Quantitative MRI in obesity & reno-cardiovascular function

Dekkers, I.A.

6

The effect of glycemic control on renal triglyceride content assessed by proton-spectroscopy in patients with type 2 diabetes mellitus: a single-center parallel-group trial

Dekkers IA, Bizino MB, Paiman EHM, Smit JW, Jazet IM, de Vries APJ, Lamb HJ.

Submitted

ABSTRACT

Objective

Since renal steatosis is a potential driver of diabetic kidney disease, and tight glycemic control can reduce risk of diabetic nephropathy, we assessed whether glycemic control influences renal triglyceride content (RTGC). Furthermore, we compared GLP-1 receptor agonist liraglutide versus standard glucose-lowering therapy.

Methods

In this single-center parallel-group trial T2DM patients were randomized to liraglutide or placebo added to standard care (metformin/sulfonylurea-derivative/insulin). Change in RTGC after 26 weeks of glycemic control measured by proton-spectroscopy and difference in RTGC between treatment groups was analyzed.

Results

Fifty T2DM patients were included in the baseline analysis (mean age of 56.5 ± 9.1 years; range 33–73 years; 46% males). Seventeen patients had baseline and follow-up measurements. Mean HbA1c was 61.6 ± 8.4 mmol/mol, which changed to 56.3 ± 9.5 mmol/mol after 26 weeks of glycemic control irrespective of treatment group (P=0.046). Log-transformed RTGC was -0.68 ± 0.30%, and changed to -0.83 ± 0.32% after 26 weeks of glycemic control irrespective of treatment group (P=0.049). 26-weeks ' to ' baseline RTGC ratio (95% CI) was significantly different between liraglutide (-0.30 [-0.50,-0.09]) and placebo added to standard care (-0.003 [-0.34,0.34]) (P=0.04).

Conclusions

In this exploratory study we found that twenty-six weeks of glycemic control resulted in lower RTGC, in particular for liraglutide, however larger clinical studies are needed to assess whether these changes reflect a true effect of glycemic control on renal steatosis.

INTRODUCTION

Roughly a third of patients with type 2 diabetes mellitus (T2DM) will develop diabetic kidney disease (DKD) depending on age, ethnicity, diabetes duration and/or extent of hyperglycemia exposure (1). DKD is one of the leading causes of end-stage renal disease (ESRD) worldwide, and the UKPDS (2) and ADVANCE (3) studies showed that improved glycemic control reduces microvascular disease and ESRD. Additionally, the RENAAL (4) and IDNT (5) trials showed that treatment of hypertension and proteinuria in particular when using renin-angiotensin-aldosterone system inhibitors, conveyed a 30% risk reduction for ESRD. In spite of these cornerstone therapies, the incidence of ESRD by DKD continues to rise (6), indicating possible involvement of other (non-proteinuric) pathways related to e.g. hyperfiltration and metabolic regulation (7).

In particular, the combination of T2DM and obesity has been linked to ectopic lipid accumulation in non-adipose tissues such as liver, heart, and kidney, which can interfere with the cellular function of the respective organ (8,9). With regard to the kidney, ectopic lipid accumulation has been linked to structural changes including glomerular hypertrophy (10), and maladaptive functional responses such as hyperfiltration and albuminuria (9). Renal steatosis has been associated with renal gluconeogenesis in experimental models (9), however it is unknown whether glycemic control is conversely linked to ectopic lipid accumulation in kidney. The LEADER trial (11) showed that the glucagon-like peptide-1 receptor agonists (GLP1-RA) liraglutide was renoprotective in DKD compared to current standard glycemic care, which could possibly be related to improved glycemia, amended blood pressure regulation and reduction of weight and/or ectopic fat depots such liver fat and visceral fat. Alternatively, direct actions of GLP1-RA on the kidney have been proposed (12). Currently, it is unknown to which extent improved glycemic control, either by standard glycemic care or via additional direct effects of GLP1-RA, relates to renal steatosis in vivo. Experimental studies have shown that liraglutide might have a renoprotective effect via restoring renal metabolism by inhibiting renal lipid accumulation (13,14).

Clinical studies on renal lipid metabolism have been hampered by the absence of a non-invasive technique to measure renal lipid content. Magnetic resonance spectroscopy (^{1}H-MRS) is considered the gold standard technique to measure hepatic lipid content in vivo (15), however the application of ^{1}H-MRS to the kidney is technically challenging due to respiratory motion and low signal-to-noise ratio related to the low quantities of renal lipids and limited voxel size (16). Recently, we validated and assessed the reproducibility of renal triglyceride content (RTGC) measured using ^{1}H-MRS (16,17). Assessment of RTGC using ^{1}H-MRS offers the possibility to study the potential influence of glucose control on renal lipid metabolism, including novel drugs such as GLP1-RA, in a clinical trial. Here, we aimed to study whether glycemic control influenced RTGC as a secondary outcome of

a 26 week clinical trial of liraglutide versus placebo, added to standard glucose-lowering therapy using metformin, sulphonylurea derivatives (SUD) and/or insulin. A secondary aim was to investigate whether these two treatment groups differed in reduction of renal steatosis.

