. The mean ROI value of each parameter on each part of the pancreas was considered for analysis. An example of perfusion CT image processing is shown in Figure 1.

Statistical analysis

Data are expressed as mean (± SD). The Bland-Altman statistic was used in order to measure both intra and interobserver agreement. Lin's concordance correlation coefficients (CCC) were measured to estimate reliability between reader 1 and reader 2 (0-0.20, poor correlation; 0.21–0.40, fair; 0.41–0.60, moderate; 0.61–0.80, substantial; 0.81–1.00, almost perfect). P-value <0.05 was considered statistical significant.

Results

A total of 39 patients [men: n = 21 (53.8 %)] were included, with a mean age of 64 years. Seventeen patients had DM2 and 22 did not. Two patients were excluded due to technical problems, which lead to difficulties to measure pancreatic perfusion parameters (large ascites and improper contrast media injection).

In Bland-Altman analysis, there was no significant intra-observer variability for both reader 1 and reader 2 (Table 2). Regarding interobserver variability, there were significant differences between reader 1 and reader 2 for BV in pancreatic head (mean difference: 0.63mL/100L ±1.5; 95%CI: -2.4 to 3.6, p= 0.015), BV in pancreatic body (mean difference: 0.82mL/100L ±2.1; 95%CI: -3.3 to 5.0, p= 0.021) and BV in whole pancreas (mean difference: 0.71mL/100L ±1.3; 95%CI: 1.9 to 3.3, p= 0.002). For the other parameters, no interobserver variability was observed (Table 3). Bland-Altman plots for mean difference between reader 1 and reader 2 regarding BF, BV, MTT and TTP in the whole pancreas are shown in Figure 2.

All variables evaluated had a statistical significant Lin's CCC, when agreement between readers was quantified. The correlation was substantial to almost perfect for the majority of the parameters (Table 4), with the exception of MTT in pancreatic body and tail (fair correlation) and BF in tail and MTT in whole pancreas (moderate correlation). BV, which did not agree between readers in Bland-Altman analysis, had substantial (BV in body and tail) and almost perfect Lin's CCC (BV in head and in whole pancreas).

Discussion

Analysis of intra and interobserver agreement is essential in imaging methods. Our results show an overall good intra and interobserver agreement for parameters of pancreatic perfusion by CT. However, a poor interobserver agreement for BV was observed in pancreatic head, in pancreatic body and in whole pancreas. Interestingly, all four parameters measured in pancreatic head showed substantial or almost perfect correlation, suggesting that in studies focused on evaluation of pancreatic perfusion this part of the organ should be preferred for measurements. Of note, interobserver correlation for TTP was almost perfect in all regions of the pancreas, supporting its use

on pancreatic perfusion CT. In turn, MTT had an overall correlation from fair to moderate; consequently, this parameter might be less useful. Regarding BF, even if the mean difference between reader 1 and reader 2 was just 4.9mL/100mL/s, the upper and the lower limits of agreement were too far apart (respectively 60.86mL/100mL/s and -51.03mL/100mL/s) showing that despite the mean difference was acceptable, difference between readings of each subject was excessive.

Measurement reproducibility and accuracy are of particular interest in radiology, as important clinical decisions are often based on CT measurements (35, 36). Accordingly to McErlean *et al* (37), "lesion measurements on images should be accurate, reproducible, and performed in a standardized fashion with low rates of intra- and interobserver variability". Li *et al* (33) reported an inter-observer correlation higher than 0.9 for BF and BV in normal pancreas. Xie *et al* (34) also observed substantial agreement (0.85), but these authors only tested subjective image quality scores for pancreatic perfusion CT.

Herein we evaluated interobserver agreement by two methods: Bland-Altman and Lin's CCC. These methods are alternatives rather than complementary. Bland-Altman plot is useful for checking differences between measures and the limits of agreement, while Lin's CCC is an index of reliability instead of difference.

This study has some limitations. We tested intra and interobserver with base on readings of only two radiologists, which is a limitation of our work. This small number of observer may not truly represent the actual community of radiologists.

In conclusion, our data support the use of CT perfusion by radiologists of different levels of experience. Pancreatic head is the preferred region for measurements to be performed. Caution must be taken for measurement of BV, as interobserver agreement was poor in Bland-Altman analysis.

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Table 1. Protocol of CT acquisition

CT Scanning parameters	**Precontrast**	**Perfusion**	**Venous**
Voltage (kVp)	90	80	90
Mean scanning delay after contrast injection (s)		test*	70
Collimation (mm)	192 x 0.6	48 x 1.2	128 x 0.6
Rotation time (s)	0.5	0.25 (full rotation) 1.5 (cycle time)	0.5
Pitch	0.6	0.6	0.8
Kernel	Br36	Br32	Br40
Slice thickness reconstructed (mm)	3	5	3
Contrast agent dose (mL)		50	70
Contrast injection rate (mL/s)		4	4
Number of acquisitions	helical	31	helical
Bolus NaCl (mL)		21	21

*depends on test phase

Table 2. Intra-observer variability. Paired differences for pancreatic perfusion parameters for each reader on both reading sessions

	Reader 1 (n = 37)			Reader 2 (n = 37)		
	Mean±SD	**95%CI**	**p**	**Mean±SD**	**95%CI**	**p**
BF head	-2.81±24.3	-50.4;44.8	.486	-1.78±23.5	-47.9;44.3	.657
BF body	6.37±21.7	-36.2;48.9	.083	4.42±19.2	-33.2;42.0	.182
BF tail	4.80±46.7	-86.7;96.3	.535	0.04±21.6	-42.3;42.4	.992
BF whole pancreas	2.79±24.5	-43.3;48.8	.475	0.89±16.7	-32.4;34.2	.757
BV head	-0.14±1.9	-3.9;3.6	.657	-0.01±1.8	-3.5;3.5	.425
BV body	0.22±1.7	-3.1;3.5	.425	-0.23±1.9	-4.0;3.6	.475
BV tail	0.22±2.1	-4.0;4.4	.546	0.24±2.2	-4.3;4.3	.947
BV whole pancreas	0.10±1.1	-2.0;2.9	.567	0.09±1.4	-2.8;2.6	.701
MTT head	0.05±1.4	-2.6;2.7	.808	-0.003±1.2	-2.3±2.3	.985
MTT body	-0.28±0.1	-2.2;1.6	.093	-0.18±1.2	-2.5±2.2	.366
MTT tail	-0.19±1.4	-3.0;2.6	.415	-0.16±1.1	-2.3±2.0	.375
MTT whole pancreas	-0.17±0.9	-2.0;1.6	.267	-0.11±0.9	-1.9±1.7	.450
TTP head	-0.28±1.4	-2.8;2.7	.904	0.08±1.8	-3.4;3.6	.785
TTP body	-0.23±1.7	-3.5;3.1	.407	-0.04±1.4	-2.8;2,7	.864
TTP tail	0.08±1.5	-2.9;3.1	.744	0.47±1.6	-2.6;3.5	.079
TTP whole pancreas	-0.06±0.8	-1.7;1.6	.671	0.17±0.9	-1.6;2.0	.269

BF: blood flow in mL/100mL/min; BV: blood volume in mL/100mL; MTT: mean transit time in s; TTP: time to peak in s.

