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Whole-body insulin clearance in people with type 2 diabetes and normal kidney function: Relationship with glomerular filtration rate, renal plasma flow, and insulin sensitivity☆

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A B S T R A C T

Objective: Kidney insulin clearance, proposed to be the main route of extra-hepatic insulin clearance, occurs in tubular cells following glomerular filtration and peritubular uptake, a process that may be impaired in people with type 2 diabetes (T2D) and/or impaired kidney function. Human studies that investigated kidney insulin clearance are limited by the invasive nature of the measurement. Instead, we evaluated relationships between whole-body insulin clearance, and gold-standard measured kidney function and insulin sensitivity in adults with T2D and normal kidney function.

Research design and methods: We determined insulin, inulin/iohexol and para-aminohippuric acid (PAH) clearances during a hyperinsulinemic-euglycemic clamp to measure whole-body insulin clearance and kidney function. Insulin sensitivity was expressed by glucose infusion rate (M value). Associations between whole-body insulin clearance, kidney function and insulin sensitivity were examined using univariable and multivariable linear regressions models.

Results: We investigated 44 predominantly male (77%) T2D adults aged 63 ± 7, with fat mass 34.5 ± 9 kg, lean body mass 63.0 ± 11.8 kg, and HbA1c 7.4 ± 0.6%. Average whole-body insulin clearance was 1188 ± 358 mL/min. Mean GFR was 110 ± 22 mL/min, mean ERPF 565 ± 141 mL/min, and M value averaged 3.9 ± 2.3 mg/min. Whole-body insulin clearance was positively correlated with lean body mass, ERPF and insulin sensitivity, but not with GFR. ERPF explained 6% of the variance when entered in a nested multivariable linear regression model op top of lean body mass (25%) and insulin sensitivity (15%).

Conclusions: In adults with T2D and normal kidney function, whole-body insulin clearance was predicted best by lean body mass and insulin sensitivity, and to a lesser extent by ERPF. GFR was not associated with whole-body insulin clearance. In contrast to prior understanding, this suggests that in this population kidney insulin clearance may not play such a dominant role in whole-body insulin clearance.

1. Introduction

Insulin metabolism is disturbed in people with type 2 diabetes (T2D). Several insulin-sensitive organs including liver, skeletal muscle, and adipose tissue become resistant to insulin, while pancreatic beta-cells first hypersecrete and in later stages of the disease hyposecrete insulin.[1–4] In addition, T2D causes several changes in insulin clearance.

After secretion, insulin clearance starts in the liver, which eliminates 40–80% of portal insulin at first pass, a process that is reduced in people with T2D.[5] After first pass, the kidneys are thought have a primary role in the degradation of systemic circulating insulin, working together with the liver to maintain optimal insulin levels. Of all the insulin that enters the systemic circulation an estimated 30–80% is cleared by the kidneys.[6–9] For administered exogenous insulin this percentage is thought to be even higher.[10] In people with impaired kidney function, insulin clearance is reduced leading to prolonged insulin action.[11] This may explain the lower insulin requirement as well as the higher occurrence of hypoglycemic events in people with T2D and impaired kidney function.[12]

The degradation of insulin in the kidney is proposed to occur via two separate pathways, which results in low urinary excretion of about 1–2% of the filtered insulin load. First, following glomerular filtration insulin is reabsorbed by the proximal tubule, and subsequently degraded in intracellular lysosomes.[9,13] This reabsorption is nonspecific and independent of insulin receptors Second, in post-glomerular peritubular capillaries, insulin enters kidney tubular cells from the abluminal side

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following binding to basolateral insulin receptors, after which intracellular degradation takes place in lysosomes.[9,14] Experimental evidence from arterial and kidney vein sampling indicated that glomerular filtration is the more dominant pathway and contributes to roughly 60% of kidney insulin clearance.[11]

Several aspects of the relative contribution of the kidneys to whole-body insulin clearance in people with T2D remain uncertain. Kidney insulin clearance characteristics and the capability of the two kidney pathways might be different because of pathophysiological processes. For instance, the role of impaired insulin sensitivity in relation to whole-body and kidney insulin clearance has only scantly been investigated in this population.

