Noninvasive Quantification of Pancreatic Fat in Humans

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Objective: To validate magnetic resonance spectroscopy (MRS) as a tool for non-invasive quantification of pancreatic triglyceride (TG) content and to measure the pancreatic TG content in a diverse human population with a wide range of body mass index (BMI) and glucose control.

Methods: To validate the MRS method, we measured TG content in the pancreatic tissue of 12 lean and 12 fatty ZDF rats (ages 5–14 weeks) both by MRS and the gold standard biochemical assay. We used MRS to measure pancreatic TG content in vivo in 79 human volunteers. Additionally, to assess the reproducibility of the method, in 33 volunteers we obtained duplicate MRS measurements 1–2 weeks apart.

Results: MRS quantifies pancreatic TG content with high reproducibility and concordance to the biochemical measurement (Spearman's rank correlation coefficient = 0.91). In humans, median pancreatic TG content was as follows: (1) normal weight and normoglycemic group 0.46 f/w%, (2) overweight or obese but normoglycemic group 3.16 f/w%, (3) impaired fasting glucose or impaired glucose tolerance group (BMI matched with group 2) 5.64 f/w%, and (4) untreated type 2 diabetes group (BMI matched with group 2) 5.54 f/w% (Jonckheere-Terpstra trend test across groups $p < 0.001$).

Conclusions: Human pancreatic steatosis, as measured by MRS, increases with BMI and with impaired glycemia. MRS is a quantitative and reproducible non-invasive clinical research tool which will enable systematic studies of the relationship between ectopic fat accumulation in the pancreas and development of type 2 diabetes. (***J Clin Endocrinol Metab*** **94: 4070–4076, 2009)**

The prevalence of obesity is rising at alarming rates, and obesity-associated comorbidities are escalating. Obesity increases the risk for type 2 diabetes up to 41 fold in some groups, and type 2 diabetes is the morbidity most closely associated with the degree of obesity (1). Understanding the mechanisms that link obesity to its metabolic complications can lead to targeted interventions that will effectively prevent or treat these conditions.

Under physiologic states, excess energy is stored as triglycerides (TG) in the adipose tissue. It has been postulated that during chronic over-nutrition the capacity of adipose tissue to store the surplus TG is diminished and the TG overflow is redirected to cells of internal organs, leading to steatosis (2–4) and organ dysfunction. Intramyocellular TG accumulation is thought to be a significant determinant of the peripheral insulin resistance, one of the main pathogenic contributors to the development of type 2 diabetes. Yet the hallmark of type 2 diabetes is represented by an inadequate insulin secretion for the prevailing insulin sensitivity. In animal models of type 2 diabetes, ectopic TG overload in β-cells contributes to β-cell dysfunction (2) through the process of lipoapoptosis (5). This model of ectopic TG accumulation provides a unifying explanation of the link between obesity and both patho-

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Abbreviations: BMI, Body mass index; IFG, impaired fasting glucose; IGT, impaired glucose tolerance; MRS, magnetic resonance spectroscopy; NGT, normal glucose tolerance; pTG, pancreatic triglyceride content; T2D, type 2 diabetes; TG, triglyceride; VOI, volume of interest; ZDF, Zucker Diabetes Fatty.

genic determinants of type 2 diabetes – decreased insulin sensitivity and inadequate insulin secretion - but clinical translation of the β-cell lipotoxicity model has been hindered by lack of readily available pancreatic tissue and non-invasive methods to evaluate pancreatic or β-cell TG content in humans.

Localized proton Magnetic Resonance Spectroscopy (MRS) has been a useful non-invasive clinical tool in evaluating intra-myocellular (6), intrahepatic (7), and intramyocardial (8) TG content. Measurement of TG levels exclusively within islets *in vivo* is below the sensitivity of the MRS, but total TG content in the pancreatic parenchyma as a whole is quantifiable (9).

We performed complementary studies in rats and humans to validate MRS for quantitative non-invasive measurement of total pancreatic TG (pTG) content (*i.e.* pancreatic steatosis). First, we confirmed the presence of TG in the exocrine pancreas and validated the MRS in pancreatic tissue from Zucker rats. Second, we documented the reproducibility of the pTG content measurement in healthy human volunteers *in vivo*. Third, we examined the cross-sectional relationship between pTG content, body mass index (BMI), and glycemic status; and we evaluated clinical and biochemical determinants of pTG content in humans.

Materials and Methods

Animal studies

Animals

Animal experiments were approved by the Animal Studies Committee at the University of Texas Southwestern Medical Center, following Animal Welfare Act Guidelines.