MATERIALS AND METHODS

Study design

This study is a single-center parallel-group trial containing the baseline and follow-up data of the MAGNA VICTORIA studies in Western European (ClinicalTrials.gov NCT01761318), and South Asian (NCT02660047) T2DM patients. The present study involved RTGC as a prespecified secondary endpoint, previously published primary and secondary endpoints were e.g. left ventricular function, HbA1c, body weight and measures of body fat distribution (visceral fat, hepatic triglyceride content) (18–20). Study protocols have been described elsewhere in more detail (18–20). In short, patients were randomized (1:1 stratification for sex and insulin use in both studies separately, block size 4) to receive either liraglutide (Victoza, Novo Nordisk A/S, Bagsvaerd, Denmark) or placebo (provided by Novo Nordisk A/S, Bagsvaerd, Denmark) for 26 weeks, added to standard glucose-lowering therapy using metformin, SUD and/or insulin (18–20). Study participants, researchers, and other staff involved in the study were blinded to treatment allocation until completion of the study and analysis. Written informed consent was obtained prior to inclusion. The present study was performed according to the revised Declaration of Helsinki and was approved by the institutional review board (Leiden University Medical Center, Leiden, the Netherlands).

Participants

At start of the study, the inclusion criteria for T2DM patients were defined irrespective of ethnicity (self-identified and self-reported origin of both biological parents and their ancestors), however due to the scarcity of eligible patients of South Asian descent the inclusion criteria for this group were adjusted. Final inclusion criteria for the European and South Asian T2DM patients were, respectively: age 18-70 and 18-75 years, glycated hemoglobin (HbA1c) between ≥53.0 and <86.5 mmol/mol (≥7.0 and ≤10.0%) and ≥47.5 and <96.5 mmol/mol (≥6.5 and ≤11.0%), systolic and diastolic blood pressure between <150/85 mmHg and <180/110 mmHg, estimated glomerular filtration rate (eGFR) above >60 ml/min/1.73m^2 and >30 ml/min/1.73m^2, no history of heart failure (New York Heart Association class III-IV), no history of coronary artery disease for the European T2DM patients, and no acute coronary accident in the preceding 30 days for the South Asian T2DM patients (19,21).

Data collection

Potential participants were evaluated at a screening visit to verify eligibility for inclusion. Clinical examinations and MR scanning (including ^{1}H-MRS) were scheduled either in the morning after an overnight fast or evening (≥6 hours fasting) (for T2DM patients, the insulin dose was adjusted and study drug and other glucose-lowering medication were discontinued for maximum of 24 hours). At start and at the end of the study fasting blood samples were taken, and weight and blood pressure measurements were performed. Blood pressure was measured in seated position on the right arm after rest, using a validated automatic oscillometric device (SureSigns VS3 Vital signs monitor, Philips, Best, the Netherlands) and was the mean of two consecutive measurements. Due to logistical reasons, HbA1c was measured with boronate affinity high-performance liquid chromatography (Primus Ultra, Siemens HealthcareDiagnostics, Breda, the Netherlands) and with ion-exchange high-performance liquid chromatography (HPLC; Tosoh G8, Sysmex Nederland B.V., Etten-Leur, the Netherlands), therefore HbA1c measurements were corrected based on the correlation coefficient of a validation sample measured on both analyzers (18). Serum creatinine (SCr), triglyceride, total cholesterol, HDL-cholesterol, LDL-cholesterol (Friedewald formula) were measured on a Modular P800 analyzer (Roche Diagnostics, Mannheim, Germany), and urine samples for the measurement of urinary creatinine albumin ratio (UACR) were collected and analyzed on a Modular P800 analyzer (Roche Diagnostics, Mannheim, Germany) (18). Bioelectrical impedance analysis was used to estimate total body fat percentage (BIA; Bodystat 1500, Bodystart Ltd., Douglas, United Kingdom). Serum creatinine (mg/dl) was used to calculate the estimated GFR according to the Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI) formula (22).

MRI protocol

All participants underwent baseline and follow-up MRI and ^{1}H-MRS using a clinical 3 Tesla Ingenia whole-body MR system (Philips Medical Systems, Best, the Netherlands). All images and proton spectra were blinded for study participant and occasion.

Renal single voxel spectroscopy was performed for the quantification of RTGC using a 40 x 10 x 10 mm voxel placed in the parenchyma of the left kidney (16), and baseline voxel position served as a guide for voxel placement at follow-up. Single voxel Point Resolved Spectroscopy (PRESS) unsuppressed spectra (echo time 40ms; unsuppressed repetition time 8s; averages 8) and suppressed spectra using Multiply Optimized Insensitive Suppression Train (MOIST) (echo time 40 ms; repetition time 3s; averages 64) were acquired. Spectra were acquired during free-breathing at end-expiration with pencil beam navigator-based respiratory triggering. Reconstructed spectra were fitted to a Gaussian line shape in the time domain using Java-based MR User Interface software (jMRUI version 5.0; Katholieke Universiteit Leuven, Leuven, Belgium) (23,24).

RTGC was calculated as a percentage of the (unsuppressed) water peak using the following formula: (renal signal amplitude of methylene + methyl) / (renal signal amplitude of water) × 100%. Since renal ^{1}H-MRS is a novel method for quantification of ectopic lipid and involves triglyceride concentrations that are substantially lower than in the liver resulting in a low signal-to-noise-ratio (SNR), the following spectral quality criteria were applied: (a) variation in lipid signal amplitudes between the signal averages were analyzed to exclude potential contamination of the RTGC signal with triglyceride signal originating from renal sinus fat or perirenal fat), (b) spectra were included if Cramér–Rao lower bound (CRLB) divided by the triglyceride amplitude of <20% (to discriminate well-fitted metabolites from more poorly fitted metabolites), (c) spectra were included if linewidth of triglyceride peaks were <100 Hz, (d) exclusion of spectra and residuals with artifacts or strongly asymmetric line shapes after eddy correction (25). Technical details and validation of renal ^{1}H-MRS have been described elsewhere in more detail (16,17). An example of voxel planning and resulting ^{1}H-MRS voxel is given in **Figure 1**. Hepatic triglyceride content was assessed via single voxel spectroscopy using the PRESS unsuppressed (echo time 35 ms; repetition time 9 s; averages 4) and MOIST-suppressed spectra (echo time 35 ms; repetition time 3.5s; 32 signal averages) (19, 26). Visceral fat was calculated based on three segmented transverse slices (mDIXON sequence, repetition time 3.5 ms; first echo time 1.19 ms; second echo time 2.3 ms; flip angle 10°; spatial resolution 1.6 x 1.7 mm; slice thickness 4 mm; slice gap 2 mm) at level L4-L5 (MASS software, LUMC, Leiden, the Netherlands) (19) .