Measurement of kidney insulin clearance requires invasive arteriovenous sampling, which was not part of the our study protocol. Instead, in a cohort of 44 well-phenotyped T2D individuals we were able to examine the relationship of whole-body insulin clearance with gold-standard measurements of kidney function including glomerular filtration rate (GFR) and effective renal plasma flow (ERPF). ERPF is measured by the clearance of PAH, a substance also used to measure the anionic secretory function of the renal tubule and although these proteins are absorbed through different receptors, PAH clearance could be seen as surrogate marker of peritubular uptake due to its dependency on basolateral transportation pathways.[15,16] Moreover, we evaluated the role of insulin sensitivity in this context. We hypothesized that whole-body insulin clearance might be predicted best when taking both GFR and ERPF into account, and that impaired insulin sensitivity would restrict this relationship.

2. Research design and methods

This cross-sectional study was performed at the Amsterdam University Medical Centers, location VUMC, Amsterdam, the Netherlands and is based on the baseline data of a randomized clinical trial (NCT02682563), designed to assess the effects of 12-week dapagliflozin to gliclazide. The study protocol and all protocol-specific documents were reviewed and approved by the ethics review board of the VU University Medical Center (Amsterdam, the Netherlands) and written informed consent was obtained from all participants before any trial-related activity. The study complied with the Declaration of Helsinki and Good Clinical Practice guidelines.[17]

2.1. Study participants

Participants were recruited from our local database and by advertisements in relevant newspapers. Eligible participants were men or post-menopausal women, aged 35 to 75 years, diagnosed with T2D with an HbA1c of 6.5% to 9.0% (48–75 mmol/mol) and a body mass index (BMI) >25 kg/m^2 as described.[17,18] Participants were treated with metformin as the only glucose-lowering agent (stable dose for $\geq$3 months). Use of other anti-hyperglycemic medication was not allowed. Blood pressure was under control (i.e. $<$140/90 mmHg) and macroalbuminuria (i.e. ACR $>$300 mg/g) was not allowed; in case of previously diagnosed hypertension or albuminuria, treatment included at least a stable dose of a renin-angiotensin system (RAS) inhibitor for $\geq$3 months. Exclusion criteria included a history of unstable or rapidly progressing kidney or malignant disease (excluding basal cell carcinoma), eGFR $<$60 mL/min/1.73 m^2, urinary retention (bladder ultrasonography at screening visit was performed), (re)current urinary tract or genital infection, the use of NSAIDs or diuretics that could not be stopped 3 months prior to the study day.

2.2. Study protocol, measurements, assays and calculations

The week before testing, the participants adhered to a controlled sodium (9–12 g/day) and protein (1.5–2.0 g/kg/day) diet, in order to minimize variation in kidney physiology due to salt and protein intake. After an overnight fast, blood and urine were obtained for fasting outcome variables. Then, a renal protocol commenced by 10-min bolus infusion of 22.5 mg/kg inulin (Inutest®, Fresenius Kabi Austria GmbH, Graz, Austria) and 3 mg/kg PAH (4-Aminohippuric Acid Solution 20%, Bachem Distribution Services GmbH, Weil am Rhein, Germany), after which infusion continued at a lower rate (675 and 320 mg/h, respectively) until the end of the visit. After 33 participants completed the trial, inulin was retracted from the market because of anaphylactic reactions at another study site. Since iohexol and inulin have a similar pharmacokinetic profile, and clearances correlate almost ($r = 0.986$) perfectly,[18] inulin was substituted by iohexol (Omnipaque™, GE Healthcare B.V. Eindhoven, the Netherlands) to measure GFR in the remaining 11 participants (bolus infusion of 36 mg/kg in 10 min, followed by continuous infusion of 906 mg/h).