Animals used for MRS and biochemical studies were purchased from Charles Rivers Laboratories and were housed at 22′C on a 12-h light-dark cycle with ad libidum access to 6% fat chow (TD 7002) and water. We studied 12 obese male Zucker Diabetic Fatty (ZDF fa/fa) and 12 lean male littermates (fa/+), at ages 5, 8, 11, and 14 weeks (3 animals per age and phenotype). We selected specific ages to correspond to discrete disease stages, from normo-glycemia and normo-insulinemia (5 weeks), to normoglycemia and hyperinsulinemia (8 weeks), to hyperglycemia and hyperinsulinemia (11 weeks), and finally to early type 2 diabetes (14 weeks). Lean rats remain normoglycemic at all ages and served as a control population. The fasting blood glucose levels were determined from the tail vein blood sample one day before the experiment. Animals were anesthetized with 50 mg/kg of pentobarbital before pancreas extraction. Pancreatic tissue was examined with magnifying glass and all visible adipose tissue was thoroughly removed before further testing (Fig. 1 A, B).

MRS-based measurement of TG content in pancreatic tissue

One third of each animal's pancreas (~0.4 g) was selected for evaluation of fat content by MRS, using a 1.5 Tesla clinical MR system (Gyroscan Intera, Philips Medical Systems, North America) equipped with spectroscopy package. The pancreatic tissue was placed in a glass tube filled with a mixture of deuterated water (D_2O) and sodium chloride (Fig. 1C) at physiologic concentration (similar to normal saline). Utilization of deuterated water allowed measuring the true signal from water in pancreatic tissue, as deuterons resonate at different frequencies and are not "visible" along with hydrogens from tissue water. The sample holder was placed in the knee coil and T_1 weighted images were obtained to guide the placement of the volume of interest (VOI), which was selected to contain the entire pancreatic specimen (Fig. 1D). MR spectrum was collected using Point Resolved Spectroscopy Sequence (PRESS), and the following parameters: TR = 3 s, TE = 27 ms, 1024 data points over 1000 kHz spectral width. Spectra (Fig. 1E) were evaluated using commercial software (NUTS-ACORNNMR, Freemont, CA), a line-fit procedure, and a prior knowledge for resonance lines assignment. The water signal was used as internal standard, assuming constant tissue water content. Results were corrected for T_2 relaxation of fat and water signals assuming monoexponential signal decay. Results were reported as fat / water ratio. Although this measurement reflects the fractional content of fat in the tissue relative to the water content, the results are referred to as "TG content" in line with previous reports using the same methodology (6, 9). Data were processed in a blinded fashion and repeated on two separate occasions showing high reproducibility, with an intraclass correlation coefficient (ICC) of 0.996 (95% CI: 0.99–0.998).

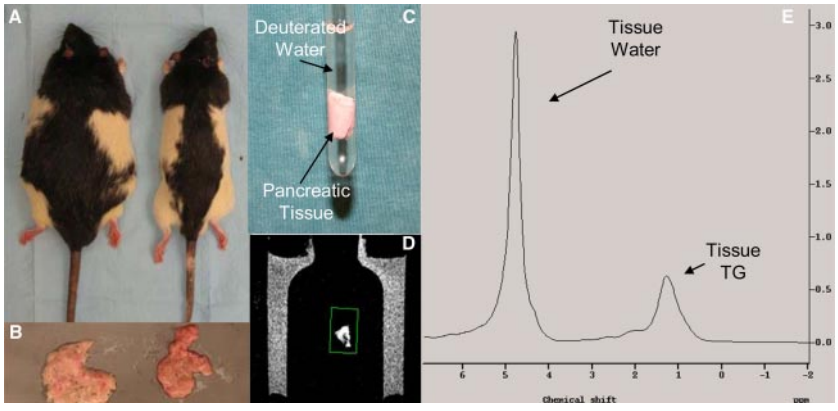


FIG. 1. A. Fatty and lean Zucker rats. B. Excised pancreas from these rats. Note the difference in color between the pancreas of the fatty and lean animal. C. Tube containing pancreatic specimen in deuterated water, which provides an "MR invisible" background, allowing data acquisition from the pancreatic specimen only. D. MR image of the custom made holder containing the tube with the pancreatic specimen. The green rectangle encompasses the entire pancreatic specimen, and represents the volume of interest from which data are collected. E. Proton spectrum from a pancreatic specimen with high fat content. MR – magnetic resonance.

Biochemical determination of TG concentration in pancreatic tissue

Immediately upon completion of the MRS experiment, pancreatic tissue was frozen and stored in liquid nitrogen for biochemical measurement of the total pTG con-

tent. Tissue was homogenized under liquid nitrogen and separated in aliquots (≈80 mg each). Neutral lipids were extracted from each aliquot using the Folch procedure (10) with slight modifications (11). Briefly, each aliquot was homogenized in 4 ml of chloroform/methanol (2/1, v/v), after which 0.8 ml of 50 mM NaCl was added, vortex and span at 1500 × *g* for 10 min. The organic phase was transferred to a new tube and washed twice with 1 ml of 0.36 M CaCl2/ Methanol (1/1, v/v); 100 μl of the organic phase was mixed with 10 μl of 50% Triton-X 100/ Chloroform. Twenty μl samples were dried and TG content was measured in triplicate using Sigma Aldrich (St Louis, MO) kit.