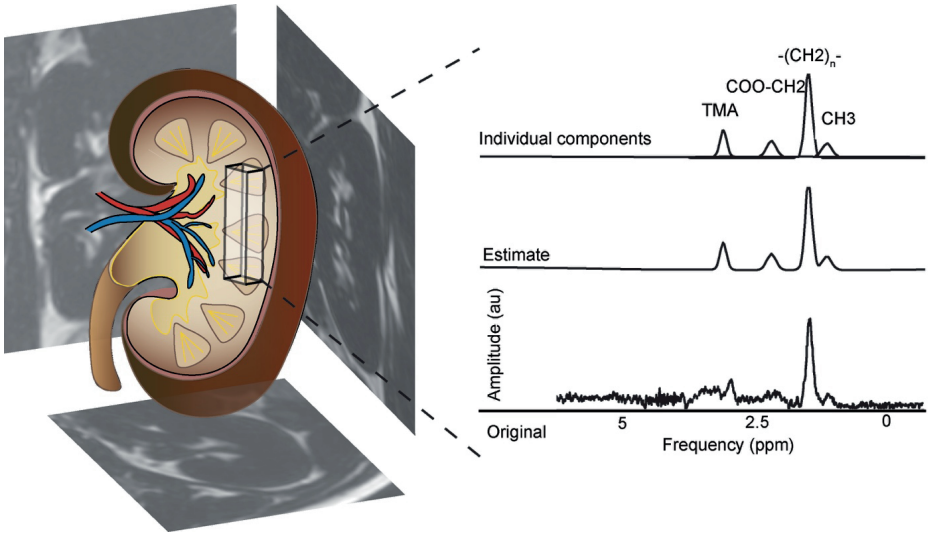

Figure 1. Planning of single voxel ^{1}H-MRS in the kidney (left), and corresponding spectra with methylene –(CH2)n– and methyl CH3 peak (right).

Statistical analysis

Data are shown as mean ± SD, or as median (25^{th}, 75^{th} percentile) when not normally distributed, and range. RTGC, UACR, SCr and eGFR were analyzed as renal outcomes. RTGC and UACR were log-transformed for normalization of their distributions. The difference (Δ) between log-transformed baseline from 26-weeks follow-up levels of RTGC and UACR respectively, are presented as 26-weeks ' to ' baseline ratio's. Correlations between RTGC and clinical determinants were assessed using Spearman correlation. Between-group differences at baseline and follow-up were analyzed using the independent samples t-test, and the paired-samples t-test was used for the within-group differences. Outcome measures were studied according to intention-to-treat analysis. Two-tailed significance levels of $P<0.05$ were considered to indicate a statistically significant difference.

RESULTS

Baseline characteristics

The parallel groups randomized to receive liraglutide or placebo consisted of 46 and 51 T2DM patients, of which 45 and 51 respectively underwent baseline MRI scanning. Due to limited scan time ^{1}H-MRS was not performed as part of the MRI scan protocol in 11 patients. Of these, 28 patients in the liraglutide group and 22 patients in the placebo group had ^{1}H-MRS spectra that met the quality criteria (excluded based on quality criteria n=35; poor fitting due to low SNR n=15, too broad linewidths n=10, likely contamination with triglyceride signal originating from extra-renal fat n=6, and artifacts n=4. In total 50 patients had baseline RTGC measurements available (mean age of 56.5 ± 9.1 years; range 33–73 years; 46% males). Baseline RTGC was not correlated with age (rs=0.08, P=0.58), BMI (rs=-0.10, P=0.47), total body fat percentage (rs=0.13, P=0.37), visceral fat (rs=0.07, P=0.63), liver fat (rs=-0.01, P=0.94), HbA1c (rs=-0.15, P=0.30), creatinine (rs=-0.14, P=0.32), eGFR (rs=-0.028, P=0.81), serum triglyceride (rs=-0.041, P=0.73) and UACR (rs=-0.10, P=0.50). Median RTGC in patients of Western European descent was 0.19% [25^{th}, 75^{th} percentile; 0.13, 0.31], and 0.21% [0.11, 0.38] in patients of South Asian descent.

At 26-weeks, 44 out of 50 patients underwent follow-up ^{1}H-MRS (25 patients in the liraglutide group and 19 patients in the placebo group). After exclusion of participants with ^{1}H-MRS that did not meet the quality criteria (n=27; poor fitting due to low SNR n=10, too broad linewidths n=8, likely contamination with triglyceride signal originating from extra-renal fat n=4, artifacts n=4, corrupted reference file n=1), nine patients of the liraglutide group and eight patients of the placebo group with both baseline and 26-weeks follow-up RTGC data were included for the intention-to-treat analysis. The trial profile is shown in **Figure 2**, and baseline characteristics in **Table 1**.

Table 1: Baseline characteristics of included patients with at least one RTGC measurement.