After 2 h of bed rest, a hyperinsulinemic-euglycemic clamp was initiated, with insulin (Novorapid, Novo Nordisk, Denmark) infusion at 40 mU/min·m^2 while maintaining plasma glucose at 5.0 mmol/L by variable glucose 20% infusion. After 90 min of clamp equilibration, urine was collected by spontaneous voiding for two 45-min periods. GFR and ERPF were quantified by the average of two gold-standard plasma clearances of inulin/iohexol and PAH respectively, and calculated by dividing infusion rate by plasma concentration.[19,20]

Insulin sensitivity was measured by glucose infusion rate (corrected for urinary glucose excretion) during the last 30 min of the clamp (M value, mg/min). Whole-body insulin clearance (mL/min) was calculated by dividing the insulin infusion rates (mU/min) by the average of two steady-state insulin concentrations (mU/mL) taken 30 min apart.

Systolic blood pressure (SBP), diastolic blood pressure (DBP) and heart rate were determined during all three phases at the brachial artery of the non-dominant arm. Measurements were performed in triplicate at 1–2 min intervals, using the mean of the last two measurements. We assessed lean body mass by single-frequency bioelectrical impedance analyzer (Maltron BF-906; Maltron International, Essex, U.K.). Blood glucose concentrations were measured using an YSI 2300 STAT Plus analyzer (YSI, Yellow Springs, OH). Insulin concentrations were measured using chemoluminescence immunoassays (Atellica IM, Siemens Healthcare Diagnostics, USA).

2.3. Statistical analysis

Statistical analyses were performed using SPSS software (IBM Statistics, Version 26). The results are expressed as mean $\pm$ SD when variables where normally distributed or as median with interquartile range (IQR) otherwise. Categorical variables are shown as n with %. The relationship between whole-body insulin clearance and traditional risk factors, GFR, ERPF and insulin sensitivity was assessed using Pearson correlations (all normally distributed); B and SE are shown. Multivariable linear regression models corrected for correlated traditional risk factors were built to examine the relationship between whole-body insulin clearance and GFR, ERPF and insulin sensitivity; B and SE are shown. Nested linear regression models were used to calculate the amount of variance in whole-body insulin clearance explained by the predictors (R squared). Statistical significance was defined as a two-sided p value of <0.05.

3. Results

3.1. Patient recruitment

From July 2016 to March 2018, 75 participants were screened, of whom 50 were included in the trial. Five of these withdrew consent before testing and one was excluded because of urinary retention, resulting in 44 included individuals in the current analysis. Baseline characteristics are provided in Table 1.

Table 1
Participant characteristics (n = 44).

Clinical characteristics	
Age, years	63 ± 7
Male, n (%)	34 (77)
Current smoker, n (%)	4 (9)
BMI, kg/m^2	31.1 ± 3.9
Fat mass, kg	34.5 ± 9.0
Lean body mass, kg	63.0 ± 11.8
Fasting plasma glucose, mg/dL	162 ± 28
Fasting plasma glucose, mmol/L	9.0 ± 1.5
HbA1c, %	7.4 ± 0.6
HbA1c, mmol/mol	57 ± 7
Diabetes duration, years	10.2 ± 5.8
Fasting systemic hemodynamics	
SBP, mmHg	134 ± 13
DPB, mmHg	83 ± 6
Heart rate, beats/min	67 ± 11
Insulin handling	
Plasma insulin concentration, pmol/L	
542 ± 132	
Whole-body insulin clearance, mL/min	1188 ± 358
Insulin infusion rate, pmol/min	605 ± 69
M value[a], mg/min	3.9 ± 2.3
Kidney function	
GFR, mL/min	110 ± 22
ERPF, mL/min	565 ± 141
Medication	
Metformin dose, mg	1500 (1000–2000)
Statin use, n (%)	30 (68)
Anticoagulant medication use, n (%)	6 (14)
RAS inhibitor use, n (%)	32 (73)

Participant characteristics. Values are expressed as mean ± SD, n (%) or median (IQR).