Human studies

Study population

Human experiments were approved by the Institutional Review Board at University of Texas Southwestern Medical Center and all volunteers provided study specific informed consent. We enrolled adult volunteers of various ethnicities and both genders, with normal or abnormal glucose tolerance. We used the following exclusion criteria: contraindications for imaging and spectroscopy (metallic implants, claustrophobia, pregnancy, body weight above 160 kg or body circumference exceeding the magnet bore size); use of pharmacologic agents that alter fat content or metabolism; active weight loss in the previous 6 months, history of pancreatic or liver disorders, daily consumption of more then 2 alcoholic drinks. Volunteers with type 2 diabetes were not using any pharmacologic agents for at least 6 months before the study.

Study design

Study volunteers (n = 79) underwent pTG measurement by MRS, anthropometric measurements, and clinical evaluation. We grouped volunteers by BMI and glucose tolerance using the current American Diabetes Association guidelines (12) to define Impaired Fasting Glucose (IFG), Impaired Glucose Tolerance (IGT), and type 2 diabetes: (Group 1) lean (BMI <25 kg/m^2) and normoglycemic (fasting blood glucose <100 mg/dl (5.55 mmol/dl) and 2 h post oral glucose load blood glucose <140 mg/dl (7.77 mmol/dl)); (Group 2) overweight or obese (BMI ≥25 kg/m^2) and normoglycemic; (Group 3) IGT (fasting blood glucose <100 mg/dl (5.55 mmol/dl) and 2 h post oral glucose load blood glucose 140–199 mg/dl (7.77–11.04 mmol/dl)) and/or IFG (fasting blood glucose 100–125 mg/dl (5.55–6.94 mmol/dl) and 2 h post oral glucose load blood glucose <140 mg/dl (7.77 mmol/dl)) regardless of weight; (Group 4) type 2 diabetes regardless of weight (fasting blood glucose >125 mg/dl (6.94 mmol/dl) or 2 h post oral glucose load blood glucose >199 mg/dl (11.04 mmol/dl)). A subset of these volunteers (n = 45) also underwent an Oral Glucose Tolerance Test and had hepatic TG content measured by MRS. Additionally, in 33 volunteers, we measured pTG content in duplicate, 1–2 weeks apart, to assess the reproducibility of the MRS measurement over time in the absence of clinical changes.

Anthropometric measurements and assessment of risk

Age, race/ethnicity, smoking status, physical activity, alcohol intake, weight history, first degree family history of diabetes, history of gestational diabetes, and dietary fat consumption were assessed by self-report. Moderate or vigorous exercise frequency ≤1 time/week was considered low. Alcohol intake was reported as >2 drinks/day, 1–2 drinks daily, 1–6 drinks/week, <1 drink/week. We obtained by recall weight change from 1, 5, and 10 yr before the study.

Height and weight were measured in all participants using the same doctor's scale with a measuring rod in all participants wearing only a medical gown and underwear. Waist circumference was measured at the umbilical level at end-exhalation, and the hip circumference was measured at the widest part of the hips. Blood pressure was measured with an automated Omron monitor; the average of three measurements is reported.

Diabetes risk was calculated using age, waist circumference, height, history of gestational diabetes, race/ethnicity, history of hypertension, family history of diabetes, and exercise level (13).

Oral glucose tolerance test

After an overnight fast, study volunteers ingested glucose solution containing 75 g of carbohydrates. Blood samples were obtained at baseline and 120 min after the ingestion.

Analytic assays

Blood glucose levels were measured in duplicate by the glucose oxydase technique with an automated analyzer (Yellow Springs Instrument Co, Yellow Springs, OH). HbA1c was measured by the high performance liquid chromatography method in a laboratory certified by the National Glycohemoglobin Standardization Program. The HbA1c inter-assay coefficient of variability is <2% and intra-assay variability <0.3%. Lipid profile assay was performed at a commercial laboratory (Quest Diagnostics, Irving, TX).

MRS measurement of pancreatic and hepatic TG content

MR scanning was performed in the afternoon in non-fasted state using a 1.5 Tesla clinical whole body MR system (Gyroscan Intera, Philips Medical Systems, North America). High-resolution images through the abdomen were collected to locate the pancreas with patients in supine position holding their breath at exhalation. Using three perpendicular images of the pancreas, a volume of 2cc (10*10*20 mm^3) for spectroscopic testing was selected within the body of the pancreas, avoiding any peri-visceral fat (Fig. 2). We choose to use a relatively large VOI (2 cc) to average any minor heterogeneity of pTG distribution throughout the pancreas. Spectroscopic data were collected as patients breathed freely with MR signal triggering at exhalation, using a cardiac synergy coil and PRESS sequence. Data were acquired and processed using parameters as described in the animal studies section. Hepatic TG content was determined during the same session, using our previously described protocol (7). The length of each MRI/MRS session was approximately 30 min.

