Mechanism of insulin resistance in a rat model of kidney disease and the risk of developing type 2 diabetes

François Dion¹,²*, Christopher Dumayne¹,²*, Nathalie Henley¹, Stéphanie Beauchemin¹, Edward B. Arias³, François A. Leblond¹, Sylvie Lesage¹,², Stéphane Lefrançois⁵,⁶, Gregory D. Cartee³, Vincent Pichette¹,²*

¹ Centre de recherche de l’Hôpital Maisonneuve-Rosemont, Faculté de Médecine, Centre affilié à l’Université de Montréal, Montréal, Québec, Canada, ² Département de pharmacologie, Faculté de Médecine, Université de Montréal, Montréal, Québec, Canada, ³ School of Kinesiology, University of Michigan, Ann Arbor, Michigan, United States of America, ⁴ Département de microbiologie, infectiologie et immunologie, Faculté de Médecine, Université de Montréal, Montréal, Québec, Canada, ⁵ Centre INRS-Institut Armand-Frappier, Institut National de la Recherche Scientifique, Laval, Québec, Canada, ⁶ Department of Anatomy and Cell Biology, McGill University, Montreal, Quebec, Canada

* These authors contributed equally to this work.

OPEN ACCESS

Citation: Dion F, Dumayne C, Henley N, Beauchemin S, Arias EB, Leblond FA, et al. (2017) Mechanism of insulin resistance in a rat model of kidney disease and the risk of developing type 2 diabetes. PLoS ONE 12(5): e0176650. https://doi.org/10.1371/journal.pone.0176650

Editor: Makoto Kanzaki, Tohoku University, JAPAN

Received: January 11, 2017

Accepted: April 13, 2017

Published: May 1, 2017

Copyright: © 2017 Dion et al. This is an open access article distributed under the terms of the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited.

Data Availability Statement: All relevant data are within the paper and its Supporting Information files.

Funding: Support was provided by Fondation de l’Hôpital Maisonneuve-Rosemont [http://www.fondationhrm.ca/] to VP. The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript. Support was also provided by Diabète québec [http://www.diabete.qc.ca/] to FD. The funders had no role in study design, data collection.

Abstract

Chronic kidney disease is associated with homeostatic imbalances such as insulin resistance. However, the underlying mechanisms leading to these imbalances and whether they promote the development of type 2 diabetes is unknown. The effect of chronic kidney disease on insulin resistance was studied on two different rat strains. First, in a 5/6th nephrectomised Sprague-Dawley rat model of chronic kidney disease, we observed a correlation between the severity of chronic kidney disease and hyperglycemia as evaluated by serum fructosamine levels ($p < 0.0001$). Further, glucose tolerance tests indicated an increase of 25% in glycemia in chronic kidney disease rats ($p < 0.0001$) as compared to controls whereas insulin levels remained unchanged. We also observed modulation of glucose transporters expression in several tissues such as the liver (decrease of 40%, $p < 0.01$) and muscles (decrease of $\approx 29\%$, $p \leq 0.05$). Despite a significant reduction of $\approx 37\%$ in insulin-dependent glucose uptake in the muscles of chronic kidney disease rats ($p < 0.0001$), the development of type 2 diabetes was never observed. Second, in a rat model of metabolic syndrome (Zucker Lep⁴di/fa), chronic kidney disease caused a 50% increased fasting hyperglycemia ($p < 0.0001$) and an exacerbated glycemic response ($p < 0.0001$) during glucose challenge. Similar modulations of glucose transporters expression and glucose uptake were observed in the two models. However, 30% ($p < 0.05$) of chronic kidney disease Zucker rats developed characteristics of type 2 diabetes. Thus, our results suggest that downregulation of GLUT4 in skeletal muscle may be associated with insulin resistance in chronic kidney disease and could lead to type 2 diabetes in predisposed animals.
Introduction

The prevalence of chronic kidney disease (CKD) is steadily increasing [1], and its metabolic and hormonal complications have been the subject of many studies [2, 3]. In particular, type 2 diabetes (T2D), a leading cause of end-stage renal disease (ESRD), is an important and growing health problem [4, 5]. The convergence of these two conditions could lead to mutual exacerbation and worsening of patient health [6, 7].

