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

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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

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Objective: Ectopic lipid accumulation in the kidney (fatty kidney) 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 glucagon-like peptide-1 receptor agonist liraglutide versus standard glucose-lowering therapy.

Design and Methods: In this single-center parallel-group trial, patients with type 2 diabetes mellitus were randomized to liraglutide or placebo added to standard care (metformin/sulfonylurea derivative/insulin). Changes in RTGC after 26 weeks of glycemic control measured by proton spectroscopy and difference in RTGC between treatment groups were analyzed.

Results: Fifty patients with type 2 diabetes mellitus were included in the baseline analysis (mean age, 56.5 ± 9.1 years; range, 33-73 years; 46% males). Seventeen patients had baseline and follow-up measurements. Mean glycated hemoglobin was 7.8 ± 0.8%, which changed to 7.3 ± 0.9% after 26 weeks of glycemic control irrespective of treatment group ($P = .046$). 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 ($P = .049$). A 26-week-to-baseline RTGC ratio (95% confidence interval) was significantly different between liraglutide (−0.30 [−0.50, −0.09]) and placebo added to standard care (−0.003 [−0.34, 0.34]) ($P = .04$).

Conclusion: In this exploratory study, we found that 26 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 fatty kidney.

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 kidney disease (ESKD) worldwide, and the UKPDS[2] and ADVANCE[3] studies showed that improved glycemic control reduces microvascular disease and ESKD. In addition, the Reduction of Endpoints in Non-insulin Dependent Diabetes Mellitus with the Angiontensin II Antagonist Losartan (RENAAL)[4] and Irbesartan Diabetic Nephropathy Trial (IDNT)[5] showed that treatment of hypertension and proteinuria in particular when using renin-angiotensin-aldosterone system inhibitors conveyed a 30% risk reduction for ESKD. In spite of these cornerstone therapies, the incidence of ESKD by DKD continues to rise,[6] indicating possible involvement of other (nonproteinuric) pathways related to, e.g., hyperfiltration and metabolic regulation.[7]

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In particular, the combination of T2DM and obesity has been linked to ectopic lipid accumulation in nonadipose tissues, such as liver, heart, and kidney, which can interfere with the cellular function of the respective organ.[8,9] Ectopic lipid accumulation in the kidney, also referred to as fatty kidney,[9] has been linked to structural changes, including glomerular hypertrophy,[10] and maladaptive functional responses, such as hyperfiltration and albuminuria.[9] Fatty kidney 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 Liraglutide Effect and Action in Diabetes: Evaluation of Cardiovascular Outcome Results (LEADER) trial[11] showed that the glucagon-like peptide-1 receptor agonist (GLP1-RA) liraglutide had a protective effect on the kidney in DKD compared with 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 as 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 ectopic lipid accumulation in the kidney in vivo. Experimental studies have shown that liraglutide might have a protective effect on the kidney by inhibiting ectopic lipid accumulation in the kidney.[13,14]

Clinical studies on lipid metabolism in the kidney have been hampered by the absence of a noninvasive technique to measure kidney lipid content. Magnetic resonance (MR) spectroscopy (^{1}H-MRS) is considered the gold standard technique to measure hepatic lipid content in vivo[15]; however, application of ^{1}H-MRS to the kidney is technically challenging because of respiratory motion and low signal-to-noise ratio (SNR) related to the low quantities of 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 kidney 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, and/or insulin. A secondary aim was to investigate whether these 2 treatment groups differed in reduction of RTGC.

Methods

Study Design

This study is a single-center parallel-group trial containing the baseline and follow-up data of the MAGNetic resonance Assessment of VICTOza efficacy in the Regression of cardiovascular dysfunction In type 2 diAbetes mellitus (MAGNA VICTORIA) studies in Western European (ClinicalTrials.gov NCT01761318) and South Asian (ClinicalTrials.gov NCT02660047) patients with T2DM. The present study involved RTGC as a prespecified secondary endpoint. The previously published primary and secondary endpoints were, e.g., left ventricular function, glycated hemoglobin (HbA1c), body weight, and measures of body fat distribution (visceral fat and 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 of 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, sulphonylurea derivatives, 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 before inclusion. The present study was performed according to the revised Declaration of Helsinki and was approved by the institutional review board of the Leiden University Medical Center.