Statistical methods and considerations

We used Spearman's rank correlation coefficient to assess the concordance of the MRS measurements *vs.* the "gold standard" (biochemical) measurements, which use different scales. We interpret the Spearman's correlation coefficient as a measure of how well the MRS method preserves the order implied by biochemistry. To evaluate the reproducibility of MRS for human pTG content, we calculated measurement error and repeatability from the duplicate MRS measurements. Measurement error, as described by Bland and Altman (14), is defined as the within

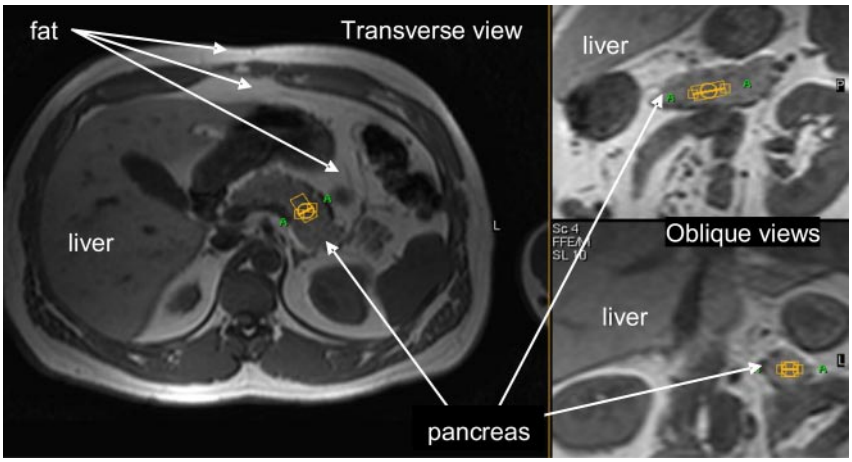


FIG. 2. The experimental setup for the measurement of pancreatic triglyceride content using localized Magnetic Resonance Spectroscopy in humans. Images through the abdomen were acquired in transverse and oblique views, to identify the location of the pancreas. The volume of interest (VOI - yellow box) defined by the magnetic field gradients was placed entirely within the pancreatic tissue. Spectra are collected from the VOI only; the signal from outside of this volume is dephased and does not contribute to collected data.

subject SD and the repeatability coefficient is 2.77 times the measurement error. ICC's were also calculated between pairs of measurements.

We applied the logarithm transformation on the pTG measurements to stabilize the variance. We conducted a two-way ANOVA to evaluate the effect of genotype and age on the animal total pTG content (using both the biochemical and MRS measurements).

The Jonckheere–Terpstra test was used to assess the trend in pTG content across the four groups with varying glycemic status. The human pTG content was further analyzed as a dependent variable in multivariate regression models using the following potential independent variables: fasting and post-challenge blood glucose level, serum TG level, HbA1c, history of weight gain, dietary fat intake, alcohol intake, smoking status, various anthropometric measurements (waist/hip ratio, BMI, weight), age, and number of risk factors for diabetes. The set of covariates achieving the highest adjusted R-square were included in the final model. We used Spearman correlation coefficient to evaluate the association between pancreatic and hepatic TG content. We reported means and SD (unless otherwise indicated) and considered a p value < 0.05 statistically significant.

Results

Total pTG content in ZDF and lean control rodents

The two-way ANOVA analyses showed total pTG content (measured by MRS) was significantly different between the lean and fatty animals ($P < 0.0001$). There was a significant increase in pTG content with age in the fatty group. At 14 weeks of age, fatty animals had a 4.5-fold higher pTG content compared with the age-matched lean counterparts (Fig. 3A). There was no significant difference in the pTG content among the different age groups of the lean animals.

Validation of pTG quantification by MRS

To validate the MRS-based quantification of pTG content, we assessed the concordance of pTG measurement by MRS against the bioassay considered the gold standard in