Table 3. Interobserver variability. Paired differences for pancreatic perfusion parameters between readers 1 and 2 considering both reading sessions

	Mean±SD (n = 37)	**95%CI**	**p**
BF head	2.81±24.0	-47.0;52.6	.497
BF body	0.58±28.8	-57.1;58.2	.903
BF tail	11.35±40.9	-70.5;93.2	.100
BF whole pancreas	4.91±28.0	-51.0;60.9	.292
BV head	0.63±1.5	-2.4;3.6	.015
BV body	0.82±2.1	-3.3;5.0	.021
BV tail	0.67±2.0	-3.3;4.7	.050
BV whole pancreas	0.71±1.3	-1.9;3.3	.002
MTT head	0.29±0.9	-1.4;2.0	.050
MTT body	0.43±1.4	-2.3;3.2	.065
MTT tail	0.12±1.4	-2.7;2.9	.601
MTT whole pancreas	0.27±1.0	-1.8;2.3	.124
TTP head	-0.13±1.2	-2.5;2.3	.528
TTP body	0.20±1.0	-1.8;2.2	.228
TTP tail	0.21±1.1	-2.0;2.4	.257
TTP whole pancreas	0.94±0.6	-1.2;1.4	.382

Blood flow in mL/100mL/min; blood volume in mL/100mL; mean transit time in s; time to peak in s.

Table 4. Interobserver correlation of pancreatic perfusion parameters.

	CCC	95%CI	p
BF head	0.76	0.62;0.90	<.001
BF body	0.72	0.56;0.88	<.001
BF tail	0.42	0.16;0.68	.002
BF whole pancreas	0.68	0.51;0.86	<.001
BV head	0.87	0.80;0.95	<.001
BV body	0.79	0.67;0.91	<.001
BV tail	0.79	0.67;0.91	<.001
BV whole pancreas	0.89	0.80;0.95	<.001
MTT head	0.71	0.54;0.87	<.001
MTT body	0.39	0.13;0.65	.004
MTT tail	0.38	0.10;0.66	.007
MTT whole pancreas	0.45	0.20;0.71	<.001
TTP head	0.88	0.80;0.95	<.001
TTP body	0.92	0.87;0.96	<.001
TTP tail	0.89	0.82;0.96	<.001
TTP whole pancreas	0.96	0.94;0.99	<.001

Blood flow in mL/100mL/min; blood volume in mL/100mL; mean transit time in s; time to peak in s.

CCC: Lin's concordance correlation coefficient

Figure 1 (A). Perfusion CT image processing.

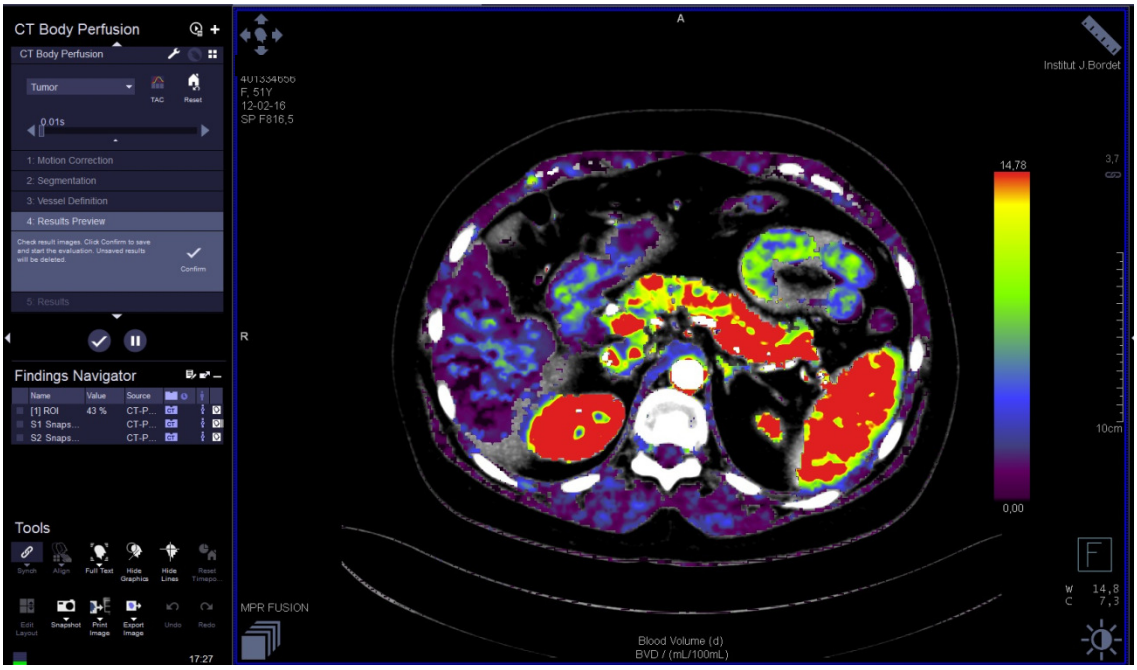


Figure 1 (B). CT ROI positioning in pancreatic tail.

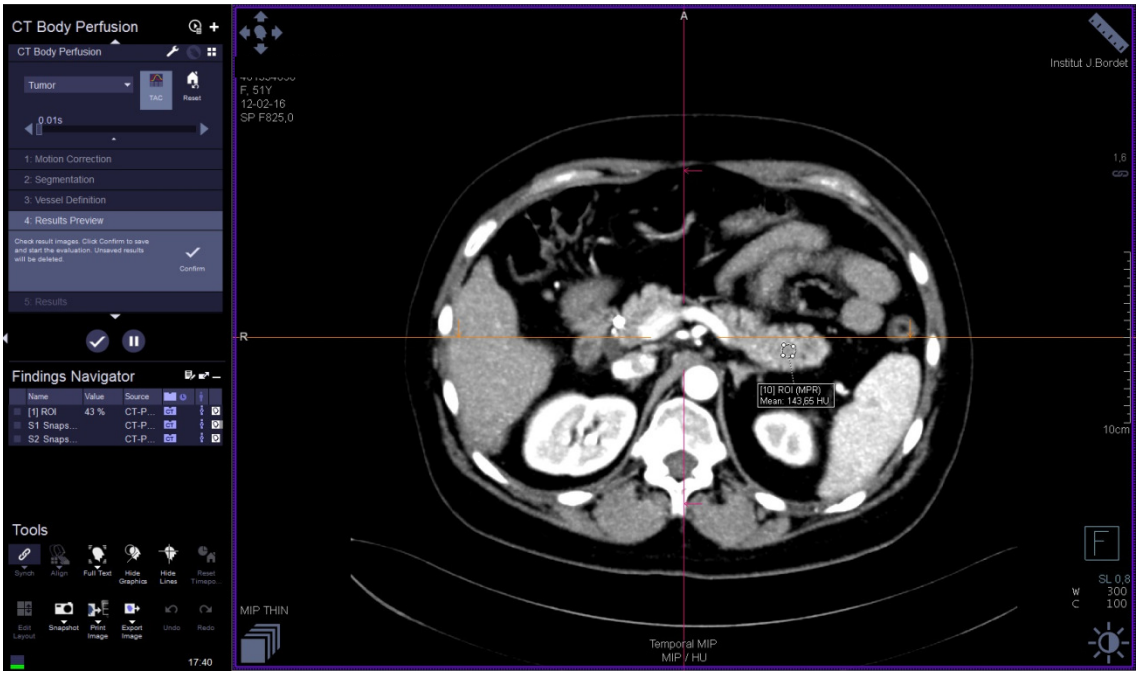


Figure 1 (C). Perfusion CT curves for BF, BV, MTT and TTP.