Demographics	**T2DM patients (n=50)**	
Treatment arm	**Liraglutide (n=22)**	**Placebo (n=28)**
Age (years)	55.6 (10.7)	57.2 (7.8)
Sex (male)	11 (50%)	12 (43%)
Ethnicity		
Western European	9 (41%)	15 (54%)
South Asian	13 (59%)	13 (46%)
Diabetes duration (years)	17.1 (10.0)	14.6 (9.9)
Diabetes complications		
Retinopathy	10 (46%)	7 (25%)
Nephropathy*	5 (24%)	7 (25%)
Neuropathy	10 (46%)	8 (29%)
Macrovascular†	5 (23%)	5 (18%)
Clinical parameters		
Body-mass index (kg/m^2)	31.1 (4.4)	31.3 (4.0)
Total body fat (%)	35.2 (9.3)	39.1 (9.3)
Hepatic triglyceride content (%)	11.9 (11.9)	17.5 (13.2)
Visceral fat (cm^2)	198.2 (61.6)	177.7 (64.0)
Systolic blood pressure (mmHg)	140.9 (21.1)	139.7 (16)
Diastolic blood pressure (mmHg)	83.2 (6.3)	85.1 (9.3)
HbA1c (mmol/mol)	67.0 (10.9)	67.1 (12.4)
HbA1c % (SD)	8.3 (1.0)	8.3 (1.1)
Triglycerides (mmol/L)	2.0 (1.6)	2.2 (1.3)
Total cholesterol (mmol/L)	4.3 (1.2)	4.8 (1.0)
HDL-c (mmol/L)	1.1 (0.3)	1.3 (0.4)
LDL-c (mmol/L) ‡	2.3 (1.0)	2.5 (1.0)
Smoking history		
Never smoked, n (%)	12 (55%)	18 (64%)
Current smoker, n (%)	4 (18%)	3 (11%)
Ex-smoker, n (%)	6 (27%)	7 (25%)
Concomitant drug use		
Metformin, (yes)	22 (100%)	27 (96%)
Metformin dose g/day	1.9 (0.6)	1.8 (0.4)
Sulfonylurea (yes)	4 (18%)	8 (29%)
Sulfonylurea dose mg/day	150 (107)	177 (338)
Insulin (yes)	15 (68%)	19 (68%)
Insulin (IU/day, average over last 2 weeks)	88 (58)	59 (30)
Statins (yes)	16 (73%)	20 (71%)
Anti-hypertensives (yes)	18 (82%)	21 (75%)
Angiontensin II recepter antagonists (yes)	7 (32%)	19 (32%)
ACE-inhibitors (yes)	9 (41%)	9 (32%)

Table 1: Baseline characteristics of included patients with at least one RTGC measurement. (continued)

Demographics	**T2DM patients (n=50)**	
Participants with baseline and follow-up ^{1}H-MRS		
Treatment artm	**Liraglutide (n=9)**	**Placebo (n=8)**
Baseline		
Log-transformed RTGC (%)	-0.67 (0.32)	-0.68 (0.30)
Log-transformed UACR (ug/umol)	14.2 (24.8)	2.1 (1.6)
SCr (umol/L)	69.6 (21.3)	64.8 (13.5)
eGFR (ml/min/1.72m²)	92.7 (23.1)	99.8 (10.6)
HbA1c (mmol/mol)	61.8 (9.4)	61.5 (7.8)
Week 26		
Log-transformed RTGC (%)	-0.97 (0.16)	-0.68 (0.40)
Log-transformed UACR (ug/umol)	16.7 (24.8)	4.1 (3.8)
SCr (umol/L)	66.9 (21.3)	64.3 (13.3)
eGFR (ml/min/1.72m²)	94.9 (23.9)	99.2 (13.5)
HbA1c (mmol/mol)	55.9 (10.4)	56.8 (9.0)

Data presented are n (%), and mean (SD). *nephropathy was defined as urinary albumin creatinine ratio ≥ 2.5 ug/umol in men and ≥ 3.5 ug/umol in women. †macrovascular complications were cerebrovascular or peripheral artery disease and not cardiovascular. ‡LDL-c was calculated using the Friedewald formula. ACE, angiotensin-converting-enzyme inhibitor; HbA1c, Glycated haemoglobin A1c; HDL-c, high-density lipoprotein-cholesterol; LDL-c, low-density lipoprotein-cholesterol; UACR, urinary-creatinine ratio; RTGC, renal triglyceride content;SCr, serum creatinine.

Results of 26 weeks of glycemic control on RTGC

An overview of renal outcomes and HbA1c at baseline and after 26 weeks of glycemic control irrespective of randomized treatment group is provided in **Table 2** and **Figure 3**. Seventeen patients had baseline and follow-up RTGC measurements available irrespective of treatment group allocation. Baseline HbA1c was 61.6 ± 8.4 mmol/mol, which changed to 56.3 ± 9.5 umol/L at follow-up (*P*=0.046). Median RTGC at baseline was 0.23% (0.13, 0.34), and 0.14% (0.10, 0.21) at follow-up. Log-transformed RTGC was significantly lower after 26-weeks of glycemic control compared to baseline (*P*=0.049). Baseline median UACR was 1.8 ug/umol (0.6, 4.1), and 1.8 ug/umol (0.7, 9.8) at follow-up. Log-transformed UACR at 26-weeks follow-up was not significantly different from baseline (*P*=0.77). Mean SCr was 67.3 ± 17.7 umol/L, which was 65.6 ± 17.5 umol/L at follow-up (*P*=0.26). Mean eGFR at baseline was 96.1 ± 18.4 ml/min/1.73m², and 96.9 ± 19.2 ml/min/1.73m² at follow-up (*P*=0.49).