[a] M value corrected for urinary glucose excretion. Abbreviations: BMI body mass index; HbA1c glycated hemoglobin A1c; SBP systolic blood pressure; DPB diastolic blood pressure; GFR glomerular filtration rate; ERPF effective renal plasma flow; RAS renin-angiotensin system.

3.2. Whole-body insulin clearance, kidney function and insulin sensitivity

During the hyperinsulinemic-euglycemic clamp, the mean whole-body insulin clearance was 1188 ± 358 mL/min with an average plasma insulin concentration of 542 ± 132 pmol/L. Mean insulin infusion rate was 605 ± 69 pmol/min. Mean GFR and ERPF were 110 ± 22 mL/min and 565 ± 124 mL/min respectively. The mean glucose rate needed to maintain plasma glucose at 5 mmol/L (90 mg/dL) was 3.9 ± 2.3 mg/min (M value) (Table 1).

3.3. Relationships with whole-body insulin clearance

Whole-body insulin clearance was positively associated with ERPF (r = 0.52, p < 0.001), but not with GFR (Fig. 1, panel i and ii). Lean body mass was positively associated with whole-body insulin clearance (r = 0.50, p = 0.001), while other characteristics such as age, sex (male/female) and body fat mass were not related to whole-body insulin clearance (Table 2). In multivariable analyses, the association between ERPF and whole-body insulin clearance remained significant after adjustment for lean body mass (Table 2, model 1). Whole-body insulin clearance was positively related to insulin sensitivity (r = 0.34, p = 0.026; Fig. 1, panel iii), which remained significant after adjustment for lean body mass (Table 2, model 2).

When entered in a nested linear regression model on top of body mass, ERPF added 8% of variance (R squared) in whole-body insulin clearance. When corrected for the amount of variables entered, adding ERPF significantly improved the model (adjusted variance from 0.23 to 0.30; p = 0.030) (Table 3, model 1). M value added 15% of variance in whole-body insulin clearance when entered in the nested linear regression model on top of lean body mass. The addition of M value to the model also gave a significant improvement (adjusted variance from 0.23 to 0.37; p = 0.002) (Table 3, model 2).