ROI/VOI	Legend	MIP	BFD	BVD	MTTD	FED
[2] ROI		154,85	85,74	13,68	9,58	82,72
[3] ROI		154,51	89,30	14,48	9,73	76,13
[4] ROI		155,73	89,28	14,43	9,69	84,37

Figure 2 (A). BF (mL/100mL/s) in whole pancreas: Bland-Altman plot for mean difference between reader 1 and reader 2. ULA: 95% upper limit of agreement. LLA: 95% lower limit of agreement.

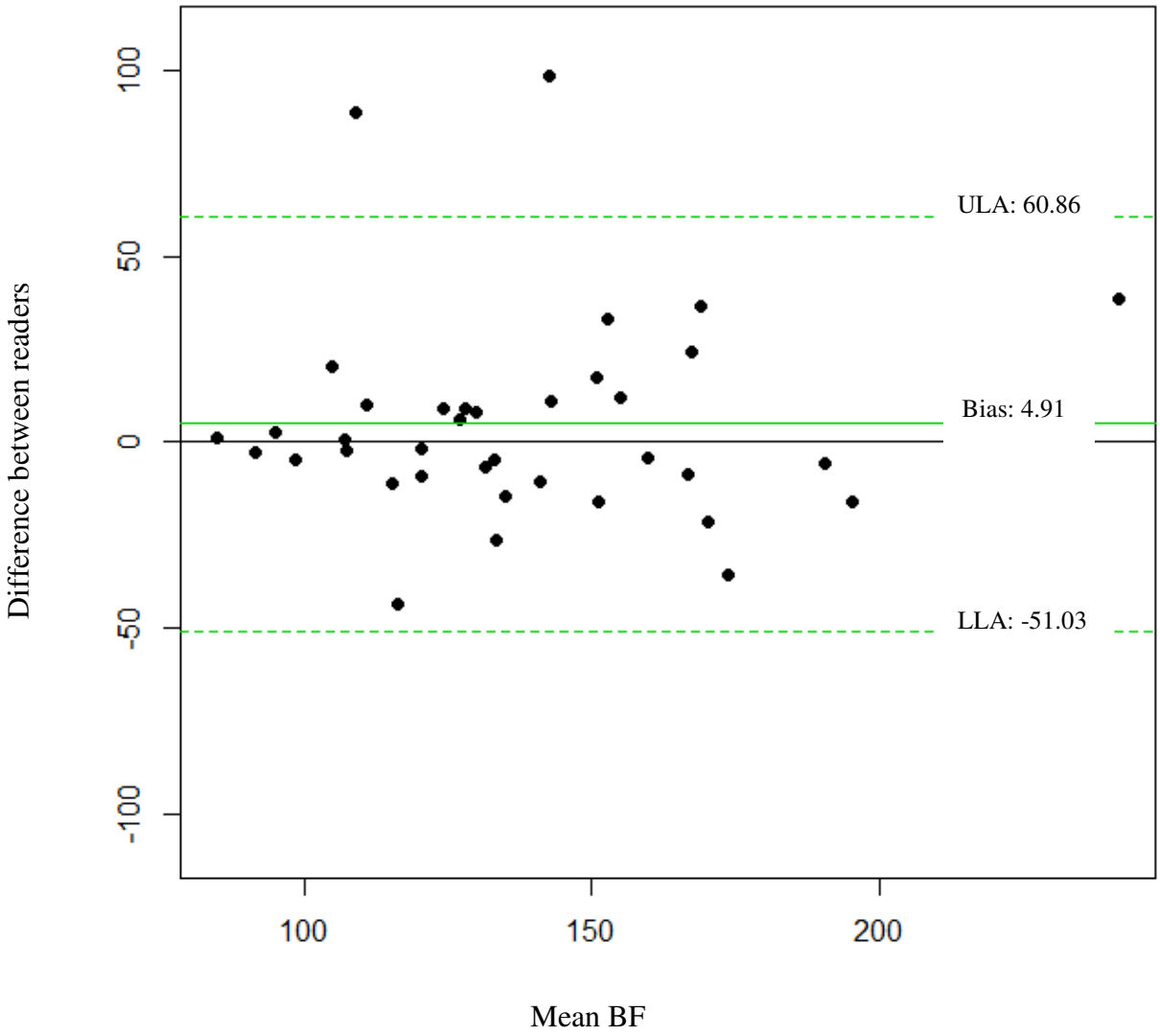


Figure 2 (B). BV (mL/100mL) in whole pancreas: Bland-Altman plot for mean difference between reader 1 and reader 2. ULA: 95% upper limit of agreement. LLA: 95% lower limit of agreement.