Results of liraglutide versus standard glycemic control on RTGC

Nine T2DM patients randomized to liraglutide, and eight T2DM patients randomized to placebo, added to usual glycemic care had both baseline and follow-up ^{1}H-MRS data available that met the quality criteria. An overview of the outcomes at baseline and after

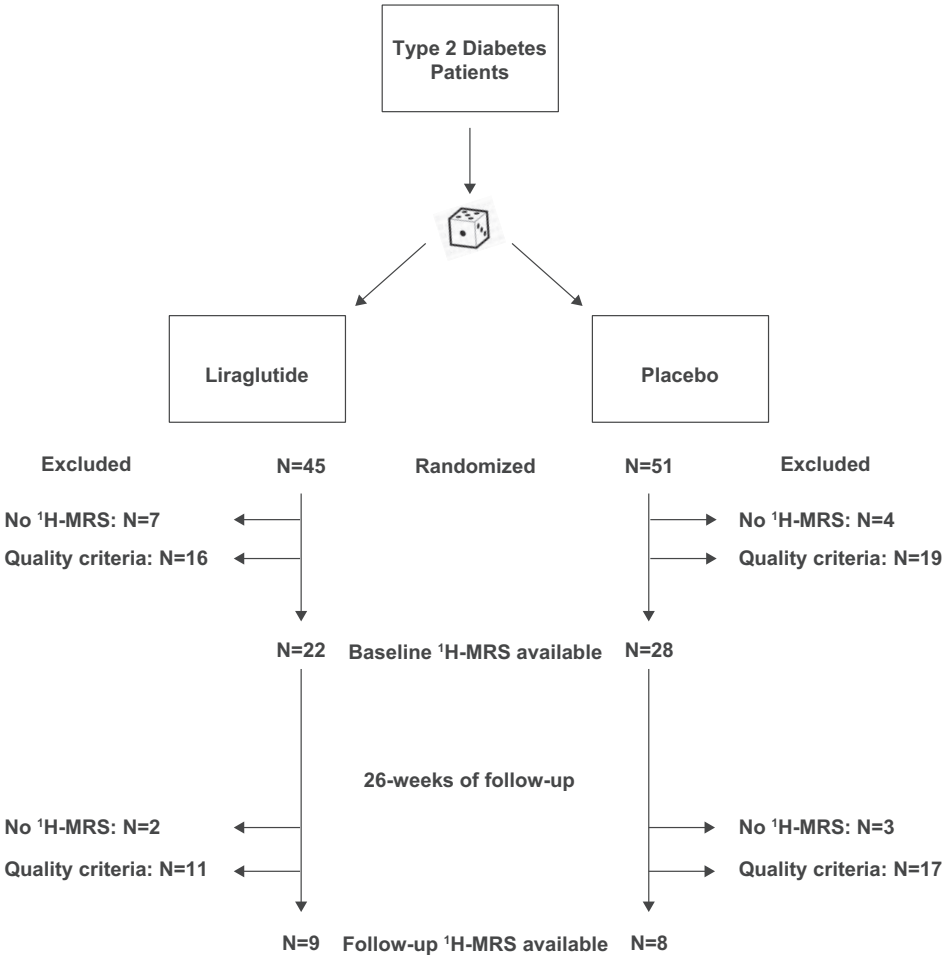

Figure 2. Flow chart. Patients were randomized to either liraglutide or placebo with stratification according to sex and insulin use.

Table 2. Outcomes at baseline and after 26-weeks of glycemic control irrespective of randomized treatment group

Outcome	Baseline, mean (SD) (n=17)	Week 26, mean (SD) (n=17)	*P*-value
Log-transformed RTGC (%)	-0.68 (0.30)	-0.83 (0.32)	0.049
Log-transformed UACR (ug/umol)	8.5 (18.7)	10.8 (26.0)	0.77
SCr (umol/L)	67.3 (17.7)	65.6 (17.5)	0.26
eGFR (ml/min/1.73m²)	96.1 (18.4)	96.9 (19.2)	0.49
HbA1c (mmol/mol)	61.6 (8.4)	56.3 (9.5)	0.046

Table includes outcome comparisons for study participants with RTGC values at both baseline and follow-up (n=17). HbA1c, Glycated haemoglobin A1c; SCr, serum creatinine; UACR, urinary-creatinine ratio; RTGC, renal triglyceride content.

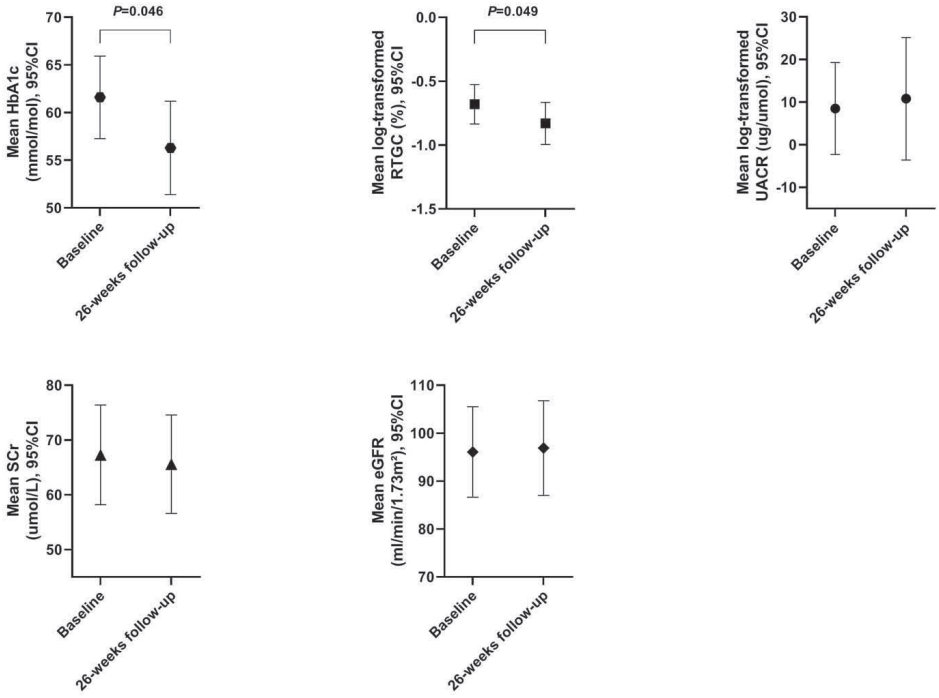

Figure 3. Treatment effect of glycemic control on Glycated haemoglobin A1c (HbA1c), renal triglyceride content (RTGC), urine-albumin-creatinine ratio (UACR), serum creatinine (SCr), and estimated glomerular filtration rate (eGFR) irrespective of randomized treatment group (n=17).