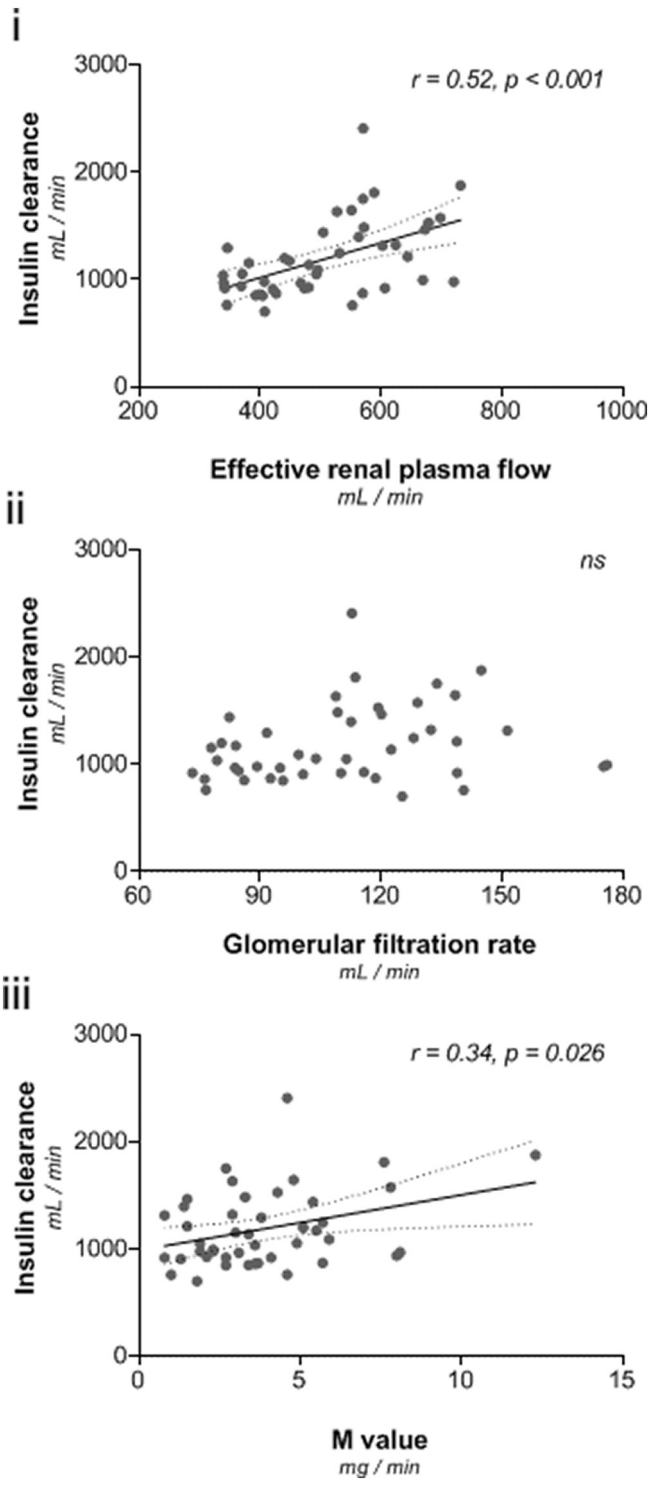

Fig. 1. Associations with insulin clearance.
Correlations between insulin clearance, kidney function, and insulin sensitivity. i: effective renal plasma flow, ii: glomerular filtration rate, iii: M value. Best fit value linear regression line and 95% confidence intervals are represented by solid and dotted lines respectively. Pearsons correlations are shown.

When the relationship between whole-body insulin clearance and ERPF was corrected for both lean body mass and insulin sensitivity, the association remained significant and the addition of ERPF on top of lean body mass and insulin sensitivity in a nested model added 6% of variance (Tables 2 and 3, model 3).

Table 2
Univariable and multivariable analyses with insulin clearance.

Variable	Univariable analysis *B ± SE*	Multivariable analysis		
		Model 1 *B ± SE*	Model 2 *B ± SE*	Model 3 *B ± SE*
Age years	−9.13 ± 7.83 p = 0.250			
Sex m/f	−176.77 ± 127.54 p = 1.73			
Body fat mass kg	7.43 ± 6.05 p = 0.226			
GFR mL/min	3.26 ± 2.07 p = 0.124			
Lean body mass kg	**15.04 ± 4.08 p = 0.001**	8.95 ± 4.75 p = 0.067	**16.31 ± 3.70 p < 0.001**	**11.00 ± 4.37 p = 0.016**
M value mg/min	**1.62 ± 0.41 p < 0.001**		**60.22 ± 18.65 p = 0.002**	**55.94 ± 18.04 p = 0.004**
ERPF mL/min	**33.99 ± 15.19 p = 0.031**	**1.09 ± 0.49 p = 0.030**		**0.94 ± 0.45 p = 0.042**
R squared (R^2)		**0.57; p < 0.001**	**0.63; p < 0.001**	**0.68; p < 0.001**

Univariable and multivariable model analyses of insulin clearance. Univariable analyses with traditional risk factors and variables of interest are shown. Multivariable models 1–3 assess whether GFR, ERPF and M value are still significantly associated with insulin clearance when corrected for lean body mass. Model 1 includes lean body mass and ERPF. Model 2 included lean body mass and M value. Model 3 includes lean body mass, ERPF and M value. Significant correlations are highlighted in bold font. B ± SE are listed with corresponding p value. Abbreviations: ERPF effective renal plasma flow; GFR glomerular filtration rate.

