

This is the overview page

Metabolic Imaging of Fatty Kidney in Diabesity: Validation and Dietary Intervention

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Complete List of Authors:	<p>Jonker, Jacqueline; Leids Universitair Medisch Centrum, Department of Medicine, Division of Nephrology de Heer, Paul; Leids Universitair Medisch Centrum, Department of Radiology, C.J. Gorter Center for High Field MR Engelse, Marten; Leids Universitair Medisch Centrum, Department of Medicine, Division of Nephrology van Rossenberg, Evelien; Leids Universitair Medisch Centrum, Department of Medicine, Division of Nephrology Klessens, Celine; Leids Universitair Medisch Centrum, Department of Pathology Baelde, Hans J; Leids Universitair Medisch Centrum, Department of Pathology Bajema, Ingeborg M.; Leids Universitair Medisch Centrum, Department of Pathology Koopmans, Sietse Jan; Wageningen Universiteit en Researchcentrum, Animal Sciences Group Coelho, Paulo ; New York University College of Dentistry, Department of Biomaterials and Biomimetics Streefland, Trea; Leids Universitair Medisch Centrum, Department of Medicine, Division of Endocrinology Webb, Andrew; Leids Universitair Medisch Centrum, Department of Radiology, C.J. Gorter Center for High Field MR Dekkers, Ilona; Leids Universitair Medisch Centrum, Department of Radiology Rabelink, Ton; Leiden University Medical Center, Nephrology Rensen, Patrick; Leids Universitair Medisch Centrum, Department of Medicine, Division of Endocrinology; Leids Universitair Medisch Centrum, Eindhoven Laboratory for Experimental Vascular Medicine Lamb, Hildo; Leids Universitair Medisch Centrum, Department of Radiology De Vries, Aiko; Leids Universitair Medisch Centrum, Department of Medicine, Division of Endocrinology</p>
Keyword list:	Fatty kidney, Hepatic triglyceride content, Proton magnetic resonance spectroscopy, Renal triglyceride content, Type 2 diabetes Mellitus, Obesity

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For Peer Review

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3 Leiden, 19th of May 2017
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7 Dear Mr. Fouque,
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10 Please find enclosed the revised version of our manuscript "Metabolic Imaging of Fatty
11 Kidney in Diabetes: Validation and Dietary Intervention".
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13
14 We appreciate the constructive comments by the reviewers, which helped to improve
15 our manuscript.
16

17 All changes in the manuscript are highlighted. Please find attached point-to-point
18 answers to the comments of the reviewers as well as the changes in the revised
19 manuscript.
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22 If the manuscript is accepted for publication, we would like to have Figure 5 printed in
23 black and white and in colour online.
24

25
26 We hope that the revised manuscript is acceptable for publication in NDT.
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29 On behalf of all authors,
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31 Yours sincerely,
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34 Jacqueline Jonker
35 Paul de Heer
36 Leiden University Medical Center
37 Leiden, the Netherlands
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Response to comments of the reviewer:

We thank the reviewer for his/her comments that helped us to improve the manuscript.

"De Heer et al. present a manuscript in which they report on non-invasive measurement of renal triglyceride content by $^1\text{H-MRS}$, and compare this with tissue analyses. The manuscript is well-written and the data are clearly presented. I have the following questions and suggestions:

1) The authors have shown relatively strong associations between renal lipid accumulation quantified by MRS and enzymatic assays. However, this is not the same as good agreement between the two techniques. I suggest they provide Bland-Altman plots to address this

In accordance with the comment of the reviewer, we added a Bland-Altman plot to Figure 2 to address this point.

2) How do the authors explain the discrepancy between the effects on body weight gain and lipid accumulation (Fig 3a vs 3b/c)?

We thank the reviewer for this relevant comment. Indeed, this is a very interesting observation. In insulin resistant states, like the pigs in the cafeteria + streptozocin group, there is an increase in lipolysis (because of the relative shortage of insulin), leading to redistribution of triglycerides from adipose tissue to the organs (ectopic fat). Furthermore, the increased inflammation may lead to additional triglyceride deposition in the organs and especially the kidney, beyond mere obesity.

We added this to the discussion:

`Interestingly, pigs in the cafeteria+STZ group, albeit that they had a lower baseline weight and equal increase in body weight during the study, did have increased renal triglyceride content compared to the controls. In diabetes, an increase in lipolysis and in inflammation might lead to additional triglyceride redistribution into organs beyond mere obesity and thus accentuate the findings (2) `.

3) The fact that no significant differences between the groups were present for the MRS-based renal lipid measurements (despite $n=15/\text{group}$), although a trend was clearly present, suggests that some animals apparently displayed high lipid accumulation in their kidneys whereas other animals were very similar to controls. Could the authors provide additional data clarifying what could explain the inter-individual variation within the groups?