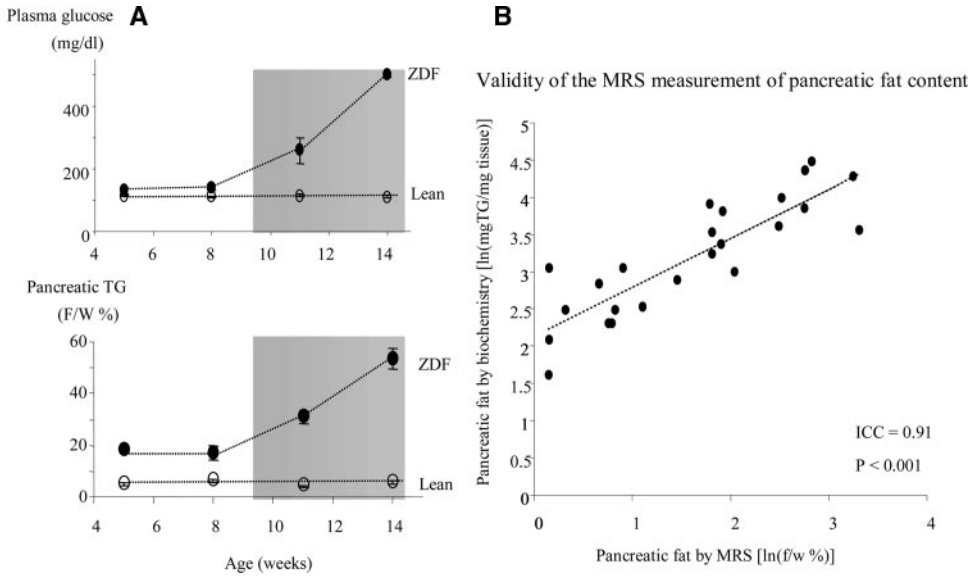


FIG. 3. Results of the animal experiments. A, Blood glucose level (upper panel) and pancreatic TG content measured by MRS (lower panel) from lean and fatty ZDF rats. As the fatty ZDF rats develop hyperglycemia, the pancreatic TG content rises. Pancreatic TG content does not significantly change due to aging alone (lean controls). Data are mean and SE. B, Comparison of pancreatic triglyceride content measured by the "gold standard" biochemical method (y-axis) and by the MRS technique (x-axis). ZDF – Zucker Diabetic Fatty; TG – triglyceride; f/w% – fat to water ratio; MRS – Magnetic Resonance Spectroscopy.

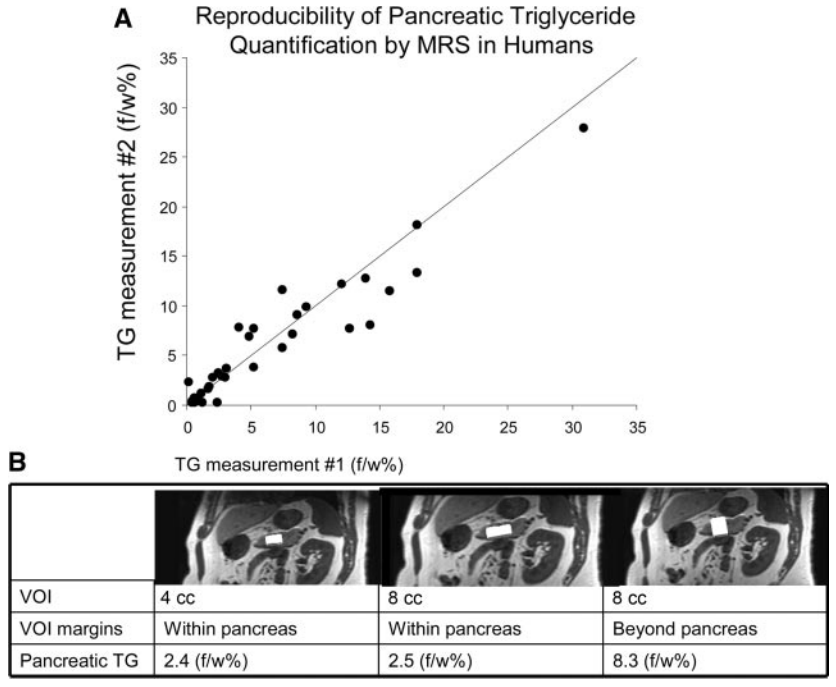


FIG. 4. Reproducibility of the pancreatic TG content measurement by the MRS method. A, High test-retest reproducibility of the measurement of pancreatic TG content by the MRS method in humans. The pancreatic TG content was measured in duplicate in 33 individuals, 1–2 weeks apart. Identity line included. B, Testing volume selection is an important consideration for the accuracy and reproducibility of the measurement of pancreatic TG content. We present three testing volumes (white boxes) in the same individual. The first image illustrates a volume of interest (VOI) of 4 cc, placed entirely within the pancreas. The measured TG content in this volume was 2.4%. The second image illustrates a VOI twice as large (8 cc), again placed entirely within the pancreas. The measured TG content in this volume did not change. Finally, a VOI of 8 cc was intentionally positioned to include visceral fat. Contamination of the VOI by visceral fat lead to gross overestimation of the pTG content (8.3%). TG – triglyceride, MRS – Magnetic Resonance Spectroscopy, VOI – volume of interest, f/w% – fat to water ratio.

animal pancreatic tissue and the reproducibility of the MRS pTG measurement in human volunteers. The Spearman's rank correlation coefficient between the MRS measurement and the biochemical assay of the animal total pTG content was 0.91 ($P < 0.0001$) (Fig. 3B).