Table 3
Variance of models explained.

Variable	Variance	Added variance	Adjusted variance	Change (p value)
Model 1 – insulin clearance (mL/min) and ERPF (mL/min)				
+ Lean body mass, kg	**0.25**	**25%**	**0.23**	**0.001**
+ ERPF	**0.33**	**8%**	**0.30**	**0.030**
Model 2 – insulin clearance (mL/min) and M value (mg/min)				
+ Lean body mass, kg	**0.25**	**25%**	**0.23**	**0.001**
+ M value, mg/min	**0.40**	**15%**	**0.37**	**0.002**
Model 3 – insulin clearance (mL/min), PAH clearance (mL/min), and M value (mg/min)				
+ Lean body mass, kg	**0.25**	**25%**	**0.23**	**0.001**
+ M value, mg/min	**0.40**	**15%**	**0.37**	**0.002**
+ ERPF	**0.46**	**6%**	**0.42**	**0.004**

Variance explained (R squared). Explained variance using linear regression analysis with entered variables. To correct for the amount of entered variables, an adjusted R squared is also given. An adjusted R squared only improves if the additional predictor improves the model more than expected on chance alone. Significant correlations are highlighted in bold font. Abbreviations: ERPF effective renal plasma flow; GFR glomerular filtration rate; Cl clearance.

4. Discussion

In this study we report on 3 novel findings. In adults with well controlled T2D and normal kidney function; 1. whole-body insulin clearance was positively related to ERPF, which is in accordance with previous observations that peritubular uptake is one of the two pathways in kidney insulin clearance. However, despite the fact that glomerular filtration and subsequent reabsorption is thought to be the more dominant pathway in kidney insulin clearance, whole-body insulin clearance was not related to GFR, 2. in accordance with previous studies[21–26] whole-body insulin clearance was positively related to whole-body insulin sensitivity and 3. although peritubular uptake contributes to whole-body insulin clearance, the percentage that was explained by ERPF was relatively low, indicating that the role of the kidneys in systemic insulin clearance may be more limited than previously estimated.

In contrast to the glomerular pathway, the peritubular pathway of kidney insulin clearance is suggested to be dependent of insulin receptors,[2] and therefore could be affected by impaired insulin sensitivity.[27] The precise distribution between these two pathways and their dependency on whole-body insulin sensitivity in people with T2D is unknown. ERPF is measured by the clearance of PAH, a solute that is also used to measure the secretory function of the renal tubule. Almost all PAH (90%) in the postglomerular circulation crosses the basolateral membrane using specific organic anion transporters. Peritubular uptake of insulin also occurs via basolateral pathways and as such, PAH clearance (EPRF) could function as surrogate marker of peritubular uptake.[15,16]

Regarding the distribution of the two pathways, we did not find an association between GFR and whole-body insulin clearance, indicating that other factors might be more relevant. In contrast, ERPF was related to whole-body insulin clearance, which persisted in multivariable models. As such, it might be that in our study population, peritubular uptake is the dominant pathway of kidney insulin clearance, and not glomerular filtration of insulin.

Studies have indicated that the insulin receptor is likely to play a role in the uptake of insulin through the basolateral pathway.[9,14] Since we could link whole-body insulin sensitivity to whole-body insulin clearance, we were able to address this relationship. After correction for lean body mass, whole-body insulin sensitivity was positively related to whole-body insulin clearance, while whole-body insulin sensitivity explained 15% of the variance in whole-body insulin clearance. This correlation could support the proposed involvement of insulin receptors in the kidney clearance of insulin through basolateral pathways. On the other hand, this correlation is driven by other insulin-sensitive tissues (e.g. liver, skeletal muscle, adipose tissue) that are involved in whole-body insulin clearance.