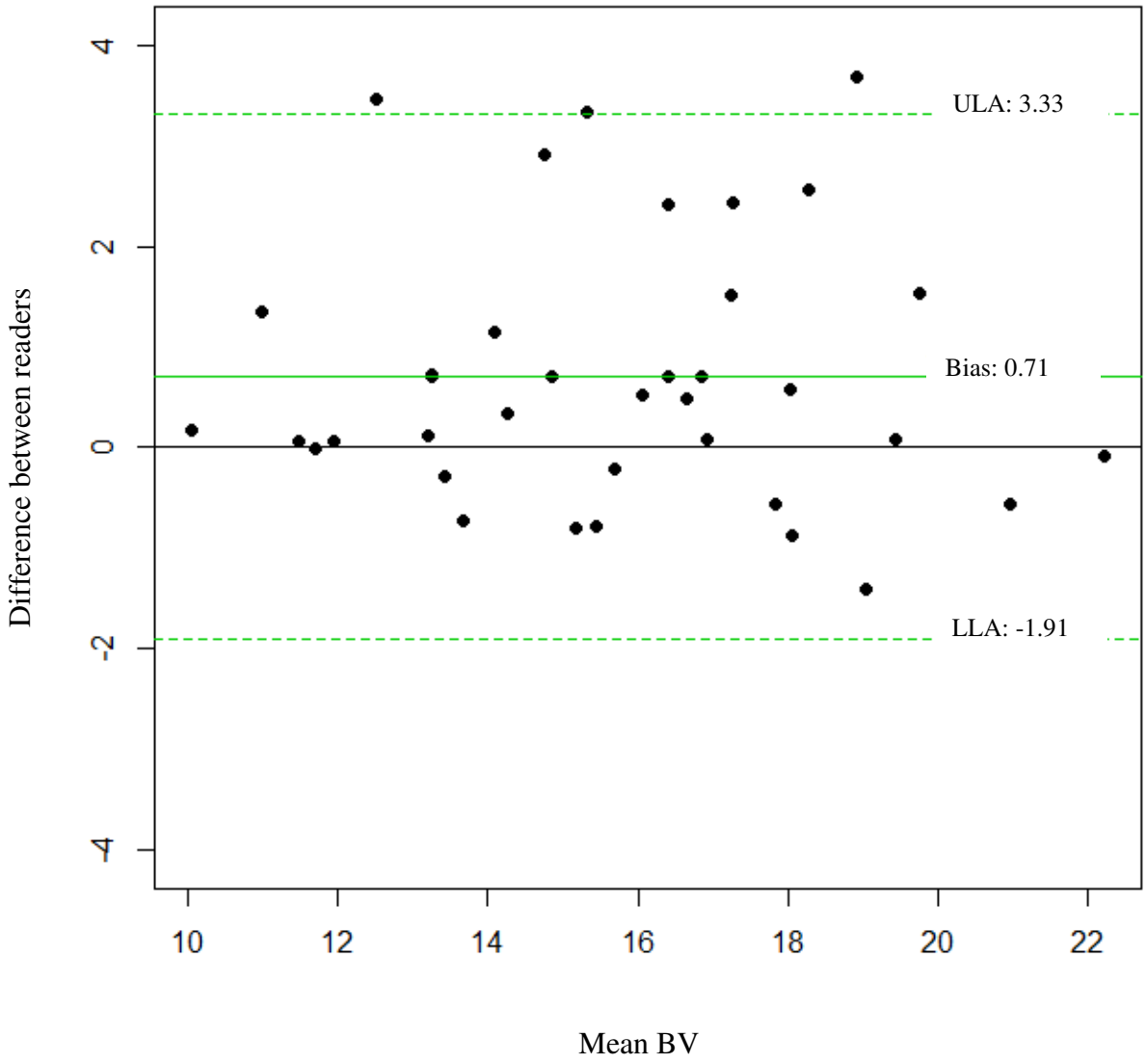


Figure 2 (C). MTT (s) in whole pancreas: Bland-Altman plot for mean difference between reader 1 and reader 2. ULA: 95% upper limit of agreement. LLA: 95% lower limit of agreement.

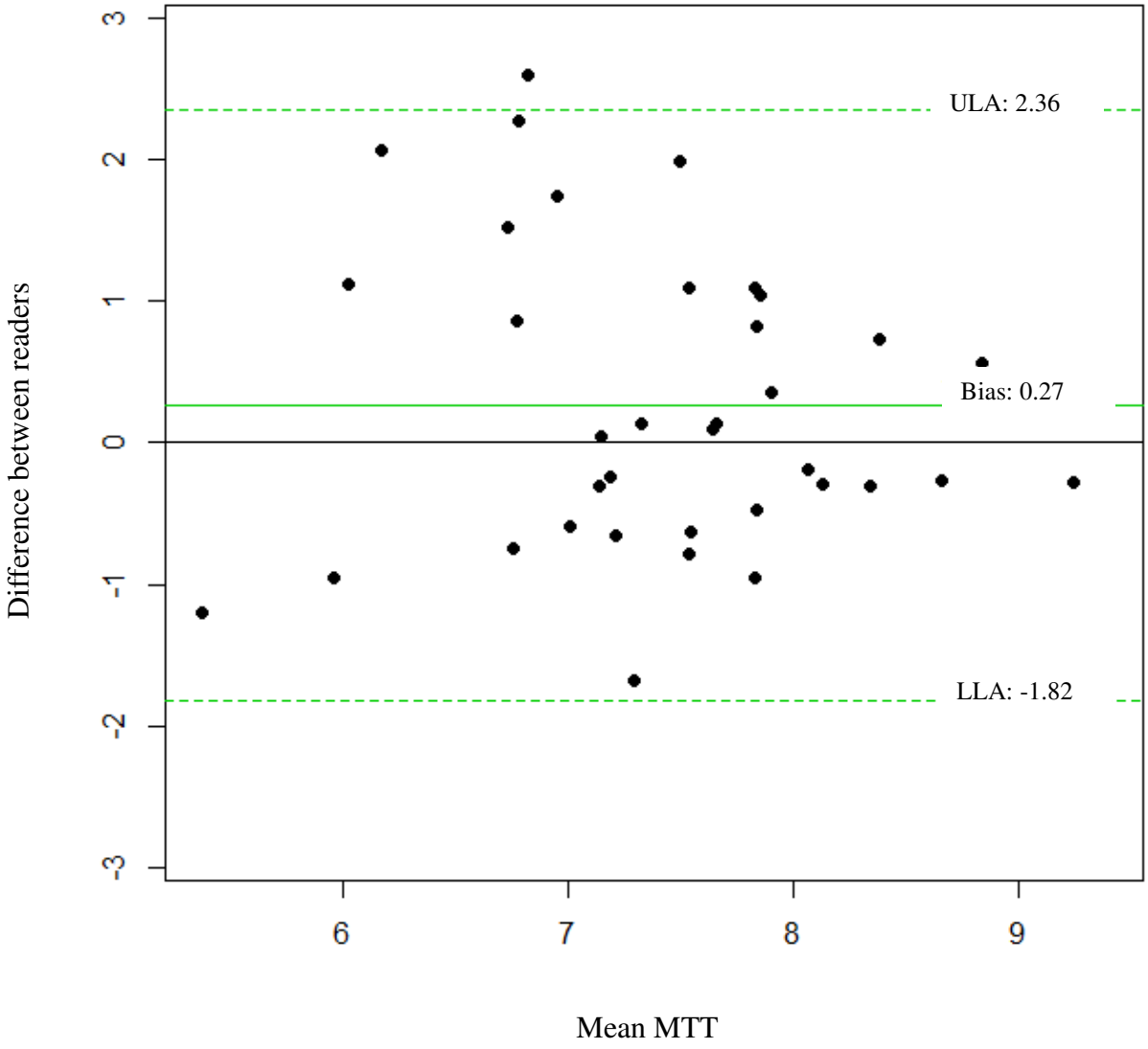
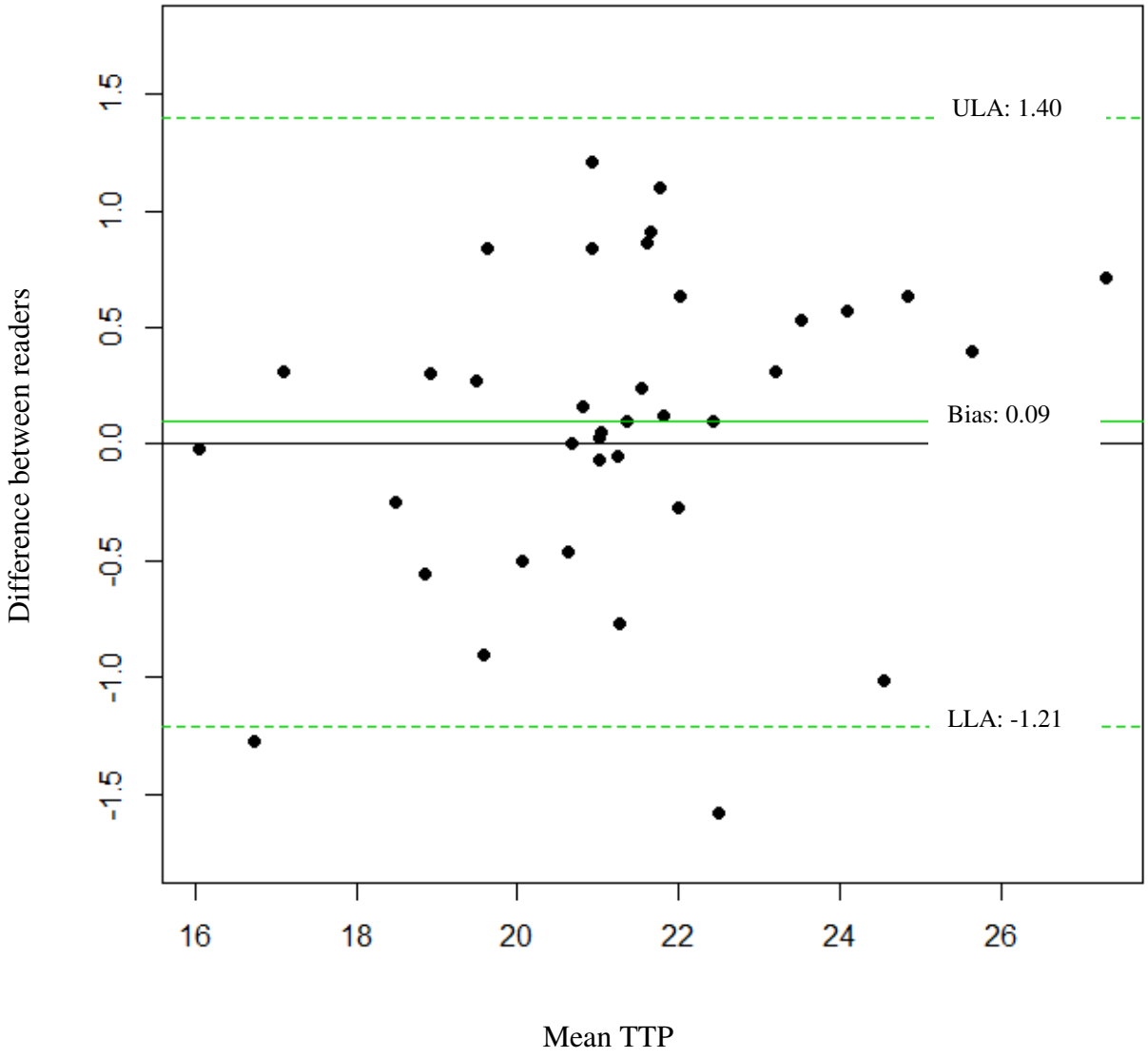