Thirty-three volunteers underwent duplicate measurement of the pTG content 1–2 weeks apart (Fig. 4A). The measurement error derived from the 33 pairs of measurements was 1.62 f/w%, with a coefficient of repeatability of 4.5 f/w%, which indicates that 95% of pairs of measurements would differ by less than 4.5 f/w% apart. The ICC was 0.94 (95%CI, 0.88–0.97).

Selection of the testing volume (volume of interest – VOI) is an important consideration for the accuracy and reproducibility of the MRS-based measurement of pTG content. VOI size does not alter the measurement as long as it only contains pancreatic tissue. As demonstrated in Figure 4B, the values for pTG content are consistent if VOI is not contaminated by abdominal fat. If the VOI margin extends beyond the pancreas and includes abdominal fat, the resulting TG measurement is increased and no longer reflects the actual pancreatic TG content.

Pancreatic steatosis in humans

The pTG content of 79 volunteers, grouped by BMI and glycemic status, is shown in Figure 5 (Jonckheere-Terpstra Trend Test across the 4 groups $P < 0.001$). Median (inter-quartile range) pTG content was lowest in lean normoglycemic volunteers [Group 1: pTG = 0.46 (0.88) f/w%]. Pancreatic TG content was 7-fold higher in overweight or obese normoglycemic volunteers [Group 2: pTG = 3.16 (3.55) f/w%], and further increased in volunteers with similar BMI but abnormal glucose levels (IFG/IGT) or type 2 diabetes [Group 3: pTG = 5.64 (6.38) f/w%; Groups 4: pTG = 5.54 (4.22) f/w%].

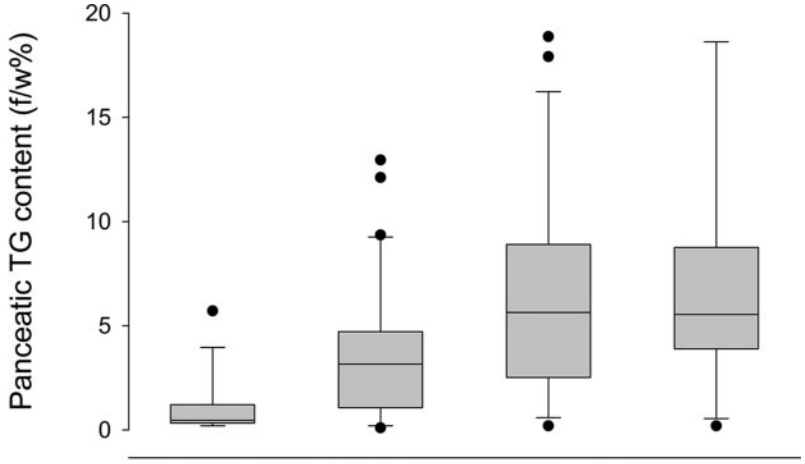
In this group of 15 healthy, lean, and normo-glycemic volunteers the range of pTG was 0.2–5.71 f/w%, with 14 of 15 volunteers having a pTG content <2.8 f/w%. We are currently considering the value of 2.8 f/w% as the upper limit of normal for human pTG content, but acknowledge that further testing in normal volunteers is needed to define the normal pTG range.

To evaluate independent determinants of pTG content, we performed multiple regression analysis on the data with complete biochemical evaluation (n = 45). The adjusted R-square statistic for the most parsimonious regression model was 0.48, and the three selected covariates were: blood glucose level at 2 h during a standard oral glucose tolerance test, waist to hip ratio, and amount of weight gain over the previous 10 yr (Table 1). The first covariate, an indicator of glycemic state, was by far the strongest contributor to the model. All other variables and combination of variables yielded no significant relationship (HbA1c, serum TG level, number of diabetes risk factors, smoking status, alcohol intake), or a much weaker relationship (age, BMI, fasting blood glucose, exercise level, dietary fat intake).

The Spearman's rank correlation coefficient for pancreatic and hepatic TG content was 0.33 ($P = 0.04$).

Discussion

We have shown that MRS is a non-invasive and quantitative method that reliably measures pTG content *in vivo*. Measurement of pTG by MRS in humans is sensitive to detect differences between groups of volunteers from lean



	NGT BMI<25	NGT BMI≥25	IGT/IFG	T2D
N	15	30	23	11
Age (Years)	31 ± 5	39 ± 10	42 ± 9	48 ± 11
BMI (kg/m²)	22.2 ± 1.6	32.4 ± 6.1	32.4 ±5 .6	32.9 ± 5.0
Gender (M)	6	8	8	2
Ethnicity (W/H/AA/A)	8/6/0/1	14/8/8/0	5/14/3/1	5/4/2/0