This is an interesting observation and there were indeed large inter-individual differences between the pigs. However we don't think it is possible to draw any conclusions on the underlying mechanisms, as the groups consisted of 5 pigs per diet group. There seems to be a trend toward higher fructosamin levels (as a proxy for insulin resistance) in the pigs with the highest renal TG content, however this is not

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3 statistically significant. Therefore, it is important to repeat these studies in-vivo with
4 larger groups.
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7 4) What is the relationship between MRS findings and renal triglyceride accumulation
8 quantified by the Oil Red O staining?
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10 The Pearson correlation between the renal triglyceride content measured by ¹H-MRS
11 and the lipids measured by Oil Red O staining is 0.67 (P=0.006). We did not include this
12 comparison in our manuscript, as Oil Red O measures all lipids in the biopsy, while ¹H-
13 MRS measures specifically triglycerides.
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16 5) How can the authors be sure that this is actually renal lipid accumulation, and not
17 clearance we are looking at? From Figure 5 it seems that the majority of the lipids are
18 present in vesicles within the tubuli. This makes the reviewer wonder whether fatty
19 kidney is actually a pathophysiological phenomenon or rather a reflection of high-fat
20 intake?"
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22

23 This is a very important remark indeed. We think both suggestions of the reviewer are
24 true. Tubular uptake of non esterified fatty acids (NEFA) occurs proportional to
25 glomerular albuminuria and the plasma NEFA concentration. Some of the NEFA
26 undergo oxidation for formation of ATP for high energy processes, the rest is stored into
27 lipid droplets as triglycerides (TG). As accounts for other organs (heart, muscle), a little
28 TG storage will indeed not interfere with organ function. However, in animal studies, it
29 has been shown that an excess in renal TG deposition can lead to tubulointerstitial
30 injury. Furthermore the accumulation of TG also leads to an increase in renal
31 gluconeogenesis and in type 2 diabetes, the kidney contributes to postprandial and
32 fasting hyperglycemia. Therefore, the amount of renal TG accumulation depends on
33 high fat intake and insulin sensitivity.
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36 (Refs: D'Agati VD et al. (2016) Obesity-related glomerulopathy: clinical and pathologic
37 characteristics and pathogenesis. *Nat Rev Nephrol* Aug;12(8):453-71; de Vries et al.
38 Ruggenenti P, Ruan XZ, et al (2014) Fatty kidney: emerging role of ectopic lipid in
39 obesity-related renal disease. *Lancet Diabetes Endocrinol* 5:417-426).
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Abstract

Background

Obesity and type 2 diabetes have not only been linked to fatty liver, but also to fatty kidney and chronic kidney disease. Since noninvasive tools are lacking to study fatty kidney in clinical studies, we explored agreement between proton magnetic resonance spectroscopy ($^1\text{H-MRS}$) and enzymatic assessment of renal triglyceride content (without and with dietary intervention). We further studied the correlation between fatty kidney and fatty liver.

Methods

Triglyceride content in the renal cortex was measured by $^1\text{H-MRS}$ on a 7-Tesla scanner in 27 pigs, among which 15 minipigs had been randomized to a 7-month control diet, cafeteria diet (CAF), or CAF with low-dose streptozocin (CAF-S) to induce insulin-independent diabetes. Renal biopsies were taken from corresponding MRS-voxel locations. Additionally, liver biopsies were taken and triglyceride content in all biopsies was measured by enzymatic assay.

Results

Renal triglyceride content measured by $^1\text{H-MRS}$ and enzymatic assay correlated positively ($r=0.86$, $P<0.0001$). Compared to control diet-fed minipigs, renal triglyceride content was higher in CAF-S fed minipigs (137 ± 51 nmol/mg protein, mean \pm SEM, $P<0.05$), but not in CAF-fed minipigs (60 ± 10 nmol/mg protein) compared to controls (40 ± 6 nmol/mg protein). Triglyceride content in liver and kidney biopsies were strongly correlated ($r=0.97$, $P<0.001$).

Conclusions

Non-invasive measurement of renal triglyceride content by $^1\text{H-MRS}$ closely predicts triglyceride content as measured enzymatically in biopsies, and fatty kidney appears to develop parallel to fatty liver. $^1\text{H-MRS}$ may be a valuable tool to explore the role of fatty kidney in obesity and type 2 diabetic nephropathy in humans in-vivo.

Keywords:

Fatty kidney, hepatic triglyceride content, obesity, proton magnetic resonance spectroscopy, renal triglyceride content, type 2 diabetes mellitus, ectopic fat

Short summary:

Obesity and type 2 diabetes have been linked to renal triglyceride accumulation (fatty kidney) and chronic kidney disease. Current study shows that proton magnetic resonance spectroscopy may be a valuable, non-invasive tool to further explore the role of fatty kidney in obesity and type 2 diabetic nephropathy in humans in-vivo.

Introduction

Over the past decades, the prevalence of obesity and type 2 diabetes has grown to epidemic proportions (1). Obesity, in particular central obesity, is associated with metabolic dysfunction, which drives the development of insulin resistance leading to type 2 diabetes and ultimately end organ damage. The combination of obesity and type 2 diabetes, also referred to as diabetes, is often accompanied by other cardiovascular risk factors, including hypertension and dyslipidemia. Common early markers of renal disease like glomerular hyperfiltration and increased urinary albumin are more prevalent in both obesity and type 2 diabetes (2). Furthermore, renal pathology has shown considerable overlap between obesity and type 2 diabetes (3).

How (early) diabetes may lead to incipient chronic kidney disease remains to be understood, but ectopic lipid accumulation in the kidney (fatty kidney) has gained appreciation as a novel potential pathway (2,4-6). Diabetes is associated with lipid accumulation in non-adipose tissue such as liver, skeletal muscle, and heart, and this so-called ectopic fat may interfere with cellular function in the respective organ (7-10). Notably, obesity and type 2 diabetes have been associated with renal lipid accumulation in both human and porcine kidneys with differences in anatomical distribution between glomeruli and tubuli, as well as cortex and medulla (11,12). Increased renal lipid content has also been linked to functional and structural renal hyperfiltration (12), obesity-related glomerulopathy (2), and type 2 diabetic nephropathy (13). Various rodent models have shown that intervention in cellular lipid pathways attenuated obesity-related glomerulopathy or diet-induced chronic kidney disease (14). To date, translation of such experimental evidence to the clinical arena has been hampered by lack of a non-invasive diagnostic tool to sequentially monitor renal lipid accumulation in obesity and type 2 diabetes mellitus.