Figure 2 (D). TTP (s) in whole pancreas: Bland-Altman plot for mean difference between reader 1 and reader 2. ULA: 95% upper limit of agreement. LLA: 95% lower limit of agreement.



CAPÍTULO 4

Pancreatic perfusion in patients with type 2 diabetes mellitus using perfusion computed tomography

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Abstract

Objective: to compare quantitatively the pancreatic perfusion by CT in type 2 diabetes (T2DM) and non-diabetic subjects.

Research design and methods: 17 patients with T2DM and 22 non-diabetic controls were examined with a dynamic 192-slices perfusion CT (Siemens, Munich, Germany) between October 2015 and September 2016 for estimating pancreatic blood flow (BF), blood volume (BV), time to peak (TTP) and mean transit time (MTT). Variables were compared by student t test and x^2 between subjects with and without T2DM. Correlations between CT perfusion parameters and clinical and laboratory characteristics were performed by Pearson correlation coefficients.

Results: patients with T2DM had lower BV in pancreatic head (with T2DM: 14.0mL/100L ± 3.4 vs. without T2DM: 16.1mL/100L ± 2.4; p=0.033), in pancreatic tail (with: 14.4mL/100L ± 3.6 vs. without: 16.8mL/100L ± 2.5; p=0.023), and in the whole pancreas (with: 14.2mL/100L ± 3.2 vs. without: 16.2mL/100L ± 2.5; P=0.042). Similar results were observed for MTT in pancreatic head (with: 7.0s ± 1.0 vs. without: 7.9s ± 1.2; p=0.018), in pancreatic tail (with: 6.6s ± 1.3 vs. without: 7.7s ± 0.9; P=0.005), and in the whole pancreas (with: 6.8s ± 1.0 vs. without: 7.7s ± 0.9; p=0.016). BV was inversely correlated with age (head – r: -0.352, p=0.032; tail – r: -0.421, p=0.031; whole pancreas – r: -0.439, p=0.007) and with fasting plasma glucose (head – r: -0.360, p=0.031; tail – r: -0.483, p=0.003; whole pancreas – r: -0.447, p=0.006). BV in pancreatic head showed also negative correlation with HbA1c (r: -0.067, p=0.021).

Conclusion: pancreatic BV and MTT were significantly lower in T2DM patients. These data suggest the possibility of microvascular changes in the pancreas of T2DM subjects.

Introduction

Diabetes mellitus (DM) is characterized by chronic hyperglycemia resulting from increased peripheral insulin resistance and/or decreased insulin secretion (1). Type 1 diabetes mellitus (T1DM) is caused by autoimmune destruction of pancreatic beta cells, while in diabetes mellitus type 2 (T2DM) the pathogenic mechanism is related to an increase in insulin resistance and a relative deficiency in insulin secretion (2). There are other types of diabetes, including the monogenic MODY (maturity onset diabetes of the young), which may be associated with pancreatic morphological changes (3, 4). This observation lead to interest in defining if pancreatic volume, shape and blood flow would vary in the more common forms of diabetes (T1DM and T2DM).

Reduction of beta-cell mass and function in patients with diabetes type 1 and 2 has been documented, as has the reduction of pancreatic volume in these patients (3, 5-9). This volume reduction is more pronounced in patients treated with insulin (5). A meta-analysis showed that the pancreatic volume is on average 38cm^3 smaller in patients with T1DM, and 12cm^3 smaller in patients with T2DM, as compared to healthy individuals (unpublished data).

Despite accounting for only 1-2% of pancreatic mass, islets of Langerhans receive around 10-23% of the total pancreatic blood flow (10, 11). Ultrasound, CT and MRI may be used in tissue perfusion studies. CT, however, has a linear relationship between concentration of iodinated contrast media and the recorded density in Hounsfield units, and may be considered the preferred technique for acquisition of perfusion images (12). Several studies have assessed normal values of pancreatic perfusion by CT (13, 14), pancreatic perfusion impairments in pancreatic (12, 15-21) and hepatic (22, 23) diseases and the modifications of pancreatic perfusion after

oncologic therapy (24, 25). However, the majority of the studies focus on perfusion CT of pancreatic cancer, and pancreatic perfusion in diabetic subjects has not been properly evaluated. The only study on pancreatic perfusion CT in patients with diabetes type 2 showed differences between patients according to the duration of diabetes; even though, no control group was included in this study (26). No differences in pancreatic perfusion between health volunteers and T1DM patients were observed by arterial spin labeling (ASL) magnetic resonance imaging, but this imaging technique may be not sensitive enough to assess pancreatic perfusion (27).

Therefore, the aim of this study is to compare quantitatively the pancreatic perfusion by CT in T2DM and non-diabetic subjects.

Research Design and Methods

Study Design and Setting

We prospectively investigated 6 patients with T2DM and 6 non-diabetic subjects who were referred to abdominal CT scan at Jules Bordet Institute (Brussels, Belgium) for reasons not related to pancreatic symptoms or disease, from October 2015 to September 2016. Eleven patients with T2DM and 16 non-diabetic patients were additionally included from a CT archive. All included subjects were from the oncology clinic of the Hospital. The study was performed in accordance to the Helsinki Declaration and was approved by the Ethics Committee of Jules Bordet Institute, and informed consent was obtained from all participants that were prospectively included. Exclusion criteria were pregnancy, history of allergic reaction to iodinated contrast media, renal insufficiency and history of pancreatic disease.