Participants

At the start of the study, the inclusion criteria for patients with T2DM were defined irrespective of ethnicity (self-identified and self-reported origin of both biological parents and their ancestors); however, because of 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 patients with T2DM were, respectively, aged 18 to 70 and 18 to 75 years, HbA1c between ≥7.0 and ≤10.0% and ≥6.5 and ≤11.0%, systolic and diastolic blood pressures between <150/85 mm Hg and <180/110 mm Hg, estimated glomerular filtration rate (eGFR) based on Chronic Kidney Disease Epidemiology Collaboration formula above >60 mL/minute/1.73 m^2 and >30 mL/minute/1.73 m^2, no history of heart failure (New York Heart Association Class III-IV), no history of coronary artery disease for the European patients with T2DM, and no acute coronary accident in the preceding 30 days for the South Asian patients with T2DM.[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 patients with T2DM, the insulin dose was adjusted, and study drug and other glucose-lowering medications were discontinued for a maximum of 24 hours). At the start and 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 2 consecutive measurements. Because of 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 (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, high-density lipoprotein cholesterol, low-density lipoprotein cholesterol (Friedewald formula) were measured on a Modular P800 analyzer (Roche Diagnostics, Mannheim, Germany), and urine samples for the measurement of urinary albumin-to-creatinine 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 (Bodystat 1500; Bodystart Ltd, Douglas, United Kingdom). SCr (mg/dL) was used to calculate the estimated GFR according to the Chronic Kidney Disease Epidemiology Collaboration formula.[22]

MRI Protocol

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

Single-voxel spectroscopy was performed for the quantification of RTGC using a 40 × 10 × 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 unsuppressed spectra (echo time, 40 milliseconds; unsuppressed repetition time, 8 seconds; average, 8) and suppressed spectra using multiply optimized insensitive suppression train (echo time, 40 milliseconds; repetition time, 3 seconds; average, 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: (signal amplitude of methylene + methyl)/(signal amplitude of water) × 100%. Because ^{1}H-MRS of the kidney 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 SNR, the following spectral quality criteria were applied: (1) variation in lipid signal amplitudes between the signal averages was analyzed to exclude potential contamination of the RTGC signal with triglyceride signal originating from renal sinus fat or perirenal fat, (2) spectra were included if Cramér-Rao lower bound divided by the triglyceride amplitude of <20% (to discriminate well-fitted metabolites from more poorly fitted metabolites), (3) spectra were included if linewidth of triglyceride peaks was <100 Hz, and (4) exclusion of spectra and residuals with artifacts or strongly asymmetric line shapes after eddy correction.[25] Technical details and

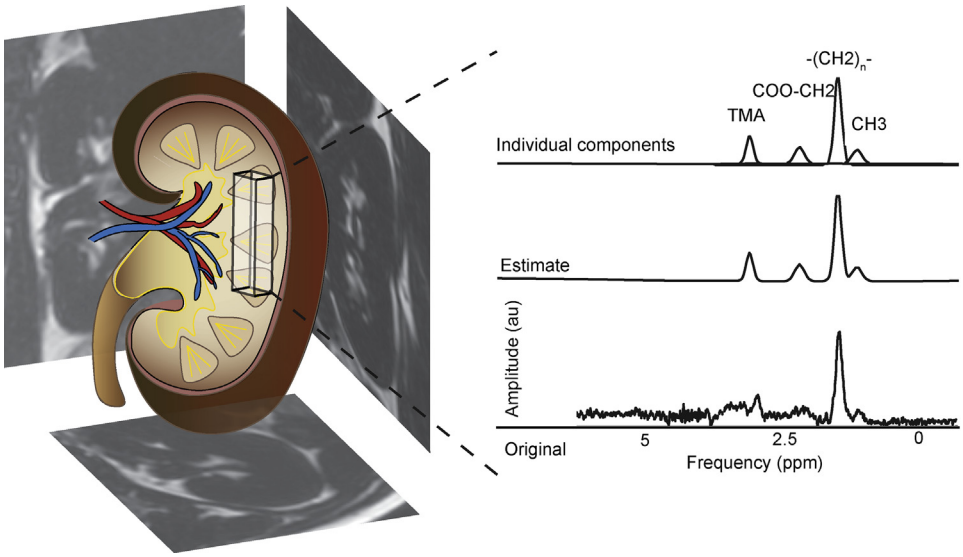

Figure 1. Planning of single-voxel ^{1}H-MRS in the kidney (*left*) and corresponding spectra with methylene –$(CH2)_n$– and methyl CH3 peak (*right*). TMA, trimethylamines.