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5 Proton magnetic resonance spectroscopy (^1H -MRS) is a non-invasive and reproducible
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Proton magnetic resonance spectroscopy (^1H -MRS) is a non-invasive and reproducible technique which has been used successfully to quantify lipid content in heart, liver, and muscle (15-17). Recently, feasibility and reproducibility of renal ^1H -MRS in-vivo was shown (18). However, few ^1H -MRS protocols have been validated against tissue biopsies.

Therefore, we explored the agreement of the non-invasive ^1H -MRS renal triglyceride measurement, using a 7-Tesla Magnetic Resonance (MR) scanner, against a biochemical assay (as gold standard) to determine lipid accumulation in of porcine kidneys. Secondly, we measured renal triglyceride content after a dietary intervention study in minipigs. We investigated the effects of a high-fat, high-cholesterol cafeteria diet with and without low-dose streptozocin to induce non-insulin dependent diabetes mellitus, compared to standard diet on renal and hepatic triglyceride content and distribution.

Subjects and Methods

Group A

Fourteen left-sided porcine kidneys were harvested from two Dutch pig slaughter lines. The kidneys were harvested within 30 minutes of termination to limit warm ischemia time and were placed on ice. Kidneys were flushed with University of Wisconsin (UW) fluid and were scanned upon arrival. Tissue biopsies were taken from both the upper and lower pole of each kidney, immediately after scanning. The biopsy locations were visually matched to the areas where the ^1H -MRS measurements were performed. Biopsies were snap frozen in liquid nitrogen and stored at -80°C .

Group B

Fifteen Göttingen minipigs (19) were studied after they had been randomized to two different diets. The control group received a diet consisting primarily of barley, wheat, and soya bean oil. The cafeteria group was fed a cafeteria diet (20), with a high content of lard, fructose, sucrose, and added cholesterol. Five of the cafeteria diet fed pigs were additionally treated with low-dose streptozocin (STZ) after 5 months. The dose of STZ was individually adjusted to induce non-insulin dependent diabetes to model type 2 diabetes. After seven months of diet, the pigs were euthanized. Directly after termination, blood was drawn and a liver biopsy was taken. The whole study was executed at Ecole Veterinaire D'Alfort, France and was approved by the local ethics committee for animal experiments. The same harvesting and analyses protocols performed for Group A were followed: left sided kidneys were harvested with a maximum of 30 minutes warm ischemia time, kidneys were flushed with UW fluid and transported to the hospital in the Netherlands on ice, for subsequent 7-Tesla MR scanning. After each MR scan, renal biopsies were taken from the corresponding MRS-voxel location of the upper and lower kidney poles. Biopsies were snap-frozen and stored at -80°C. For both groups A and B, all kidneys had a maximum cold ischemia time of 20 hours before ¹H-MRS was performed.

Measurements

Proton Magnetic Resonance Spectroscopy

A 7-Tesla Philips Magnetic Resonance Imaging (MRI) scanner (Philips Healthcare, Best, The Netherlands) was used to measure renal triglyceride content by ¹H-MRS. A Nova quadrature transmit and 32-channel receive head coil (Nova Medical, Wilmington, MA, USA) was used for transmission and reception. A survey together with a Dixon water-fat scan was performed to position the MRS voxel (10x10x10 mm³) within the cortex of the kidney trying to avoid

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3 the medulla as much as possible (21). On the Dixon scan fat image (Figure 1) correct
4 placement was confirmed, carefully avoiding extracellular (sinus or perirenal) lipids in the
5 voxel. Secondly, measurements were performed in the upper and lower pole of each kidney.
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7 Stimulated-echo acquisition mode (STEAM) spectra were acquired with an echo time of 8.2
8 ms without water suppression (repetition time (TR) 9 s, 3 averages) and with Multiply
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10 Optimized Insensitive Suppression Train (MOIST) water suppression (TR 3.5 s, 96 averages)
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12 (22,23). The MRS acquisition had a bandwidth of 3000 Hz and 4096 samples were acquired
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14 resulting in spectral resolution of 0.73 Hz / sample. All spectra were fitted in the time-domain
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16 using the Java-based MR User Interface (jMRUI) (24). The advanced method for accurate,
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18 robust and efficient spectral fitting (AMARES) algorithm was used to fit the resonances to a
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20 Gaussian line shape. Triglyceride content was calculated as a percentage of the
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22 (unsuppressed) water peak using the following equation:
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$$32 \quad TG = \frac{TG \text{ methyl } (CH_3) + TG \text{ methylene } (CH_2n)}{Water + TG \text{ methyl } (CH_3) + TG \text{ methylene } (CH_2n)} \times 100\% \quad (\text{Equation 1})$$

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35 where, TG = renal triglyceride content [%]

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37 TG methyl = Area of the methyl resonance at 0.9 ppm [arbitrary unit]

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39 TG methylene = Area of the methylene resonance at 1.3 ppm [arbitrary unit]