Glucose intolerance is a common finding in patients and animals affected by renal impairment [8, 9]. Numerous studies suggest that peripheral insulin resistance and/or impaired insulin secretion are causative factors for diminished carbohydrate metabolism [10–13]. As insulin resistance in CKD is associated with diabetes, kidney disease could also lead to a deterioration of insulin sensitivity [11, 14]. The morbidity and mortality associated with CKD are predominantly attributable to cardiovascular complications, as more than half of the reported cases of death in patients with ESRD are linked to heart disease [15, 16]. Consequently, given that insulin resistance may contribute to the development of cardiovascular disease, insulin resistance is an important target to prevent such complications in patients with CKD.

Although insulin resistance in CKD is usually regarded as a post-receptor defect [16, 17], the mechanisms underlying this disorder are still poorly understood. Several lines of emerging evidence have demonstrated that CKD leads to modulation of the expression and activity of several drug transporters including those of the ATP-Binding Cassette and Solute Carriers families in the liver, intestine, kidneys and brain [18–20]. It therefore seems plausible that glucose transporters, members of the Solute Carriers family, may also be altered. To date, however, there is limited and conflicting data concerning alterations in the expression of glucose transporters in CKD and their role in insulin resistance [17, 21–23].

The purpose of this study was to determine whether: 1) CKD leads to insulin resistance in a nephrectomised rat model of CKD, 2) CKD modulates key transporters involved in glucose homeostasis which could ultimately lead to insulin resistance, 3) CKD is associated with impaired glucose uptake in insulin-sensitive organs and 4) insulin resistance in CKD leads to T2D. To address these aims, we used the 5/6th nephrectomy model to induce CKD in both Sprague-Dawley (SD) and Zucker Lepr<sup>fa/fa</sup> rats, where the latter rat strain present a metabolic syndrome and, therefore, exhibit insulin resistance without developing T2D. These studies allowed us to evaluate the potential of kidney disease to precipitate the onset of T2D in predisposed patients.

Materials and methods

Ethical statement

All experiments were conducted according to the Canadian Council on Animal Care guidelines for the care and use of laboratory animals and were specifically approved by our local animal care committee (Comité de protection des animaux du Centre de recherche de l’Hôpital Maisonneuve-Rosemont; Protocol #2015–26). Isoflurane was used for anaesthesia during surgeries and all precautions were taken to minimize discomfort. At the end of the study, rats were euthanized by decapitation to avoid pharmacological interference with experiments.

Animal models

Male Sprague-Dawley (SD) rats (Charles River Laboratories, St-Constant, QC, Canada), weighing 176 to 225 g, 6 to 8 weeks of age, and Zucker ZUC-Lepr<sup>fa/fa</sup> rats (Charles River Laboratories, Raleigh, NC), weighing 180-200g, 6 weeks of age, were housed in the animal facility at...
the Hôpital Maisonneuve-Rosemont Research Center and were provided with Harlan Teklad
rodent diet #2014 (Envigo, Lachine, QC, Canada) and water ad libitum.

Surgery and experimental protocol
CKD was induced by a two-stage 5/6 nephrectomy as previously described [24]. A timeline of
the experimental procedures is provided in supporting information S1 Fig. Chronic kidney
disease (CKD) and control (CTL) rats were matched according to their weight and age at the
start of the experiment. CKD rats underwent a 2/3 nephrectomy of the left kidney (Day 0) and
7 days later, a right total nephrectomy. CTL rats underwent two sham laparotomies. The
amount of chow fed to control pair-fed rats was adjusted daily, based on the amount of chow
that the CKD rats ate the day before. After Day 21, rats were monitored once a week for glycos-
uria. At Day 41, rats were housed in metabolic cages and urine was collected for 24 hours to
determine creatinine clearance. Rats were sacrificed at Day 42 for organ and blood collection,
unless stated otherwise. Liver, kidneys, Biceps Femoris and epididymal white adipose tissue
were immediately excised, rinsed in phosphate buffered saline (PBS) and flash-frozen in liquid
nitrogen. Blood was collected for further biochemical characterization. Rats used for glycosuria
assessment were kept alive for up to 15 weeks. All days are calculated from 1st nephrectomy.
Rats with a fasting glycemia over 11 mmol/L, with the presence of glycosuria as defined below,
were defined as T2D.