All patients were scanned in a Siemens Somatom® Force 192-slices scanner (Munich, Germany). The perfusion-CT examination was performed in the interval

between unenhanced and portal phases. A test phase, in order to define a correct delay for the perfusion CT, was performed after injection of 10mL of nonionic contrast medium (Iomeron 400) and a bolus of 21mL of saline solution, with a delay of 8s. For this test phase, a region of interest (ROI) was placed on distal thoracic aorta and 15 images were acquired (1 every 2s, rotation time: 0.25s, 40Mas, 100Kvp), so a curve of aortic enhancement could be obtained (software DynEva®). The time needed to achieve the peak of aortic enhancement was used to define the delay for the perfusion CT. For the perfusion CT, 50mL of a nonionic contrast medium (Iomeron 400) were injected through a 18-gauge catheter in an antecubital vein using a flow rate of 4.0 mL/s, followed by a chaser bolus of 21mL of saline solution. The tube voltage was 80 kilovolt peak (kVp). The dynamic imaging sequence consisted of 31 acquisitions of 0.25-second duration (rotation time) with a time interval of 1.5 s (cycle time), resulting in a total examination time of 45.45 seconds. The perfusion sequence covered a cranio-caudal width of 24 cm (collimation of 48 x 1,2 mm) (Table 1). A portal phase was acquired with a delay of 70s after the beginning of the IV injection of 70mL of contrast media at the end of perfusion CT. Images were analyzed by two radiologists, with sixteen (T.S.G. – reader 1) and twenty-five years of experience in abdominal imaging (J.L.E. – reader 2) blinded to the clinical information. Reader 2 participated in a training program on abdominal perfusion CT. The following parameters were measured: blood flow (BF), blood volume (BV), mean transit time (MTT) and time to peak (TTP). BF is defined as the volume of flowing blood moving through a given volume of tissue in a specific amount of time. BV is defined as the volume of flowing blood for a given volume of tissue. MTT is defined by the formula: MTT = BV/BF, corresponding to the average amount of time that blood takes to transit through a given volume of tissue. TTP is defined as the time elapsed to reach the peak of enhancement in a given tissue (28).

Each radiologist placed three circular ROI in each part of the pancreas (head, body and tail) to measure these parameters. The mean value of each parameter on each part of the pancreas was considered for analysis. These reading sections were performed twice by each of the readers (session 1 and 2). For analysis, we considered the mean value from both sessions of each reader and the mean of both sessions from both readers.

Clinical and laboratory evaluations

Patients prospectively included underwent an interview and their hospital charts were reviewed for collection of demographic and anthropometric data. The diagnosis of T2DM was based on history (age >30 years at onset of diabetes, no previous episodes of ketoacidosis or documented ketonuria) and glycemic profile.

Fasting plasma glucose was measured by the hexokinase method and HbA1c by a high-performance liquid chromatography (HPLC) assay (Merck-Hitachi 9100, normal range 4–6%). Total cholesterol, HDL cholesterol and triglycerides were measured by the colorimetric method, and LDL was calculated with the Friedewald formula. Fasting C-peptide was measured by double antibody-radioimmunoassay (COBAS®, Roche).

Statistical analysis

Data are expressed as mean (± SD) or absolute and relative frequencies. Variables were compared by student t test and x^2 between subjects with and without T2DM. Correlations between CT perfusion parameters and clinical and laboratory characteristics were performed by Pearson correlations coefficients. A model of multivariate regression was carried out for CT perfusion parameters and clinical and laboratory characteristics. This sample is powered (beta 80% and alpha <5%) to find a difference of 20mL/100mL/min in the BF perfusion parameter between subjects with

and without T2DM (18). P value <0.05% was considered statistically significant (SPSS 18.0).

Results

A total of 39 patients [men: n = 21 (53.8%)] were included, with a mean age of 64 years old and a body mass index of 27.9 kg/m^2. Seventeen patients had T2DM, while 22 did not. One patient from each group was excluded due to technical difficulties, which lead to impossibility in measuring pancreatic perfusion parameters (large ascites and improper contrast media injection).

Clinical and laboratory characteristics of patients, according to the DM status, are presented in Table 2. T2DM subjects were older and had higher fasting plasma glucose levels than those without diabetes, as expected. There were more men in T2DM group than in control group. Of note, LDL-cholesterol was lower in T2DM group.

Pancreas volume was similar in patients with and without T2DM (with: 64.3 ± 28.1 vs. without: 63.6 ± 23.1; p=0.929). Considering the mean values of BF, BV, MTT and TTP of both readers in both sessions, we found no significant differences between pancreatic perfusion parameters in T2DM patients and controls (Table 3). Even though, considering only readings from the most experienced radiologist (reader 2), differences between patients with and without T2DM were observed. BV in pancreatic head (with: 14mL/100L ± 3.4 vs. without: 16.1mL/100L ± 2.4; p=0.033), tail (with: 14.4mL/100L ± 3.6 vs. without: 16.8mL/100L ± 2.5; p=0.023), and in the whole pancreas (with: 14.2mL/100L ± 3.2 vs. without: 16.2mL/100L ± 2.5; p=0.042) were lower in the patients with T2DM than in controls. As well, MTT in pancreatic head (with: 7.0s ± 1.0 vs. without: 7.9s ± 1.2; p=0.018), tail (with: 6.6s ± 1.3 vs. without: 7.7s ± 0.9; p=0.005), and in the whole pancreas (with: 6.8s ± 1.0 vs. without: 7.7s ± 0.9; p=0.016) was lower

in patients with T2DM in comparison to controls (Table 4). No differences between T2DM subjects and controls were observed for BV and TTP. An example of perfusion CT images is shown in figure 1.

BV in head, tail and whole pancreas had an inverse correlation with age (head – r: -0.352, p=0.032; tail – r: -0.421, p=0.031; whole pancreas – r: -0.439, p=0.007) and with fasting plasma glucose (head – r: -0.360, p=0.031; tail – r: -0.483, p=0.003; whole pancreas – r: -0.447, p=0.006). BV in pancreatic head showed also negative correlation with HbA1c (r: -0.607, p=0.021). MTT in pancreatic head had negative correlation with C-peptide (r: -0.682, p=0.043), and MTT in tail and in whole pancreas had negative correlation with fasting plasma glucose (tail – r: -0.441, p=0.007; whole pancreas – r: -0.417, p=0.011).

In a multivariate linear regression model, which included BV in whole pancreas as the dependent variable, and age, sex and DM status as the independent ones, T2DM was no longer associated with BV in whole pancreas (β: 1.108; CI95%: -1.282 to 3.498; p= 0.353). Interestingly, in another model where DM status was replaced by HbA1c, the glycemic control was independently associated with BV in whole pancreas (β: -0.884; CI95%: -1.750 to -0.017; p= 0.046).

Discussion

In this sample of subjects undergoing pancreatic perfusion CT, BV and MTT were decreased in those with T2DM in comparison with controls, and no significant differences in pancreatic BF and TTP was observed between these two groups. Additionally, we demonstrated that there is a negative correlation between pancreatic BV and MTT and some clinical aspects, such as age, fasting plasma glucose, HbA1c

and C-peptide. Finally, BV in whole pancreas was inversely associated with HbA1c, even after adjustments for age and sex.