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42 Water = Area of the water resonance [arb. unit]
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46 *Enzymatic measurement of lipids in renal and liver biopsy*

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48 The biopsies from the upper and lower pole of each kidney, as well as the liver biopsies were
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50 snap-frozen in liquid nitrogen, homogenized, and total lipids were extracted according to a
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52 modified protocol from Bligh and Dyer (25). Total triglycerides were measured by an
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54 enzymatic kit (no. 11488872, Roche Diagnostics). The triglyceride content per biopsy was
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3 divided by the protein content (nmol triglycerides/mg protein) to correct for the size of the
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5 biopsy.
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8 9 *Oil Red O staining*

10 Frozen kidney sections (10- μ m thickness) were cut on a Reichert cryostat microtome. Oil
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12 Red O (ORO) staining was performed by incubation of the slides with an ORO solution (2
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14 mg/ml in 40% isopropanol). Sections were rinsed with ethanol, covered with Aqua-Mount
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16 mounting media and digitized with a Philips Ultra-Fast 1.6 RA Scanner (Philips, The
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18 Netherlands). The ORO positive surface area was measured using the ImageJ (U. S. National
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20 Institutes of Health, Bethesda, Maryland, USA) on 5 random selected areas of the renal
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22 cortex, and the percentage of the positive area was calculated.
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28 29 *Other laboratory measurements*

30 Concentrations of plasma triglycerides and creatinine (both total coefficient of variation
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32 (CV): 1.0-1.2%, determined by vendor), urinary creatinine (total CV: 0.5-0.7%) and urinary
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34 protein (total CV: 0.2-0.6%) were measured on a Modular P800 analyzer (Roche, Basel,
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36 Switzerland). Plasma fructosamine (total CV 1.2-1.5%) was measured using a Cobas 6000
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38 analyzer (Roche, Basel, Switzerland).
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45 **Statistics**

46 All statistical analyses were performed using SPSS version 22.0 (IBM SPSS Statistics for
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48 Windows. Armonk, NY: IBM Corp). Plots were created using Graph Pad (Graph Pad
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50 Software, La Jolla California USA). All but one analysis were performed with the average
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52 triglyceride content from the upper and lower pole of each kidney. **Bland-Altman analysis**
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54 **was performed to evaluate the agreement between renal TG content by ¹H-MRS and renal**
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3 lipid content by enzymatic assay, after normalization of the data. In one case the triglyceride
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5 content of a single pole was taken due to a corrupt MRS data file. Agreement between the
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7 triglyceride measurement techniques was evaluated by linear regression with a 95%
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9 prediction interval. Group differences between the three experimental groups were analyzed
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11 using analysis of variance (ANOVA). Least significant difference (LSD) post-hoc tests were
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13 used in case of a significant difference. Non-normally distributed data were log-transformed
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15 and checked for normality after transformation. Data are expressed as mean values \pm standard
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17 error of the mean (SEM) and a probability value of 0.05 or less was considered statistically
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19 significant.
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Results

Validation of renal triglyceride quantification by $^1\text{H-MRS}$

Renal triglyceride content was measured in 29 left sided porcine kidneys by $^1\text{H-MRS}$ and by enzymatic assay. Two measurements had to be excluded from further analysis due to a failed measurement in the enzymatic assay and one due to a corrupt MRS data file, resulting in a total of 27 measurements (n=12 from group A, n=15 from group B).

In group A (slaughter pigs), the mean renal triglyceride content was $0.23 \pm 0.03\%$ (mean \pm SEM) as measured by $^1\text{H-MRS}$ and 111 ± 15 nmol/mg protein as measured by enzymatic assay. The mean renal triglyceride content in group B (minipigs) was $0.15 \pm 0.03\%$ as measured by $^1\text{H-MRS}$ and 79 ± 20 nmol/mg protein as quantified by enzymatic assay.

Data from groups A and B were pooled and triglyceride content measured by $^1\text{H-MRS}$ and enzymatic assay showed a positive correlation ($r=0.86$, $P<0.0001$, Figure 2). The regression equation was as follows: triglycerides measured by $^1\text{H-MRS}$ (%) = $0.03 + 0.0017 \times$ triglycerides measured by enzymatic assay (nmol/mg). The triglyceride content for the pooled data ranged from 0.06% to 0.8% as measured by $^1\text{H-MRS}$ and from 30 to 331 nmol/mg protein as measured by enzymatic assay.

The Bland-Altman analysis using the normalized data showed good agreement with a bias of 0.00 (limits of agreement -0.8; 0.8) for $^1\text{H-MRS}$ and enzymatic assay measurements of renal lipid content over the metabolic spectrum (Figure 2).