Biochemical parameters
Serum creatinine, urea, fructosamine and urinary creatinine were analyzed on an ARCHI-
TECT c16000 Clinical Chemistry Analyzer (Abbott Diagnostics, Lake Forest, IL, USA).
Glycemia was measured using an AlphaTRAK blood glucose monitoring system (Abbott Lab-
oratories, North Chicago, IL, USA). Serum insulin was assayed by a Rat ultrasensitive ELISA
kit (ALPCO, Salem, NH, USA). The presence of glycosuria was defined by a positive Diastix™
(Bayer Corp. Diagnostics, Tarrytown, NY, USA) test (>14 mmol/L) on two consecutive
mornings.

Intraperitoneal glucose tolerance test
An intraperitoneal glucose tolerance test (IPGTT) was performed 4 weeks after the first
nephrectomy (Day 28). After an overnight fast, rats were injected with an intraperitoneal bolus
of D-glucose (BDH, Westchester, PA) (2 g/kg of body weight for SD rats [25, 26] and 1.5 g/kg
for Zucker rats [26, 27]). Blood samples (200 μl) were collected from the saphenous vein imme-
diately before and 15, 30, 60, 90, and 120 minutes after glucose administration. Great care was
taken to minimize stress in animals during blood retrieval. Glycemia was measured using an
AlphaTRAK blood glucose monitoring system. Serum insulin levels were determined at each
time point as described above, according to manufacturer’s protocol.

Ex vivo glucose uptake in muscles
The glucose uptake capacity of two skeletal muscles, soleus (predominantly slow-twitch mus-
cle, myosin heavy chain isoform (MHC) Type I) and epitrochlearis (predominantly fast-twitch
muscle, MHC Type IIb) [28] were determined using a previously published method [29].
Briefly, after a 4 hours fast, rats were anesthetized with an intraperitoneal injection of sodium
pentobarbital (60 mg/kg). After 10–15 minutes, the epitrochlearis and soleus muscles were
carefully removed and rapidly rinsed in PBS. Soleus muscles were delicately cut into two longi-
tudinal strips to ensure equal diffusion across the muscle and reduce potential hypoxia. No
significant differences were observed between CKD and CTL muscle weights, in both rat strains (Data not shown). Muscles were then pre-incubated in a Krebs-Henseleit buffer (KHB) solution containing 2mM pyruvate and 6mM Mannitol under constant oxygenation (95% O\textsubscript{2} - 5% CO\textsubscript{2}) at 35°C. After a 30 min pre-incubation period, muscles were incubated 20 min in KHB containing 1mM of [\textsuperscript{3}H]-2-deoxy-D-glucose (2-DG) (American Radiolabeled Chemicals, St. Louis, MO), 9mM [\textsuperscript{14}C]-mannitol (American Radiolabeled Chemicals) and 0 or 2mU/ml of insulin (Humulin R, Eli Lilly, Toronto, ON) for SD rats and 0 or 10mU/ml of insulin for Zucker rats. After incubation, muscles were snap frozen in liquid nitrogen. Frozen muscles were weighed, transferred to prechilled glass tissue grinder tubes (Kontes, Vineland, NJ), and homogenized in ice-cold perchloric acid using a glass pestle attached to a motorized homogenizer. 200μl aliquots of supernatants were mixed with 8mL scintillation cocktail (Fisher Scientific, Ottawa, Canada) and used for scintillation counting on a Hidex 300 SL beta counter (Hidex Oy, Turko, Finland) [30].

**Tissue preparation for Western blot analysis**

Frozen biopsies of rat liver, kidney, skeletal muscle and fat tissue were homogenized using an overhead stirrer in RIPA buffer (150mM sodium chloride, 1% Triton X-100, 0.5% sodium deoxycholate, 0.1% SDS, 50 mM Tris, pH 8) containing 0.1 mM PMSF at a ratio of 0.2g of tissue per 1mL buffer. Delipidation was performed on adipose tissue homogenate by chloroform extraction [31]. Protein content was determined using the Lowry method [32], using bovine serum albumin as reference protein.

**mRNA expression**

Total RNA was extracted from frozen tissue using TRIzol reagent (Invitrogen, Burlington, ON, Canada). RNA from the aqueous fraction was then purified using the RNeasy Mini kit (Qiagen) according to the manufacturer’s protocol including the optional DNA digestion step. One microgram of total RNA was used to prepare cDNA by reverse transcription using the SuperScript VILO cDNA Synthesis kit (Invitrogen). mRNAs encoding for GLUT1 (Slc2a1), GLUT2 (Slc2a2), GLUT4 (Slc2a4), SGLT1 (Slc5a1), SGLT2 (Slc5a2), GAPDH, β-actin and Villin-1 were measured by quantitative real-time polymerase chain reaction using appropriate primers on an ABI 7500 Real-Time PCR System (Applied Biosystems, Foster city, CA). Taqman Gene Expression Assays used for the quantification of mRNA for each transporter can be found in Table 1. PCR products were analyzed using the \( \Delta \Delta CT \) method [33] using GAPDH, β-actin and Villin-1 as housekeeping genes as described above.