Few studies have compared pancreatic perfusion parameters in patients with diabetes and normal subjects. Miles *et al* (13), in 1995, reported reduced BF in one patient with diabetes; this patient, however, was the only one with diabetes from a total of 12 individuals included. Cui *et al* (26) found significant differences in pancreatic perfusion parameters (MTT and BF) among patients with diabetes according to the duration of T2DM (1-5 years, 6-10 years and >10 years); however authors did not included a control group. Our study advanced in the evaluation of pancreatic perfusion in patients with T2DM. By comparing a group of T2DM patients with a control group, we demonstrated that BV and MTT are lower in patients with T2DM.

In patients with T1DM, the initial insulitis is characterized by changes in pancreatic microvasculature and during the progression of T1DM and T2DM, significant changes in islet vascularization, caused by several factors, may lead to vascular dysfunction (29-31). We observed reduced BV in T2DM patients, which might be explained by ischemia in the microvascular network of the pancreas or by the absence of trophic effect of insulin in pancreatic microcirculatory system. As defined previously, MTT is directly related to BV and inversely to BF. As no differences in BF were detected in this sample, and BV was decreased in the T2DM group, lower MTT was expected in T2DM patients. Pancreatic BV is also reduced in other diseases of the pancreas, such as pancreatic adenocarcinoma (18-20) and in acute and chronic pancreatitis (19). In the liver, Zhu *et al* (32) observed significant decreases in BV within hepatocarcinoma tissues after therapy with anti-vascular agents. As well, abnormalities in BV have been described in other diseased tissues, demonstrating the relationship between altered perfusion and microvascular disease. Cerebral BV is decreased in

subjects with moderate-to-severe white matter changes secondary to chronic microvascular ischemia compared with those with mild changes; interestingly, no changes in MTT was detected between these two groups (33). Similar microvascular changes in BV and BF has been described in the heart (35-37).

Tal (34) hypothesized that pancreatic microvascular endothelial dysfunction and subsequent islet ischemia is the cause of initial dysfunction and subsequent apoptosis of beta cells seen in T2DM. As diabetic patients frequently suffer from microangiopathy in retina, kidney, peripheric nerves and accelerated atherosclerosis, it is likely that the same vascular endothelial dysfunction also affects blood vessels within the pancreas. To our knowledge, we showed for the first time differences in the pancreatic perfusion CT in patients with T2DM in comparison to non-diabetic patients, probably related to microvascular changes in the pancreas parenchyma.

Our study had some limitations. First, significant differences in pancreatic perfusion parameters were found only in reader 2 sessions, which limits reproducibility of our results. This could be partly related to the lack of a preliminary training session for the less experienced reader (reader 1). Second, this sample was powered to detect differences in BF and some of the negative results may be due to lack of power for the other variables. Third, CT examinations were performed in the same CT scanner with the same acquisition parameters. Further studies using CT scanners from different manufacturers are required in order to enable generalization of our results.

In conclusion, pancreatic BV and MTT were significantly lower in T2DM patients in comparison to controls. These data suggest that the pancreas may be a target organ for microvascular DM chronic complications. Further studies evaluating pancreas microvascular system by histology are warranted in order to confirm this hypothesis.

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Table 1. Protocol of CT acquisition

CT Scanning parameters	**Precontrast**	**Perfusion**	**Venous**
Voltage (kVp)	90	80	90
Mean scanning delay after		test*	70
contrast injection (s)			
Collimation (mm)	192 x 0.6	48 x 1.2	128 x 0.6
Rotation time (s)	0.5	0.25 (full rotation)	0.5
		1.5 (cycle time)	
Pitch	0.6	0.6	0.8
Kernel	Br36	Br32	Br40
Slice thickness recontructed (mm)	3	5	3
Contrast agent dose (mL)		50	70
Contrast injection rate (mL/s)		4	4
Number of acquisitions	helical	31	helical
Bolus NaCl (mL)		21	21

*depends on test phase

Table 2. Clinical and laboratory characteristics of patients

	Diabetes		
	No (n = 21)	**Yes (n = 16)**	**p**
Age (years)	59±13	70±10	.004
DM duration (years)	-	11 ± 5	-
Men – n (%)	7 (33)	12 (75)	.012
BMI (kg/m2)	27±5	28±4	.434
Fasting plasma glucose	105±33	170±60	<.001
HbA1c	5.7±0.3	7.6±2.4	.065
C-peptide	1.6±1.4	1.8±0.4	.902
Cholesterol	196±46	181±55	.493
HDL	64±21	41±46	.131
LDL	112±38	73±43	.028
Triglycerides	96±28	175±147	.093
DM treatment			
Diet	-	1	-
Oral medications	-	12	-
Insulin	-	3	-
Oncologic diagnosis			
Breast cancer	8	3	-
Head and neck cancer	1	0	-
Colon cancer	6	1	-
Hepatocarcinoma	3	6	-
GIST*	1	0	-
Neuroendocrine tumor	1	1	-
Lung cancer	0	2	-
Rectal cancer	0	1	-
Cholangiocarcinoma	0	1	-
Endometrial cancer	0	1	-
Benign disease	1	0	-

Information available for BMI in 16 in each group; for fasting plasma glucose in 12 T2DM patients and in 21 controls; for HbA1c in 8 T2DM patients and in 6 controls; for C-peptide in 3 T2DM patients and in 6 controls; for cholesterol, HDL, LDL and triglycerides in 12 in each group. *GIST: gastrointestinal stromal tumor

Table 3. Mean values of BF, BV, MTT and TTP of both readers in both sessions

	Diabetes		
	No (n = 21)	**Yes (n = 16)**	**p**
BF head	133.2±33.0	133.2±38.0	1.000
BF body	139.8±37.6	136.8±35.2	.804
BF tail	151.4±37.4	143.1±30.5	.464
BF whole pancreas	141.5±34.5	137.7±33.1	.739
BV head	16.3±2.6	14.5±3.5	.098
BV body	16.0±3.1	14.8±3.4	.270
BV tail	16.7±2.7	15.1±3.4	.126
BV whole pancreas	16.4±2.6	14.9±3.1	.119
MTT head	7.9±1.2	7.3±0.9	.079
MTT body	7.6±1.2	7.3±0.9	.469
MTT tail	7.5±1.1	7.0±1.0	.241
MTT whole pancreas	7.7±1.0	7.2±0.7	.130
TTP head	21.1±2.8	22.3±1.7	.154
TTP body	20.6±2.8	21.8±1.8	.127
TTP tail	20.4±2.5	21.8±1.9	.055
TTP whole pancreas	20.7±2.6	22.0±1.7	.095

Blood flow in mL/100mL/min; blood volume in mL/100mL; mean transit time in s; time to peak in s.