Effects of cafeteria diet and streptozocin

The minipigs were 2.7 ± 0.2 years of age at termination. The pigs in the control group were heavier at the start of the study (62.7 ± 3.3 kg, $P<0.05$) than the pigs in the cafeteria group

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3 (51.4±2.0 kg) and cafeteria+STZ group (49.7±2.8 kg). The 7-month cafeteria diet induced a
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5 significant larger weight gain (39.6±3.6 kg) than the control diet (23.4±3.1 kg, P<0.05) and
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7 the cafeteria+STZ diet (23.2±5.5 kg, P<0.05, Figure 3). Serum fructosamine was higher in
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9 the cafeteria+STZ group (358±31 µmol/l, P<0.05), than in the cafeteria (264±6 µmol/l) and
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11 control group (259±11 mol/l). Serum triglycerides, serum creatinine and urine protein did not
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13 significantly differ between the groups.
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19 Renal triglyceride content was higher in the cafeteria+STZ group (137±51 nmol/mg protein)
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21 compared to the control group (40±6 nmol/mg protein, P<0.05) (Figure 3). Renal triglyceride
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23 content of the cafeteria diet group (60±10 nmol/mg protein) was not significantly different
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25 from the control group (Figure 3). Renal TG content measured by proton MR spectroscopy,
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27 showed a similar trend between the groups, however this was not statistically significant.
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29 Similarly, hepatic triglyceride content after the 7-month diet was higher in the cafeteria+STZ
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31 group (417±157 nmol/mg protein) compared to the control group (67±11 nmol/mg protein,
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33 P<0.05). The cafeteria group (141±49 nmol/mg protein) had a trend towards higher hepatic
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35 triglyceride content than the control group, but this was not significantly different (Figure 3).
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37 We observed a significant correlation between renal and hepatic triglyceride content
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39 measured by enzymatic assay (n=14, r=0.97, P<0.001, Figure 4).
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45 **Lipid distribution**

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47 We also assessed lipid localization by Oil Red O staining. The lipid content was very low in
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49 biopsies of the control diet group (percentage staining: 0.2±0.0%), and markedly higher in
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51 those of the cafeteria group (3.7±1.9%, P<0.05 versus control diet) and cafeteria+STZ group
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53 (9.6±6.3%, P<0.05 versus control diet). There was pronounced lipid accumulation in the
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55 tubuli and to a lesser extent Oil Red O staining was visible in the glomeruli (Figure 5).
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Discussion

The merit of clinical ^1H -MRS to investigate the role of ectopic lipid accumulation has been clearly established for other organs such as liver, muscle, and heart (17, 26,27). In fact, the development of ^1H -MRS of the heart has led to a better understanding of factors influencing myocardial triglyceride accumulation in relation to cardiac function in both health and diabetes (9,17). Our present study is one of the first to explore ^1H -MRS as a noninvasive tool to study fatty kidney in diabetes and to study agreement with tissue biopsies.

Thus far, only few studies reported on ^1H -MRS of the kidney, most likely owing to technical difficulties to develop and apply this technique reliably to the kidney for several reasons. Firstly, adjustment for respiratory motion and carefully avoiding contamination by perirenal and sinus fat is necessary to obtain reliable measurements from the kidney cortex only and not surrounding structures. Secondly, kidney tissue is not homogenous with substantial anatomical and functional variation between cortex and medulla as well as glomeruli and tubuli. Lastly, due to the limited space in the kidney cortex, the volume of the measured voxel has to be small, resulting in a much lower signal-to-noise-ratio compared to e.g. heart and liver.

This notwithstanding, we previously reported a feasibility and reproducibility study of renal ^1H -MRS in healthy (non-obese) volunteers in-vivo, but variation was apparent even in this relative homogenous group of subjects (18). Consequently, we aimed to improve our knowledge on triglyceride distribution across the metabolic spectrum of diabetes and across kidney anatomy with regard to voxel placement and data acquisition. Also, few spectroscopy protocols underwent validation and testing against invasive, gold standard assessment of triglycerides. Because of the impossibility to obtain enough discarded kidneys from obese

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3 and type 2 diabetic postmortem kidney donors to perform a validation study (those kidneys
4 are often not offered for transplantation), we turned to similar sized porcine kidneys to test
5 our clinical ¹H-MRS protocol. This study in porcine kidneys shows that ¹H-MRS closely
6 predicts the triglyceride content as measured by enzymatic assay of kidney biopsies (gold
7 standard) and therefore it may also be a valuable tool to study fatty kidney in humans.
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16 To evaluate the renal triglyceride content over the metabolic spectrum in conjunction with the
17 hepatic triglyceride content, we used minipigs fed a high fat, high cholesterol diet to develop
18 a metabolic syndrome phenotype including high amounts of visceral fat and insulin resistance
19 (19). Because those pigs have a large pancreatic beta cell capacity, it takes a very long time to
20 develop type 2 diabetes. Therefore the pigs were individually treated with a low dose of STZ
21 to damage a part of the pancreatic beta cell capacity, to create non-insulin dependent diabetes
22 that reflects type 2 diabetes. Using this model, we showed that renal triglyceride content
23 increased over the metabolic spectrum in conjunction with hepatic triglycerides.
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36 We observed a trend to an increase in both renal and hepatic triglyceride content in the
37 cafeteria diet only group, however this was not significant. Previous studies have shown
38 correlation between increased bodyweight and increased hepatic and renal triglyceride
39 content in both animals and humans (11,12,28,29). As our study was exploratory and the
40 animal groups small, the study may have been underpowered to detect a significant difference
41 between the control and cafeteria group. One possible confounder in this study is the
42 difference in baseline weight of the minipigs, as controls were significantly heavier than the
43 pigs in the intervention groups.
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3 Interestingly, pigs in the cafeteria+STZ group, albeit that they had a lower baseline weight
4 and equal increase in body weight during the study, did have increased renal triglyceride
5 content compared to the controls. In diabetes, the increase in inflammation or chronic kidney
6 disease might lead to additional triglyceride redistribution into organs beyond mere obesity
7 and thus accentuate the findings (2). We found a strong positive correlation between renal
8 and hepatic triglyceride content. A study in mice has shown that from a certain bodyweight,
9 the expandability of white adipose tissue becomes limited and fat starts to rapidly accumulate
10 ectopically within e.g. the liver (29). It is likely that a similar mechanism may underlie renal
11 triglyceride accumulation, which needs to be explored in future studies.
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25 By using Oil Red O staining, we observed similar trends, with increasing triglyceride content
26 in the cafeteria group and even more in the cafeteria+STZ group. We found that renal
27 triglyceride accumulation was most prominent in renal tubuli, albeit some triglyceride
28 staining was also observed in the glomeruli. This is in accordance with a recent study of
29 human nephrectomies, where triglyceride droplets were predominantly found in tubular cells
30 and to a lesser extent in glomeruli (11). Future studies should focus on even better
31 understanding of how triglyceride accumulation may lead to chronic kidney disease in
32 diabetes and whether this process can be reversed.
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45 *Conclusion*