FIG. 5. Pancreatic TG content in 79 human volunteers with different BMI and glycemia (Trend Test across the 4 groups: $P < 0.001$). All groups were significantly different ($P < 0.05$) from each other (except between groups IFG/IGT and T2D). The lower and upper limits of the box indicate the 25th and 75th percentiles, the line within the box depicts the median, and the whiskers (error bars) below and above the box indicate the 10th and 90th percentiles. BMI – body mass index, NGT – normal glucose tolerance, IGT – impaired glucose tolerance, IFG – impaired fasting glucose, T2D – type 2 diabetes; TG – triglyceride.

to obese with progressive levels of glucose intolerance. Our data suggest that post-challenge blood glucose level has the strongest relationship to pTG content, and that pancreatic and hepatic TG contents are only weakly correlated.

Our studies confirm previous findings by Tushuizen *et al.* (9) who were first to show that pTG content is elevated in human volunteers with type 2 diabetes compared with normoglycemic obese controls. We extend this seminal work in two ways: (1) by validating the MRS technique for the pancreatic application; and (2) by expanding the study population to include lean controls and volunteers with obesity and pre-diabetes. We focused the thrust of our work on high-risk and pre-diabetes population to better understand the early processes leading to the development of diabetes, as we ultimately plan to explore the role of pTG as an early bio-marker for obesity-associated diabetes.

TABLE 1. Result of the multivariate regression analysis for the dependent variable pancreatic TG content

Variable in model	β	SE β	*P* value
Adjusted $R^2 = 0.48$			
Intercept	−10.42	2.2	
Waist to hip ratio	11.58	2.4	0.0001
Weight gain, past 10 yr (kg)	0.04	0.009	0.0003
2-h glucose during OGTT (mg/dl)	0.012	0.02	0.0001

The SI conversion factor for glucose is 0.0555. β, Regression coefficient; OGTT, oral glucose tolerance test.

In line with previous studies in isolated islet cells (2), we found that in obese ZDF rodents total pTG content increases as animals develop pre-diabetes and diabetes, while there was no significant increase in pTG content in age-matched lean rodents. Based on these findings, we postulated that total parenchymal pTG content closely parallels that of islet cells, thus measurement of total pTG content could serve as a marker of β-cell steatosis. Perhaps there might also be a direct functional effect of increased TG accumulation in the peri-islet environment contributing to β-cell dysfunction.

We validated the MRS-based measurement of pTG content by (1) assessing its accuracy against the current "gold standard," the biochemical assay, in lean, obese, and diabetic rodents; and (2) establishing the intra-subject reproducibility in nondiabetic volunteers with a broad range of BMI. Thus we demonstrated that the MRS-based method precisely and reproducibly quantifies pTG content and is suitable for *in vivo* application.

Furthermore we demonstrated that VOI size does not alter the results, but it is critical to ensure that no visceral fat contamination occurs within the VOI. If visceral fat contaminates VOI, measured pTG is spuriously elevated. To avoid visceral fat contamination we guide VOI placement using high resolution MR images in three perpendicular orientations and collect data with respiratory and cardiac motion compensation.

Once the validity of the method was established, we measured pTG content in volunteers with a wide range of BMI and various degrees of glucose tolerance. We found that pTG content is significantly elevated in overweight/obese normoglycemic volunteers compared with the lean normoglycemic group. Volunteers with similar BMI but with abnormal glucose tolerance (IFG, IGT, or diabetes) had even higher pTG content. We need to emphasize that most of our volunteers in Group 2 (the overweight/obese with normal glucose tolerance) have multiple risk factors for diabetes; therefore, maladaptive processes leading to diabetes were already taking place at the time of evaluation. We hypothesize that pTG content in humans increases slightly with weight but is mostly an indicator of the abnormalities preceding and possibly causing abnormal glucose tolerance, similarly to what was reported in

the animal models of diabetes (15). To further explore this hypothesis, we evaluated several multivariate models predicting pTG content. Blood glucose level at 2 h after an oral glucose load was the strongest predictor of pTG content, suggesting an association even at very early, subclinical stage of glucose intolerance.

Our data suggests that pTG starts to accumulate early in the natural course of type 2 diabetes, perhaps even before development of "pre-diabetes." If our findings are confirmed, measurement of pTG content could potentially serve as a clinical tool to identify individuals at very high risk of diabetes development and monitor interventions aiming to prevent diabetes.

We acknowledge that the cross-sectional nature of the study is a limitation and that this hypothesis will need further testing in longitudinal studies. Additionally, the effect of pTG accumulation on β-cell function will have to be evaluated using formal measurements of β-cell function, preferably in interventional or long-term longitudinal observational studies.

An additional finding from this study is the limited correlation between pancreatic and hepatic TG contents, despite the fact that both pancreatic and hepatic TG contents are elevated in patients with IFG/IGT and type 2 diabetes. This is in line with our previous report of poor correlation between hepatic and myocardial TG contents (16), suggesting that the mechanisms promoting steatosis in various organs might be independent, but their clinical consequences are complementary.