Table 4. Mean values of blood flow, blood volume, mean transit time and time to peak of each reader in both sessions

	Reader 1			Reader 2		
	Diabetes			Diabetes		
	No	Yes	p	No	Yes	p
	(n = 21)	(n = 16)		(n = 21)	(n = 16)	
BF head	133.9±34.0	130.3±40.8	.775	132.5±33.0	129.6±36.3	.809
BF body	140.0±40.3	132.4±36.3	.559	139.6±37.7	135.4±38.1	.739
BF tail	158.5±46.1	141.5±33.2	.219	144.3±33.0	138.2±34.1	.589
BF whole pancreas	144.1±38.1	134.7±33.7	.440	138.8±32.4	134.4±34.2	.696
BV head	16.4±2.9	15.1±3.9	.247	16.1±2.4	14.0±3.4	.033
BV body	16.4±3.1	15.4±3.4	.386	15.7±3.3	14.3±3.8	.223
BV tail	16.7±3.2	16.0±3.4	.485	16.8±2.5	14.4±3.6	.023
BV whole pancreas	16.5±2.7	15.5±3.2	.308	16.2±2.5	14.2±3.2	.042
MTT head	8.0±1.3	7.6±1.1	.359	7.9±1.2	7.0±1.0	.018
MTT body	7.7±1.2	7.7±1.0	.926	7.4±1.4	7.0±1.4	.302
MTT tail	7.2±1.6	7.5±1.1	.649	7.7±0.9	6.6±1.3	.005
MTT whole pancreas	7.6±1.1	7.5±0.7	.721	7.7±0.9	6.8±1.0	.016
TTP head	21.0±3.0	22.2±1.7	.144	21.2±2.8	22.3±2.0	.193
TTP body	20.6±3.0	22.1±2.0	.084	20.6±2.6	21.6±1.7	.215
TTP tail	20.3±2.6	22.2±1.9	.016	20.5±2.5	21.5±2.0	.190
TTP whole pancreas	20.6±2.7	22.2±1.6	.050	20.8±2.5	21.8±1.8	.182

BF: blood flow in mL/100mL/min; BV: blood volume in mL/100mL; MTT: mean transit time in s; TTP: time to peak in s.

Figure 1 (A). Blood volume color-coded map.

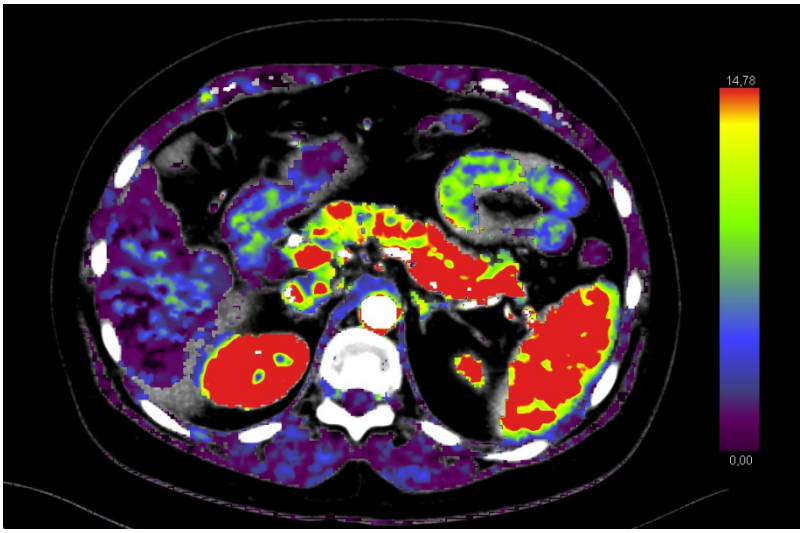

Figure 1 (B). Left: ROI placed in pancreaticread. Right: perfusion curves and values.

PERSPECTIVAS FUTURAS

Nessa tese, demonstramos que o volume e a densidade do pâncreas encontram-se reduzidos em pacientes com diabetes tipo 2 (DM2). No entanto, não há dados até o momento que permitam estabelecer se esses fenômenos já se encontram presentes nos pacientes antes do aparecimento da doença. Sendo assim, há que se investigar se a redução do volume e da densidade pancreática são fatores de risco para o desenvolvimento de DM2. Com esse intuito, desenvolvemos um projeto de um estudo de coorte retrospectiva a ser executado a partir de 2017, com o objetivo de 1) avaliar se o volume pancreático reduzido é preditor para o desenvolvimento de DM2; 2) avaliar se a densidade pancreática diminuída é preditora para o desenvolvimento de DM2 e 3) identificar um ponto de corte de volume e de densidade pancreática a partir do qual o risco de DM2 aumenta de forma clinicamente significativa. Há vários fatores de risco já conhecidos para o desenvolvimento do DM2, como história familiar de DM2, obesidade, dieta não saudável, sedentarismo e história de diabetes gestacional. Tendo em vista o alto impacto do DM2 na saúde pública, o conhecimento de outros fatores de risco, além dos já conhecidos, pode trazer novas possibilidades na prevenção da doença.

No que diz respeito à perfusão pancreática no diabetes, que observamos ser diferente entre os pacientes com DM2 e indivíduos não diabéticos no que diz respeito ao volume de sangue que perfunde o pâncreas e ao tempo de trânsito médio do sangue nesse órgão, é necessário verificar o substrato anatomopatológico dessas modificações para confirmar a hipótese de que estes resultados indicam doença microvascular pancreática. Um estudo voltado para a avaliação anatomopatológica da microvascularização pancreática em pacientes com diabetes poderia trazer mais

informações sobre as possíveis causas da redução do volume de sangue que perfunde o pâncreas nesses pacientes.

ANEXO 1

Carta submetida ao editor com comentários sobre um artigo intitulado: "Diabetes mellitus is associated with an exocrine pancreatopathy: conclusions from a review of literature" (Pancreas. 2016;45(8):1104-1110).

Letter to the Editor, *Pancreas*

Pancreatic Volume in Diabetes Mellitus

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We read with great interest the article by Dr. Sonmoon Mohapatra and colleagues (1), “Diabetes Mellitus is Associated with Exocrine Pancreatopathy: Conclusions From a Review of Literature”, published ahead of print in *Pancreas* on February 2016. In this review, authors provide an overview of studies on morphological, structural and functional changes in the exocrine pancreas in type 1 and type 2 diabetes mellitus (T1DM and T2DM). This is a relevant topic and merits further discussion. We noticed a few discrepancies in "*Table 1 –Pancreatic Volume by MR or CT in DM*", that may interfere with the interpretation of data. First, values are in disagreement with the original data published by Bilgin *et al* (2). This study describes data on five patients with T1DM and 52 patients with T2DM, instead of 28 T1DM and none T2DM, as shown in Table 1. Moreover, pancreas size, measured as the antero-posterior diameter of the organ, was compared between three groups: a) group 1: 29 patients with diabetes (four T1DM and 25 T2DM) and pancreatic exocrine insufficiency; b) group 2: 28 patients with diabetes only (one T1DM and 27 T2DM); and c) group3: 21 non-diabetic subjects with normal exocrine function. Therefore, it is not possible to assume differences in pancreas size between T1DM and controls, since T1DM and T2DM patients were mixed together in statistical analysis. Second, in the study of Saisho *et al* (3), which compared pancreatic volume in T2DM patients and healthy subjects, we believe the correct mean ± SD values for pancreatic volume in the control group of 1721 subjects is 72.4 ±25.8, instead of 74.9 ± 27. The value presented in Table 1 (74.9 ± 27) refers to a subgroup of 660 healthy subjects matched for age and body mass index with the group of 165 T2DM patients. Third, another study on the topic was published in 2015 and was not included in the systematic review (4). Macauley *et al* investigated volume, morphology and composition of the pancreas by

magnetic resonance in T2DM patients (n = 41) and healthy controls (n = 14) and found a 33% reduction in pancreas volume of diabetic patients compared to controls (55.5 ± 17.92 *vs.* 82.6 ± 17.95; $p<0.0001$), in line with other studies included in this systematic review. In conclusion, study selection and data extraction is the cornerstone of a systematic review, and might be carefully performed in order to guarantee correct interpretation of data.

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