Table 3. Outcomes at baseline and after 26-weeks of liraglutide or placebo, added to usual glycemic care

Mean change in outcomes between baseline and follow up (95% CI)	Liraglutide (n=9)	Placebo (n=8)	*P*-value
26-weeks - to - baseline RTGC ratio†	-0.30 (-0.50, -0.09)	-0.003 (-0.34, 0.34)	0.04
26-weeks - to - baseline UACR ratio†	-0.38 (-0.67,-0.08)	0.22 (0.03, 0.40)	<0.01
Mean change SCr (umol/L)	-2.7 (-7.1, 6.1)	-0.5 (-8.0, 6.1)	0.46
Mean change eGFR (ml/min/1.72m²))	-2.3 (-6.5, -5.3)	-0.6 (-4.4, 5.6)	0.27
Mean change HbA1c (mmol/mol)	-5.9 (-17.8, 6.0)	-4.7 (-13.2, -2.3)	0.82

Table includes outcome comparisons for study participants with RTGC values at both baseline and follow-up (n=17). †26-weeks - to - baseline ratio derived from the difference between the log-transformed baseline and 26-weeks levels of RTGC and UACR respectively. eGFR, estimated glomerular filtration rate; SCr, serum creatinine; UACR, urinary-creatinine ratio; RTGC, renal triglyceride content.

26-weeks of liraglutide or placebo is provided in **Table 1, Table 3** and **Figure 4**. Baseline HbA1c in the liraglutide group was 61.8 ± 9.4 mmol/mol and 61.5 ± 7.8 mmol/mol. At follow-up this changed to 55.9 ± mmol/mol and 61.5 ± 7.8 mmol/mol for the liraglutide and placebo group respectively. No significant differences were found in HbA1c between the liraglutide group and placebo group (*P*=0.82). Median RTGC at baseline was 0.23% (0.11,

0.34), and 0.19% (0.13, 0.33) in the placebo group. At 26-weeks RTGC was 0.11% (0.08, 0.14) in the liraglutide group and 0.23% (0.16, 0.39) in the placebo group. 26-weeks- to - baseline RTGC ratio (95% CI) was significantly different between liraglutide (-0.30 [-0.50, -0.09]) and placebo added to standard care (-0.003 [-0.34, 0.34]) (*P*=0.04) (**Table 3, Fig. 4**). Median UACR in the liraglutide group at baseline was 1.8 [0.7, 6.6] ug/umol, and 2.0 [0.8, 3.3] ug/umol in the placebo group. At follow-up median UACR was 1.7 [0.3, 11.5] ug/umol in the liraglutide group, and 2.5 [1.3, 6.1] ug/umol in the placebo group. 26-weeks- to - baseline UACR ratio (95% CI) was significantly different between liraglutide (0.22 [0.0.3, 0.40]) and placebo added to standard care (-0.38 [-0.67, 0.08]) (*P*=0.04). Mean change in SCr and eGFR were not statistically different between the liraglutide and placebo group (SCr: *P*=0.46; eGFR: *P*=0.27).

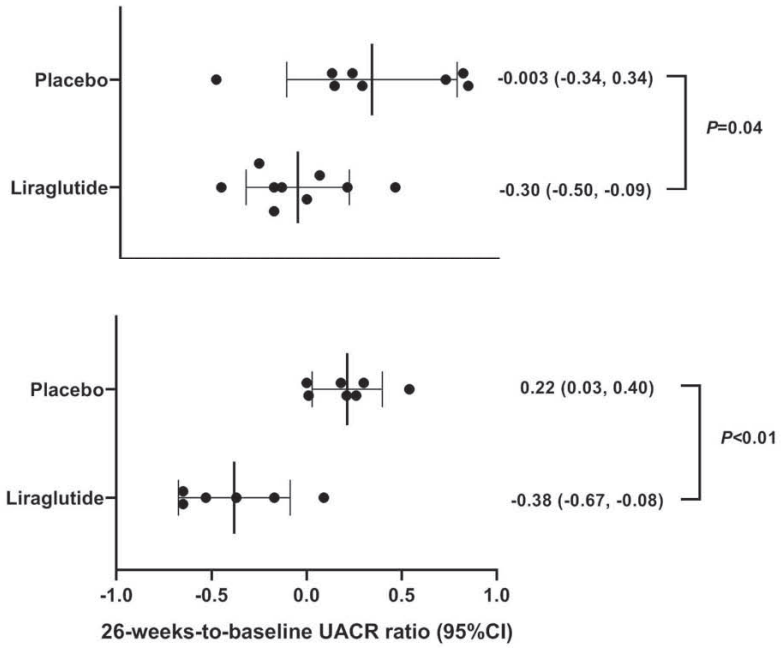

Figure 4. Treatment effect of liraglutide (n=9) versus placebo (n=8) on renal triglyceride content (RTGC) and urinary albumin-creatinine ratio (UACR).