validation of ^{1}H-MRS of the kidney 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 point resolved spectroscopy unsuppressed (echo time, 35 milliseconds; repetition time, 9 seconds; average, 4) and multiply optimized insensitive suppression train-suppressed spectra (echo time, 35 milliseconds; repetition time, 3.5 seconds; 32 signal averages).[19,26] Visceral fat was calculated based on 3 segmented transverse slices (mDIXON sequence, repetition time, 3.5 milliseconds; first echo time, 1.19 milliseconds; second echo time, 2.3 milliseconds; flip angle, 10°; spatial resolution, 1.6 × 1.7 mm; slice thickness, 4 mm; and slice gap, 2 mm) at level L4-L5 (MASS software; LUMC, Leiden, the Netherlands).[19]

Statistical Analysis

Data are shown as mean ± standard deviation or as median (25th and 75th percentile) when not normally distributed, and range. RTGC, UACR, SCr, and eGFR were analyzed as clinical outcomes. RTGC and UACR were log transformed for normalization of their distributions. The difference (Δ) between log-transformed baseline from 26-week follow-up levels of RTGC and UACR, respectively, is presented as 26-week-to-baseline ratios. Correlations between RTGC and clinical determinants were assessed using Spearman correlation (r). 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 $P < .05$ 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 patients with T2DM, of which 45 and 51, respectively, underwent baseline MRI scanning. Because of 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 because of low SNR, n = 15; too broad

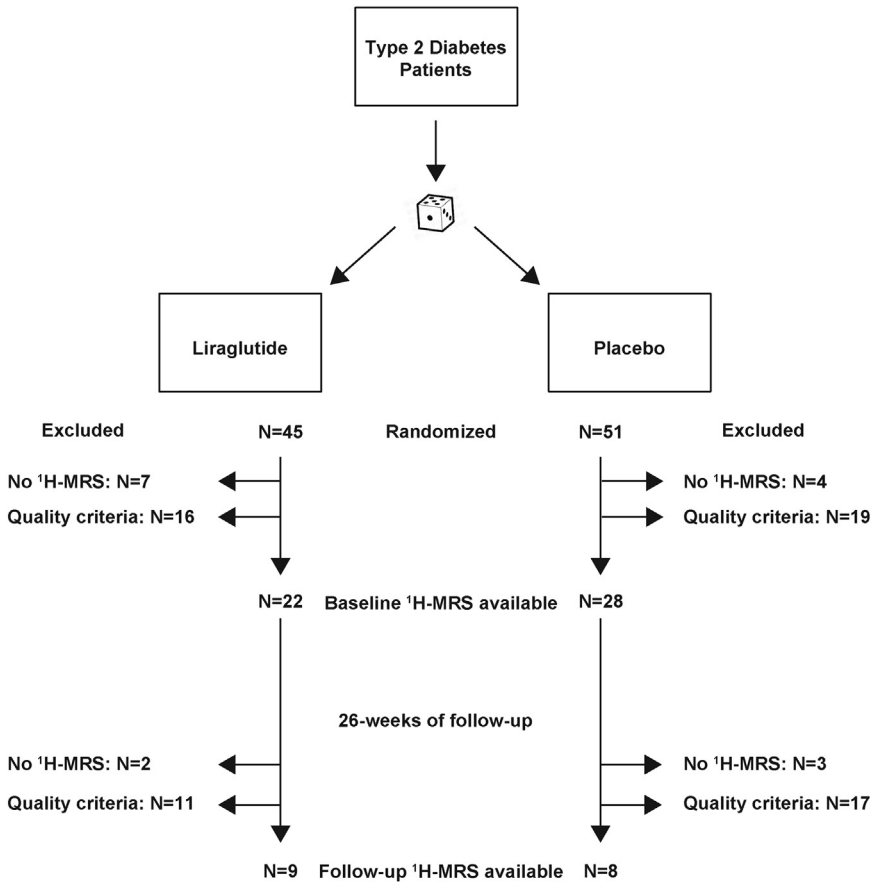

Figure 2. Flowchart. Patients were randomized to either liraglutide or placebo with stratification according to sex and insulin use. MRS, magnetic resonance spectroscopy.