In conclusion, we have demonstrated that MRS, a noninvasive and quantitative method for assessment of TG accumulation in ectopic sites, is a precise and reproducible clinical research tool to study pancreatic steatosis in humans. The method offers a unique strategy for early detection of ectopic fat accumulation in high risk individuals. Our results set the stage for future studies to determine if pancreatic steatosis leads to β-cell dysfunction and if interventions that reduce pTG levels favorably impact β-cell function.

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References

1. **Field AE, Coakley EH, Must A, Spadano JL, Laird N, Dietz WH, Rimm E, Colditz GA** 2001 Impact of overweight on the risk of developing common chronic diseases during a 10-year period. Arch Intern Med 161:1581–1586
2. **Lee Y, Hirose H, Ohneda M, Johnson JH, McGarry JD, Unger RH** 1994 β-Cell lipotoxicity in the pathogenesis of non-insulin-dependent diabetes mellitus of obese rats: impairment in adipocyte-β-cell relationships. Proc Natl Acad Sci USA 91:10878–10882
3. **Lee Y, Wang MY, Kakuma T, Wang ZW, Babcock E, McCorkle K, Higa M, Zhou YT, Unger RH** 2001 Liporegulation in diet-induced obesity. The antisteatotic role of hyperleptinemia. J Biol Chem 276:5629–5635
4. **Wang MY, Grayburn P, Chen S, Ravazzola M, Orci L, Unger RH** 2008 Adipogenic capacity and the susceptibility to type 2 diabetes and metabolic syndrome. Proc Natl Acad Sci USA 105:6139–6144
5. **Shimabukuro M, Higa M, Zhou YT, Wang MY, Newgard CB, Unger RH** 1998 Lipoapoptosis in β-cells of obese prediabetic fa/fa rats. Role of serine palmitoyltransferase overexpression. J Biol Chem 273:32487–32490
6. **Szczepaniak LS, Babcock EE, Schick F, Dobbins RL, Garg A, Burns DK, McGarry JD, Stein DT** 1999 Measurement of intracellular triglyceride stores by H spectroscopy: validation in vivo. Am J Physiol 276:E977–E989
7. **Szczepaniak LS, Nurenberg P, Leonard D, Browning JD, Reingold JS, Grundy S, Hobbs HH, Dobbins RL** 2005 Magnetic resonance spectroscopy to measure hepatic triglyceride content: prevalence of hepatic steatosis in the general population. Am J Physiol 288:E462–E468
8. **Szczepaniak LS, Dobbins RL, Metzger GJ, Sartoni-D'Ambrosia G, Arbique D, Vongpatanasin W, Unger R, Victor RG** 2003 Myocardial triglycerides and systolic function in humans: in vivo evaluation by localized proton spectroscopy and cardiac imaging. Magn Reson Med 49:417–423
9. **Tushuizen ME, Bunck MC, Pouwels PJ, Bontemps S, van Waesberghe JH, Schindhelm RK, Mari A, Heine RJ, Diamant M** 2007 Pancreatic fat content and β-cell function in men with and without type 2 diabetes. Diabetes care 30:2916–2921
10. **Folch J, Lees M, Sloane Stanley GH** 1957 A simple method for the isolation and purification of total lipides from animal tissues. J Biol Chem 226:497–509
11. **Yokode M, Hammer RE, Ishibashi S, Brown MS, Goldstein JL** 1990 Diet-induced hypercholesterolemia in mice: prevention by overexpression of LDL receptors. Science 250:1273–1275
12. **Genuth S, Alberti KG, Bennett P, Buse J, Defronzo R, Kahn R, Kitzmiller J, Knowler WC, Lebovitz H, Lernmark A, Nathan D, Palmer J, Rizza R, Saudek C, Shaw J, Steffes M, Stern M, Tuomilehto J, Zimmet P** 2003 Follow-up report on the diagnosis of diabetes mellitus. Diabetes Care 26:3160–3167
13. **Heikes KE, Eddy DM, Arondekar B, Schlessinger L** 2008 Diabetes Risk Calculator: a simple tool for detecting undiagnosed diabetes and pre-diabetes. Diabetes Care 31:1040–1045
14. **Bland JM, Altman DG** 1996 Measurement error. BMJ 313:744
15. **Unger RH** 2008 Reinventing type 2 diabetes: pathogenesis, treatment, and prevention. JAMA 299:1185–1187
16. **McGavock JM, Lingvay I, Zib I, Tillery T, Salas N, Unger R, Levine BD, Raskin P, Victor RG, Szczepaniak LS** 2007 Cardiac steatosis in diabetes mellitus: a ^{1}H-magnetic resonance spectroscopy study. Circulation 116:1170–1175