DISCUSSION

The aim of this exploratory study was to assess whether glycemic control influences renal triglyceride content, including comparing the GLP-1RA liraglutide versus placebo, added to standard glucose-lowering therapy. In this study we found a significant reduction in RTGC after 26-weeks of glycemic control (irrespective of randomized treatment group).

From DKD literature, it is known that intensive glycemic control improves renal outcomes as evidenced by reduction of proteinuria progression and reduced risk of ESRD [3]. In our secondary analysis of the MAGNA VICTORIA studies we showed that RTGC reduced significantly more with liraglutide than placebo, while the HbA1c reduction was not different between these groups. Although this subgroup analysis should be considered cautiously with regard to the size and exploratory character of our study, our findings suggest that glycemic control, via GLP-1RA or standard glucose lowering therapy, might potentially beneficially influence renal steatosis. However, it should be noted that considering the lack of a control group (participants with no treatment at all) with baseline and follow-up measurements of RTGC, the phenomenon of 'regression to the mean' as a possible explanation for the found reduction in RTGC over time cannot be excluded. Taking this into account, and considering the small sample size of the current study as well as the limited prevalence/severity of DKD in our sample (since eGFR below 60 ml/min/1.73m^2 was an exclusion criterium), further research in larger clinical trials is warranted to better delineate the association between glycemic control and renal steatosis.

Based on the findings of the LEADER trial, glycemic control via liraglutide seems to have an additional independent effect on renal outcomes when added to usual glycemic care (11). We have previously shown in the MAGNA VICTORIA study that T2DM patients treated with liraglutide, compared to placebo, lost significantly more body weight, but liraglutide did not significantly change other ectopic fat depots such as visceral fat and hepatic triglyceride content (19,20). It remains area of further research whether GLP1-RA affects renal steatosis by direct effect of GLP1-RA on the kidney considering the existence of renal GLP1 receptors (12), or alternatively whether current findings stem from the composite effects of GLP1-RA on body weight and/or HbA1c.

There are several limitations that need to be considered. First, because of the exploratory nature of this study, and considering that this study is the first clinical trial using renal ^{1}H-MRS, we applied several quality criteria for the obtained renal spectra to assure the quality of the measurements. Because of these, we excluded a substantial amount of renal spectra from the analysis. Although renal outcomes were pre-specified in the MAGNA VICTORIA studies, these studies were powered for primary endpoints involving left ventricular diastolic and systolic function and not for RTGC. We have previously assessed the reproducibility of ^{1}H-MRS for the measurement of renal triglycerides in humans (16), and performed a porcine histologic validation and dietary intervention study, which showed that ^{1}H-MRS closely predicts triglyceride content as measured enzymatically in biopsies (17). However, considering the substantial number of obtained renal spectra that did not meet the quality criteria, renal ^{1}H-MRS remains a technically challenging technique, limiting the use of RTGC as a biomarker for the evaluation of treatment effects on renal lipid metabolism. Furthermore, although we have previously found much lower levels of RTGC in healthy volunteers (median RTGC of 0.12% [0.08,

0.22]) (16), additional studies are needed to determine reference values and to assess differences in RTGC between T2DM patients and healthy volunteers while taking age and sex into account. Another limitation is that we cannot exclude the potential influence of ethnicity on RTGC reduction, albeit baseline RTGC levels were comparable for T2DM patients of Western European and South Asian descent, as well as the proportion of T2DM patients of South Asian descent in the liraglutide and placebo arm. Future studies are needed to better understand how renal lipid metabolism and DKD are interrelated. Moreover, better understanding of the interplay of other ectopic fat compartments (e.g. renal sinus fat, hepatic fat and visceral fat) with renal lipid metabolism may contribute to the development of new therapeutic strategies.

In conclusion, in this exploratory study we found that twenty-six weeks of glycemic control resulted in lower RTGC, in particular for liraglutide, however larger clinical studies are needed to assess whether these changes reflect a true effect of glycemic control on renal steatosis.

Acknowledgements

We would like to thank P. de Heer and H.J. van Eyk for their help with data acquisition. We express our gratitude to all individuals who participated in the MAGNA VICTORIA. We are grateful to all participating general practitioners and nurses (M.A. Diez Canseco Quintana, C. Overman, I. Minken, H. Laurier, E. Pleij, M. de Winde, T.N. Bonten and L. van Duijn) and the physicians and nurses of the Haaglanden Medical Center (P.H.L.M. Geelhoed, A.H. Bootsma and A.V. Kharagjitsingh, The Hague, The Netherlands) for inviting eligible participants.

Funding

Novo Nordisk (Denmark) funded this investigator-initiated study. Novo Nordisk had no role in the design of the study, data collection, data analysis, data interpretation, or writing of the report. All authors had access to all the data and final responsibility for the decision to submit for publication. Dutch Kidney Foundation (innovation grant IP11.56).