Table 1. Baseline Characteristics of Included Patients With at Least 1 RTGC Measurement

Demographics	T2DM Patients (n = 50)	
	Liraglutide (n = 22)	Placebo (n = 28)
Treatment Arm	N (%)/Mean ± SD	
Age (y)	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 (y)	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		
BMI (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 (mm Hg)	140.9 ± 21.1	139.7 ± 16
Diastolic blood pressure (mm Hg)	83.2 ± 6.3	85.1 ± 9.3
HbA1c % (SD)	8.3 ± 1.0	8.3 ± 1.1
Triglycerides (mg/dL)	177.0 ± 141.6	194.7 ± 115.0
Total cholesterol (mg/dL)	166.3 ± 46.4	185.6 ± 38.7
HDL-c (mg/dL)	42.5 ± 11.6	50.3 ± 15.5
LDL-c (mg/dL)‡	88.9 ± 38.7	96.7 ± 38.7
Smoking history, n (%)		
Never smoked	12 (55)	18 (64)
Current smoker	4 (18)	3 (11)
Exsmoker	6 (27)	7 (25)
Concomitant drug use		
Metformin (yes)	22 (100%)	27 (96%)
Metformin dose, g/d	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/d, average during last 2 wk)	88 (58)	59 (30)
Statins (yes)	16 (73%)	20 (71%)
Antihypertensives (yes)	18 (82%)	21 (75%)
Angiotensin II receptor antagonists (yes)	7 (32%)	19 (32%)
ACE inhibitors (yes)	9 (41%)	9 (32%)
Participants with baseline and follow-up ^{1}H-MRS	T2DM Patients (n = 17)	
	Liraglutide (n = 9)	Placebo (n = 8)
Treatment arm	Mean ± SD	
Baseline		
Log-transformed RTGC (%)	−0.67 ± 0.32	−0.68 ± 0.30
Log-transformed UACR (mg/g)	1.65 ± 0.78	1.23 ± 0.35
SCr (mg/dL)	0.78 ± 0.21	0.80 ± 0.23
eGFR (mL/min/1.72 m^2)	92.7 ± 23.1	99.8 ± 10.6
HbA1c (%)	7.8 ± 0.9	7.8 ± 0.7
Fasting glucose (mg/dL)	131.5 ± 23.4	138.7 ± 52.2
Weight (kg)	89.4 ± 14.7	92.0 ± 14.5
BMI (kg/m^2)	31.7 ± 5.3	32.6 ± 3.7
Week 26		
Log-transformed RTGC (%)	−0.97 ± 0.16	−0.68 ± 0.40
Log-transformed UACR (mg/g)	1.60 ± 0.93	1.40 ± 0.39
SCr (mg/dL)	0.76 ± 0.24	0.73 ± 0.15
eGFR (mL/min/1.72 m^2)	94.9 ± 23.9	99.2 ± 13.5

(*Continued*)

Table 1. Baseline Characteristics of Included Patients With at Least 1 RTGC Measurement (*Continued*)

Participants with baseline and follow-up ^{1}H-MRS	T2DM Patients (n = 17)	
	Liraglutide (n = 9)	Placebo (n = 8)
Treatment arm	Mean ± SD	
HbA1c (%)	7.3 ± 1.0	7.3 ± 0.8
Fasting glucose (mg/dL)	118.9 ± 37.8	122.5 ± 32.4
Weight (kg)	85.5 ± 15.7	92.4 ± 14.4
BMI (kg/m^2)	30.3 ± 5.8	32.8 ± 3.9

ACE, angiotensin-converting-enzyme inhibitor; BMI, body mass index; eGFR, estimated glomerular filtration rate based on Chronic Kidney Disease Epidemiology Collaboration equation; HbA1c, glycated hemoglobin; HDL-c, high-density lipoprotein cholesterol; LDL-c, low-density lipoprotein cholesterol; RTGC, renal triglyceride content; SCr, serum creatinine; SD, standard deviation; T2DM, type 2 diabetes mellitus; UACR, urinary albumin-to-creatinine ratio.