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47 Non-invasive measurement of renal triglyceride content by ¹H-MRS closely predicts the
48 triglyceride content as measured enzymatically in biopsies. Renal triglyceride content
49 increases over the metabolic spectrum of diabetes and in the current study fatty kidney
50 develops in parallel with fatty liver. Proton magnetic resonance spectroscopy seems suitable
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3 to explore the role of fatty kidney in obesity and type 2 diabetic nephropathy in humans in-
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Conflict of interest statement

All authors have fulfilled the criteria for authorship and report no conflicts of interest. A manuscript on the same or similar material has not already been published by us or has not been or will not be submitted to another journal by us or by colleagues at our institution before the work appears in Nephrology Dialysis and Transplantation. Part of this work was presented orally at the ASN Kidney Week 2016 in Chicago.

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Figure legends

Figure 1

Placement of the voxel (black box) in the kidney cortex on a transverse and coronal survey (A) and Dixon fat image (B). Below the surveys two spectra acquired from the kidney cortex of two different kidneys. Spectra have been selected to show an example of low and high renal triglyceride (TG) content and have been scaled to the water signal to be able to visually compare the two. For quantification TG content the CH_2n (1.3 ppm) and the CH_3 (0.9 ppm) resonances are used. Other visible resonances in the spectra are $\text{CH}_2\text{CH}_2\text{COO}$ marked with number 1 at 1.5 ppm, $\text{CHCH}_2=\text{CHCH}_2$ marked with number 2 at 2 ppm, and CH_2COO marked with number 3 at 2.2 ppm and a trimethylamine (TMA) resonance at 3.2 ppm.

Figure 2

Correlation (left) and Bland-Altman difference-average plot (right) of renal triglyceride (TG) content measured by ^1H -MRS and enzymatic assay in biopsies, using pooled data from both study A (12 slaughter pigs) and study B (15 minipigs) showing a positive correlation ($r=0.86$, $P<0.0001$).

Figure 3

Weight gain (A), renal triglyceride (TG) content measured by proton magnetic resonance spectroscopy (^1H -MRS, B), renal TG content from renal biopsy by enzymatic assay (C), hepatic TG content from liver biopsy by enzymatic assay (D) after 7 months of control diet, cafeteria diet or cafeteria diet with streptozocin (STZ). Data are represented as mean \pm SEM (n=15 for renal triglycerides, n=14 for hepatic triglycerides). * $P<0.05$

Figure 4

Correlation between renal and hepatic triglyceride (TG) content after 7 months of diet, measured by enzymatic assay in biopsies (n=14).

Figure 5

Three representative examples of Oil Red O staining for lipids in kidney biopsies from minipigs on a 7 months control (A), cafeteria (B) or cafeteria with streptozocin (C) diet. Percentage of biopsy with Oil Red O staining in the three diet groups (mean±SEM, n=5 per group). *P<0.05 compared to the control diet.