REFERENCES

1. Anders H-J, Huber TB, Isermann B, Schiffer M. CKD in diabetes: diabetic kidney disease versus non-diabetic kidney disease. Nat Rev Nephrol 2018;14:361–77.
2. Holman RR, Paul SK, Bethel MA, Matthews DR, Neil HAW. 10-Year Follow-up of Intensive Glucose Control in Type 2 Diabetes. N Engl J Med 2008;359:1577–89.
3. Perkovic V, Heerspink HL, Chalmers J, Woodward M, Jun M, Li Q, et al. Intensive glucose control improves kidney outcomes in patients with type 2 diabetes. Kidney Int 2013;83:517–23.
4. Brenner BM, Cooper ME, de Zeeuw D, Keane WF, Mitch WE, Parving H-H, et al. Effects of Losartan on Renal and Cardiovascular Outcomes in Patients with Type 2 Diabetes and Nephropathy. N Engl J Med 2001;345:861–9.
5. Lewis EJ, Hunsicker LG, Clarke WR, Berl T, Pohl MA, Lewis JB, et al. Renoprotective Effect of the Angiotensin-Receptor Antagonist Irbesartan in Patients with Nephropathy Due to Type 2 Diabetes. N Engl J Med 2001;345:851–60.
6. Hoekstra T, Van Ittersum FJ, Hemmelder MH. RENINE Annual Report 2017. Nederlandse Federatie voor Nefrologie.
7. Porrini E, Ruggenenti P, Mogensen CE, Barlovic DP, Praga M, Cruzado JM, et al. Non-proteinuric pathways in loss of renal function in patients with type 2 diabetes. Lancet Diabetes Endocrinol 2015;3:382–91.
8. Shulman GI. Ectopic Fat in Insulin Resistance, Dyslipidemia, and Cardiometabolic Disease. N Engl J Med 2014;371:1131–41.
9. de Vries APJ, Ruggenenti P, Ruan XZ, Praga M, Cruzado JM, Bajema IM, et al. Fatty kidney: emerging role of ectopic lipid in obesity-related renal disease. Lancet Diabetes Endocrinol 2014;2:417–26.
10. Li L, Zhao Z, Xia J, Xin L, Chen Y, Yang S, et al. A Long-Term High-Fat/High-Sucrose Diet Promotes Kidney Lipid Deposition and Causes Apoptosis and Glomerular Hypertrophy in Bama Minipigs. PLoS One 2015 Nov 16;10(11):e0142884. 2015.
11. Mann JFE, Ørsted DD, Brown-Frandsen K, Marso SP, Poulter NR, Rasmussen S, et al. Liraglutide and Renal Outcomes in Type 2 Diabetes. N Engl J Med 2017;377:839–48.
12. Muskiet MHA, Tonneijck L, Smits MM, van Baar MJB, Kramer MHH, Hoorn EJ, et al. GLP-1 and the kidney: from physiology to pharmacology and outcomes in diabetes. Nat Rev Nephrol 2017;13:605–28.
13. Wang C, Li L, Liu S, Liao G, Li L, Chen Y, et al. GLP-1 receptor agonist ameliorates obesity-induced chronic kidney injury via restoring renal metabolism homeostasis. PLoS One 2018;13:e0193473.
14. Guo H, Wang B, Li H, Ling L, Niu J, Gu Y. Glucagon-like peptide-1 analog prevents obesity-related glomerulopathy by inhibiting excessive autophagy in podocytes. Am J Physiol Physiol 2018;314:F181–9.
15. Szczepaniak LS, Nurenberg P, Leonard D, Browning JD, Reingold JS, Grundy S, et al. Magnetic resonance spectroscopy to measure hepatic triglyceride content: prevalence of hepatic steatosis in the general population. Am J Physiol Metab 2005;288:E462–8.

16. Dekkers IA, de Heer P, Bizino MB, de Vries APJ, Lamb HJ. 1 H-MRS for the assessment of renal triglyceride content in humans at 3T: A primer and reproducibility study. J Magn Reson Imaging 2018;48:507–13.

17. Jonker JT, de Heer P, Engelse MA, van Rossenberg EH, Klessens CQF, Baelde HJ, et al. Metabolic imaging of fatty kidney in diabesity: validation and dietary intervention. Nephrol Dial Transplant 2018;33:224–30.

18. Bizino MB, Jazet IM, Westenberg JJM, van Eyk HJ, Paiman EHM, Smit JWA, et al. Effect of liraglutide on cardiac function in patients with type 2 diabetes mellitus: randomized placebo-controlled trial. Cardiovasc Diabetol 2019;18:55.

19. van Eyk HJ, M Paiman EH, Bizino MB, de Heer P, Geelhoed-Duijvestijn PH, Kharagjitsingh A V, et al. A double-blind, placebo-controlled, randomised trial to assess the effect of liraglutide on ectopic fat accumulation in South Asian type 2 diabetes patients. Cardiovasc Diabetol 2019;18:87.

20. Bizino MB, Jazet IM, de Heer P, van Eyk HJ, Dekkers IA, Rensen PCN, et al. Placebo-controlled randomised trial with liraglutide on magnetic resonance endpoints in individuals with type 2 diabetes: a pre-specified secondary study on ectopic fat accumulation. Diabetologia 2019.

21. Paiman EHM, van Eyk HJ, Bizino MB, Dekkers IA, de Heer P, Smit JWA, et al. Phenotyping diabetic cardiomyopathy in Europeans and South Asians. Cardiovasc Diabetol 2019;18:133.

22. Levey AS, Stevens LA, Schmid CH, Zhang Y, Castro AF, Feldman HI, et al. A new equation to estimate glomerular filtration rate. Ann Intern Med 2009;150:604–12.

23. Naressi A, Couturier C, Devos JM, Janssen M, Mangeat C, Beer R de, et al. Java-based graphical user interface for the MRUI quantitation package. MAGMA 2001;12:141–52.

24. Stefan D, Cesare F Di, Andrasescu A, Popa E, Lazariev A, Vescovo E, et al. Quantitation of magnetic resonance spectroscopy signals: The jMRUI software package. Meas Sci Technol 2009;20.

25. Kreis R. Issues of spectral quality in clinical 1 H-magnetic resonance spectroscopy and a gallery of artifacts. NMR Biomed NMR Biomed 2004;17:361–81.

26. Van Eyk HJ, Blauw LL, Bizino MB, et al. Hepatic triglyceride content does not affect circulating CETP: lessons from a liraglutide intervention trial and a population-based cohort. Sci Rep. 2019;9(1)9996.