Data presented are n (%) and mean (SD).

*Nephropathy was defined as urinary albumin creatinine ratio ≥17 mg/g in men and ≥25 mg/g in women.

†Macrovascular complications were cerebrovascular or peripheral artery disease and not cardiovascular.

‡LDL-c was calculated using the Friedewald formula.

Table 2. Outcomes at Baseline and After 26 Weeks of Glycemic Control Irrespective of Randomized Treatment Group

Outcome	Baseline	Week 26	*P*
	Mean (SD) (n = 17)		
Log-transformed RTGC (%)	−0.68 (0.30)	−0.83 (0.32)	.049
Log-transformed UACR (mg/g)	1.4 (0.6)	1.5 (0.7)	.59
SCr (mg/dL)	0.76 (0.2)	0.74 (0.2)	.26
eGFR (mL/min/1.73 m^2)	96.1 (18.4)	96.9 (19.2)	.49
HbA1c (%)	7.8 (0.8)	7.3 (0.9)	.046

eGFR, estimated glomerular filtration rate based on Chronic Kidney Disease Epidemiology Collaboration equation; HbA1c, glycated hemoglobin; RTGC, renal triglyceride content; SCr, serum creatinine; SD, standard deviation; UACR, urinary albumin-to-creatinine ratio.

The table includes outcome comparisons for study participants with baseline and follow-up RTGC measurements (total, n = 17), who were randomized either to liraglutide (n = 9) or placebo (n = 8) added to standard care (metformin/sulfonylurea derivative/insulin).

linewidths, n = 10; likely contamination with triglyceride signal originating from extrarenal fat, n = 6; and artifacts, n = 4). In total, 50 patients had baseline RTGC measurements available (mean age, 56.5 ± 9.1 years; range, 33-73 years; 46% males). Baseline RTGC was not correlated with age (r = 0.08; P = .58), body mass index (r = −0.10; P = .47), total body fat percentage (r = 0.13; P = .37), visceral fat (r = 0.07; P = .63), liver fat (r = −0.01; P = .94), HbA1c (r = −0.15; P = .30), creatinine (r = −0.14; P = .32), eGFR (r = −0.028; P = .81), serum triglyceride (r = −0.041; P = .73), and UACR (r = −0.10; P = .50). Median RTGC in patients of Western European descent was 0.19% (25th, 75th percentile; 0.13, 0.31) and 0.21% (0.11, 0.38) in patients of South Asian descent.

At 26 weeks, 44 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 who did not meet the quality criteria (n = 27; poor fitting because of low SNR, n = 10; too broad linewidths, n = 8; likely contamination with triglyceride signal originating from extrarenal fat, n = 4; artifacts, n = 4; and corrupted reference file, n = 1), 9 patients of the liraglutide group and 8 patients of the placebo group with both baseline and 26-week follow-up RTGC data were included for the intention-to-treat analysis. The trial profile is shown in Figure 2, and baseline characteristics are shown in Table 1.

Results of 26 Weeks of Glycemic Control on RTGC

An overview of clinical outcomes and HbA1c at baseline and after 26 weeks of glycemic control irrespective of randomized treatment group is provided in Table 2. Seventeen patients had baseline and follow-up RTGC measurements available irrespective of treatment group allocation. Baseline HbA1c was 7.8 ± 0.8%, which changed to 7.3 ± 0.9% at follow-up (P = .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 with baseline (P = .049). Baseline median UACR was 15.9 mg/g (5.3, 36.2) and 15.9 mg/g (6.2, 86.7) at follow-up. Log-transformed UACR at 26-week follow-up was not significantly different from baseline (P = .59). Mean SCr was