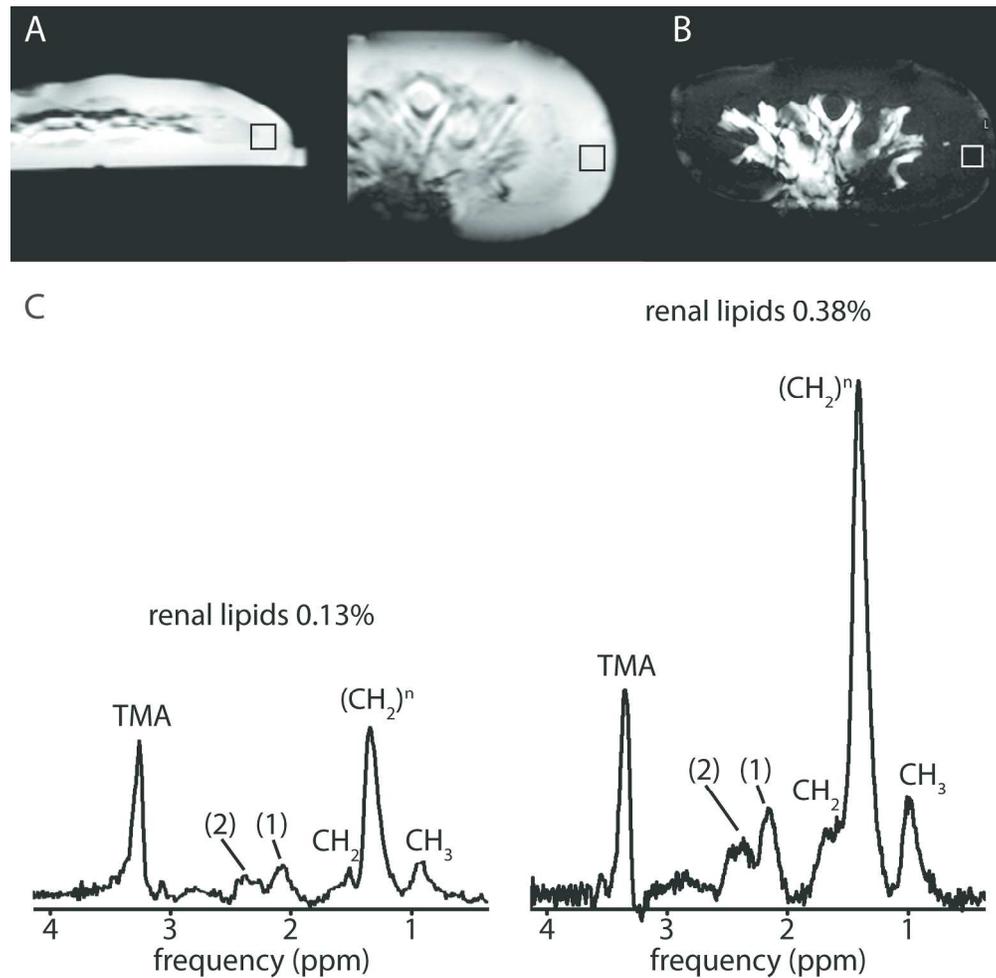


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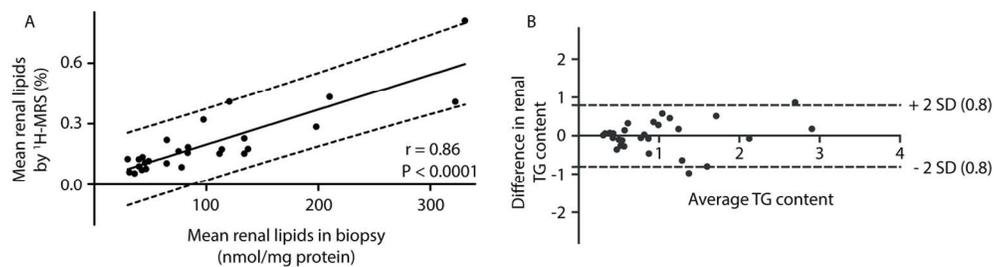


Figure 2

Correlation (left) and Bland-Altman difference-average plot (right) of renal triglyceride (TG) content measured by $^1\text{H-MRS}$ and enzymatic assay in biopsies, using pooled data from both study A (12 slaughter pigs) and study B (15 minipigs) showing a positive correlation ($r=0.86$, $P<0.0001$).

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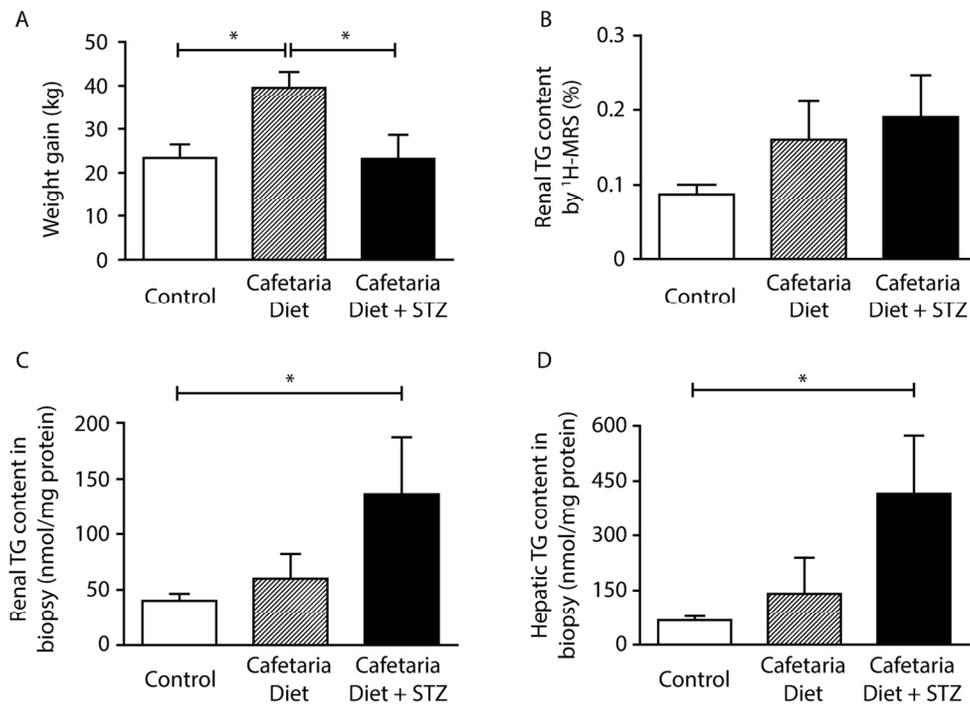


Figure 3

Weight gain (A), renal triglyceride (TG) content measured by proton magnetic resonance spectroscopy (¹H-MRS, B), renal TG content from renal biopsy by enzymatic assay (C), hepatic TG content from liver biopsy by enzymatic assay (D) after 7 months of control diet, cafeteria diet or cafeteria diet with streptozocin (STZ). Data are represented as mean±SEM (n=15 for renal triglycerides, n=14 for hepatic triglycerides). *P<0.05