Surprisingly, the total amount of variance in whole-body insulin clearance explained by ERPF was only 6–8%. Given the proposed central role for the kidneys to clear insulin from the subcutaneous or arterial compartments, this might be perceived as rather low, especially compared to the stronger correlations with lean body mass and insulin sensitivity. It might suggest that – apart from the dominant role of the liver - other insulin-sensitive peripheral tissues such as brain, adipose and skeletal muscle tissue could play a more fundamental role in whole-body insulin clearance,[2,28,29] and that a lesser role is reserved for the kidneys. This is line with findings of a recent study in people without diabetes with chronic kidney damage,[16] where the contribution of the kidneys to systemic insulin clearance was shown to be limited and in the order of magnitude shown in our nested linear regression analyses. This finding is in contrast with the clinical observation that people with T2D and impaired kidney function might require lower insulin dosage or have a higher risk of hypoglycemia and warrants further research.

This study has strengths and weaknesses. Strengths include gold-standard kidney function measurements of GFR and use of tubular secretion marker PAH, as well as gold-standard measurement of insulin sensitivity by hyperinsulinemic-euglycemic clamp test. However, we were unable to determine kidney clearance of insulin, as this would require arteriovenous sampling which was deemed too labor-intensive and invasive to fit in the study protocol. Instead, we used PAH clearance as a surrogate marker for peritubular insulin uptake. In contrast to the insulin protein, PAH is an organic anion which does not bind to the insulin receptor, and although it has been used earlier as surrogate

marker for basolateral transport,[16] it is important to keep in mind that both substances use distinct physiological mechanisms to cross the basolateral membrane. We did not include patients with low GFR making extrapolation of our data to adults with T2D and impaired kidney function difficult. Furthermore, our sample size was limited, however, due to the high precision of the performed measurements, lower numbers are sufficient compared to studies using estimated GFR (eGFR), surrogate measures for insulin sensitivity or tubular function. Finally, we had a cross-sectional design, making causal conclusions impossible.

We conclude that in adults with T2D and normal kidney function, whole-body insulin clearance is positively related to ERPF and whole-body insulin sensitivity, but not to GFR. However, the total contribution of ERPF to whole-body insulin clearance was relatively low, indicating that the role of the kidneys in whole-body insulin clearance is limited.

CRediT authorship contribution statement

The manuscript is written by Michaël JB van Baar, Erik JM van Bommel, Mark M Smits, Daan J Touw, Max Nieuwdorp, Reinier W ten Kate, Jaap A Joles, and Daniël H van Raalte. All authors agree on the content of the manuscript, which has not been submitted elsewhere.

MJBvB, MMS and DHvR performed statistical analysis. MJBvB and DHvR wrote the first draft of the manuscript, and the submitted version was approved by all authors. MJBvB, and EJMvB were involved in sample collection and/or analysis. EJMvB, MN, JAJ and DHvR designed and set up the trial. MJBvB is the guarantor of this work and, as such, had full access to all the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis.

Declaration of competing interest

This trial was funded by AstraZeneca as an investigator-initiated study. M.N. received an unrestricted investigator-initiated grant from AstraZeneca on sodium–glucose cotransporter 2 inhibitor and lipid fluxes. DHvR has acted as a consultant and received honoraria from Boehringer Ingelheim and Lilly, Merck, Novo Nordisk, Sanofi, and AstraZeneca and has received research operating funds from Boehringer Ingelheim–Lilly Diabetes Alliance, AstraZeneca, Merck, and Novo Nordisk, with all honoraria paid to his employer (Amsterdam University Medical Center, location VUmc). No other potential conflicts of interest relevant to this article were reported. The funder had no role in the study design, the analyses or interpretation of the data, or drafting the manuscript. The funder had no role in the decision to submit this manuscript for publication.

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