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*
26-week-to-baseline RTGC ratio*	−0.30 (−0.50, −0.09)	−0.003 (−0.34, 0.34)	.04
26-week-to-baseline UACR ratio*	−0.38 (−0.67, −0.08)	0.22 (0.03, 0.40)	<.01
Mean change SCr (mg/dL)	−0.03 (−0.08, 0.07)	−0.01 (0.09, 0.07)	.46
Mean change eGFR (mL/min/1.72 m^2)	−2.3 (−6.5, −5.3)	−0.6 (−4.4, 5.6)	.27
Mean change HbA1c (%)	−2.7 (−3.8, 2.7)	−2.6 (−3.4, 2.4)	.82

95% CI, 95% confidence interval; eGFR, estimated glomerular filtration rate; HbA1c, glycated hemoglobin; RTGC, renal triglyceride content; SCr, serum creatinine; UACR, urinary albumin-to-creatinine ratio.

The table includes outcome comparisons for study participants with RTGC values at both baseline and follow-up (n = 17).

*A 26-week-to-baseline ratio derived from the difference between the log-transformed baseline and 26-week levels of RTGC and UACR, respectively.

0.76 ± 0.2 mg/dL, which was 0.74 ± 0.2 mg/dL at follow-up ($P = .26$). Mean eGFR at baseline was 96.1 ± 18.4 mL/minute/1.73 m^2 and 96.9 ± 19.2 mL/minute/1.73 m^2 at follow-up ($P = .49$).

Results of Liraglutide Versus Standard Glycemic Control on RTGC

Nine patients with T2DM randomized to liraglutide, and 8 patients with T2DM 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 26 weeks of liraglutide or placebo is provided in Tables 1 and 3 as well as in Figure 3. Baseline HbA1c in the liraglutide group was 7.8 ± 0.9% and 7.8 ± 0.7%. At follow-up, this changed to 7.3 ± 1.0% and 7.3 ± 0.8% for the liraglutide and placebo groups, respectively. No significant differences were found in HbA1c between the liraglutide group and placebo group ($P = .82$). 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. A 26-week-to-baseline RTGC ratio (95% confidence interval) was significantly different between liraglutide (−0.30 [−0.50, −0.09]) and placebo added to standard care (−0.003 [−0.34, 0.34]) ($P = .04$) (Table 3; Fig. 3). Median UACR in the liraglutide group at baseline was 15.9 mg/g (6.2, 58.4) and 17.7 mg/g (7.1, 29.2) in the placebo group. At follow-up, median UACR was 15.0 mg/g (2.7, 101.8) in the liraglutide group and 22.1 mg/g (11.5, 54.0) in the placebo group. A 26-week-to-baseline UACR ratio (95% confidence interval) was significantly different between liraglutide (0.22 [0.0.3, 0.40]) and placebo added to standard care (−0.38 [−0.67, 0.08]) ($P = .04$). Mean changes in SCr and eGFR were not statistically different between the liraglutide and placebo groups (SCr, $P = .46$; eGFR, $P = .27$).

Discussion

The aim of this exploratory study was to assess whether glycemic control influences RTGC, 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

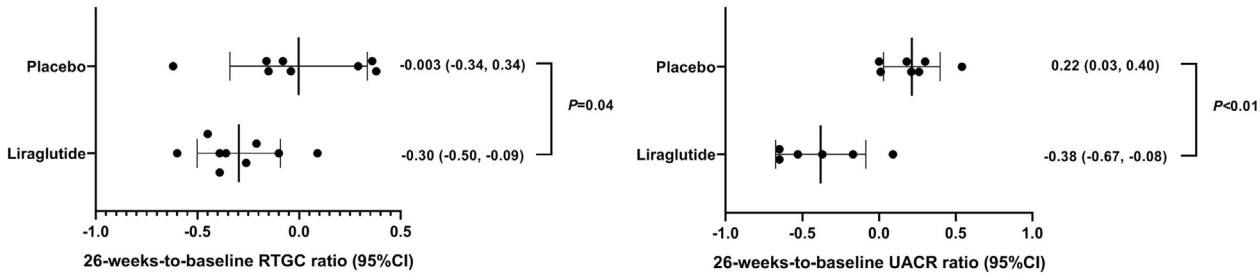

Figure 3. Treatment effect of liraglutide (n = 9) versus placebo (n = 8) on RTGC and UACR. 95% CI, 95% confidence interval; RTGC, renal triglyceride content; UACR, urinary albumin-to-creatinine ratio.