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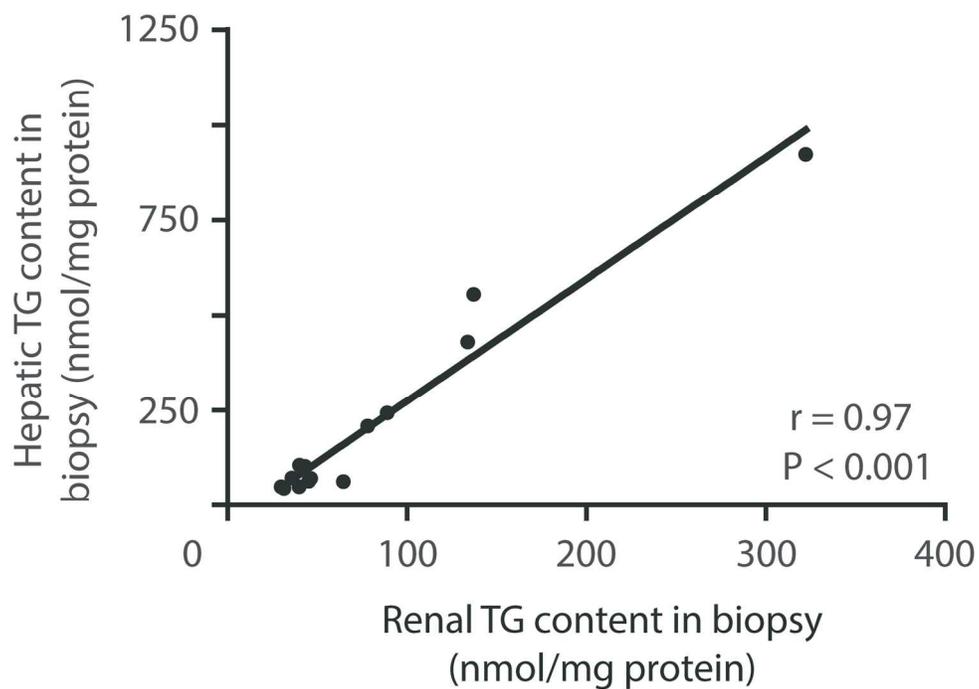


Figure 4
Correlation between renal and hepatic triglyceride (TG) content after 7 months of diet, measured by enzymatic assay in biopsies (n=14).

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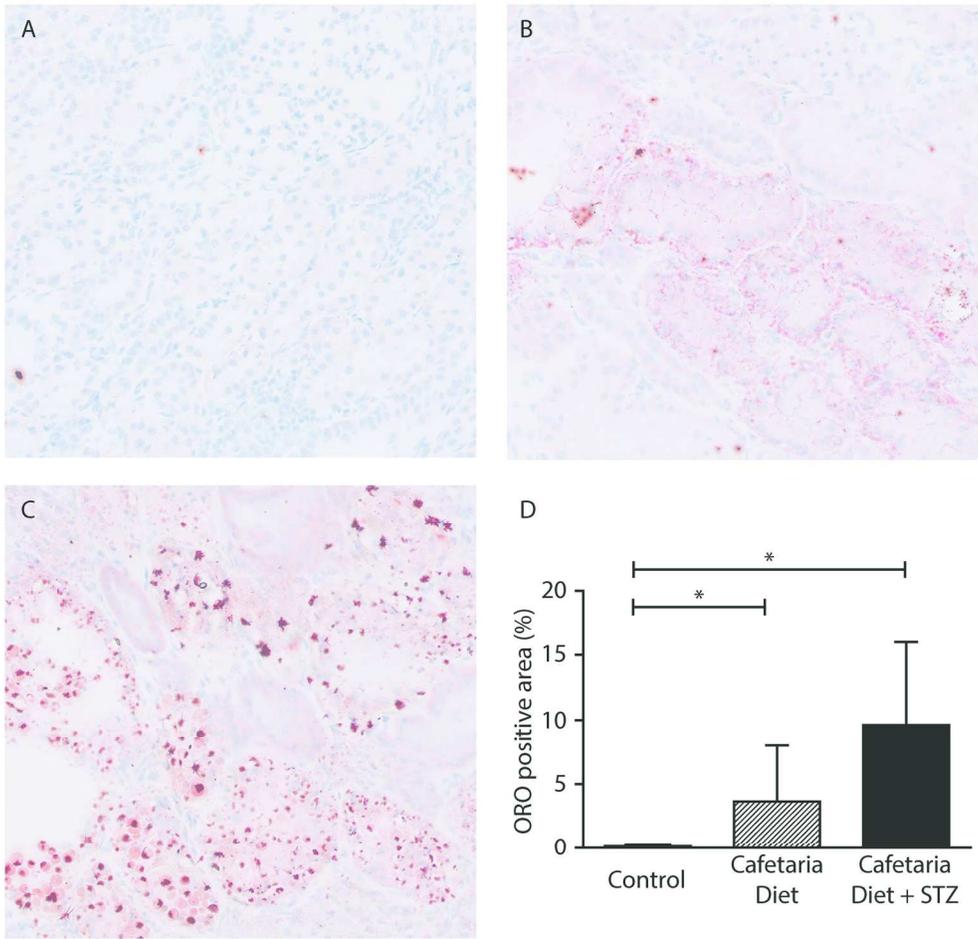


Figure 5
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