control (irrespective of randomized treatment group). From the DKD literature, it is known that intensive glycemic control improves renal outcomes as evidenced by reduction of proteinuria progression and reduced risk of ESKD.[3] In our secondary analysis of the MAGNA VICTORIA study, we showed that RTGC reduced significantly more with liraglutide than placebo, whereas 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 and beneficially influence fatty kidney. Albeit our study findings were slightly below the 2-tailed significance levels of $P < .05$, these results cannot be considered as strong evidence as the P value depends on the magnitude of the treatment effect and the size of the standard error. Moreover, it should be noted that considering the lack of a control group (participants with no treatment at all) in this study, the phenomenon of regression to the mean as a possible explanation for the found reduction in RTGC at follow-up cannot be excluded. Taking this into account, and considering the small sample size of the present study as well as the limited prevalence/severity of DKD in our sample (because eGFR below 60 mL/minute/1.73 m^2 was an exclusion criterium), further research in larger clinical trials is warranted to better delineate the association between glycemic control and fatty kidney.

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 patients with T2DM treated with liraglutide, compared with 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] The lack of a statistical significant difference in HbA1c between the liraglutide group and placebo group suggests that the difference in RTGC between the liraglutide group and placebo group is possibly related to other mechanisms than solely glycemic control. One of the possible explanations is that GLP1-RA affects fatty kidney by a direct effect of GLP1-RA on the kidney considering the existence of renal GLP1 receptors,[12] rather than that current findings merely represent the composite effects of GLP1-RA on body weight and/or HbA1c. Suggested direct pathways related to GLP1 activation are inhibition of various injurious pathways within the kidney, including oxidative stress (nicotinamide adenine dinucleotide phosphate oxidase inhibition), inflammation (reduced expression of cytokines and chemokines), and fibrosis (reduced expression of transforming growth factor-β1 and collagen IV).[27,28] Moreover, it remains uninvestigated whether concomitant use of medications such as statins and antihypertensives influence RTGC levels.

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 ^{1}H-MRS in the kidney, we applied several quality criteria for the obtained spectra to assure the quality of the measurements. Because of these, we excluded a substantial amount of the spectra from the analysis. Although the renal outcomes were prespecified in the MAGNA VICTORIA study, these studies were powered for primary endpoints involving left ventricular diastolic and systolic functions 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 spectra that did not meet the quality criteria, ^{1}H-MRS of the kidney remains a technically challenging technique, limiting the use of RTGC as a biomarker for the evaluation of treatment effects on lipid metabolism in the kidney. 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 assess differences in RTGC between patients with T2DM 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 patients with T2DM of Western European and South Asian descent, as well as the proportion of patients with T2DM of South Asian descent in the liraglutide and placebo arms. Future studies are needed to better understand how lipid metabolism in the kidney 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 26 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 fatty kidney.

Practical Application

Our study indicates that glycemic control, in particular for liraglutide, might influence ectopic lipid accumulation in the kidney. Considering tight glycemic control can reduce the risk of diabetic nephropathy, our findings support the role of fatty kidney as a potential driver of DKD.

Credit Authorship Contribution Statement

Ilona A. Dekkers: Conceptualization, Methodology, Formal analysis, Data curation, Funding acquisition, Writing - original draft, Writing - review & editing. **Maurice B. Bizino:** Conceptualization, Methodology,

Formal analysis, Data curation, Funding acquisition, Writing - review & editing. **Elisabeth H.M. Paiman:** Conceptualization, Methodology, Formal analysis, Data curation, Funding acquisition, Writing - review & editing. **Johannes W. Smit:** Conceptualization, Methodology, Formal analysis, Data curation, Supervision, Writing - review & editing. **Ingrid M. Jazet:** Conceptualization, Methodology, Formal analysis, Data curation, Supervision, Writing - review & editing. **Aiko P.J. de Vries:** Conceptualization, Methodology, Formal analysis, Data curation, Supervision, Writing - review & editing. **Hildo J. Lamb:** Conceptualization, Methodology, Formal analysis, Data curation, Writing - review & editing.

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