**Western blotting**

Glucose transporter protein levels were assessed by Western blot analysis. Samples of 5 or 20 μg of protein were separated by electrophoresis on a 9% polyacrylamide gel containing 0.1% sodium dodecyl sulfate (SDS) and were electrophoretically transferred onto Polyvinylidene fluoride (PVDF) membrane. Membranes were saturated with 5% BSA in Tris buffered saline (TBS) containing 0.1% Tween20 (TBST) and washed with TBST. GLUT1 was detected using antibody PA1-1063 (Thermo Scientific Pierce, Rockford, IL), GLUT2 using antibody ARP41706 (Aviva Systems Biology, San Diego, CA), GLUT4 using antibody PA1-1065 (Thermo Scientific Pierce), SGLT1 using antibody Ab-14686 (Abcam, Cambridge, MA), and SGLT2 using antibody AP00335PU-N (Acris Antibodies, San Diego, CA). GAPDH was used as a GLUT4 containing-tissue loading control using antibody Ab9485 (Abcam). β-Actin was used as a loading control for liver samples using antibody GTX23280 (GeneTex, Irvine, CA). Villin-1 was used as a loading control for kidney tissue using antibody R814 (Cell Signaling...
Technology, Danvers, MA). Antibodies specificities were confirmed with blocking peptides. Specific antibody binding was revealed using appropriate secondary antibodies (Goat anti-rabbit IgG or Goat anti-mouse IgG from Sigma-Aldrich, St. Louis, MO) coupled to peroxidase and with the Clarity Western ECL Substrate from Bio-Rad Laboratories (Hercules, CA). Scanned images are provided in S2 Fig. Results were analyzed by computer-assisted densitometry using ImageQuant LAS-4000 system from GE Healthcare Life Sciences (Mississauga, ON) and FUJIFILM MultiGauge V3.0.

Statistical analysis
Results are expressed as mean ± S.D.. SD rats were analyzed as a cohort using the Student’s t test. Each Zucker CKD rat was analyzed against its own control using a ratio paired Student’s t test. Survival curves were assessed using the Mantel-Cox test. The threshold of significance was \( p \leq 0.05 \). Statistical analyzes were performed with GraphPad Prism v6.05 (La Jolla, California, USA), except for Fig 1, in which non-parametric Spearman correlation analyzes were done with the use of SAS software, version 9.4 (SAS Institute, Cary, NC).

Results
Biochemical parameters and body weight of SD rats
As compared to controls, CKD rats had higher levels of serum creatinine and urea and lower creatinine clearance (Table 2). Although glucose levels were not statistically elevated in CKD animals, we nevertheless observed a hyperglycemic state. Indeed, fructosamine levels were 25% higher (\( p < 0.0001 \)) in CKD rats compared to controls. Furthermore, a direct correlation between serum fructosamine and serum creatinine (\( r_{\text{Spearman}} = 0.87, p < 0.0001 \)) was observed (Fig 1A). No significant changes in insulinemia were observed.

Intraperitoneal glucose tolerance test on SD rats
A glucose tolerance test (GTT) performed on SD rats at 4 weeks post nephrectomy (Day 28) resulted in impaired carbohydrate metabolism in the nephrectomised cohort, as indicated by higher glycemc levels at each time-point studied, in comparison to control rats (Fig 2A). Glucose-stimulated insulin secretion (GSIS) was also impaired in nephrectomised rats, as demonstrated by a 30% greater insulin AUC over a 2 hours period, compared to control rats (Fig 2B).

mRNA expression of SGLT and GLUT glucose transporters in SD rats
Analysis of mRNA encoding glucose transporters involved in carbohydrate homeostasis in key organs is presented in Fig 3. There was a significant decrease in the expression of GLUT1 and...
GLUT2 (decrease of 31% and 32%, $p<0.001$ and $p<0.0001$ respectively) in CKD rat livers as compared to controls. In addition, renal impairment induced an increase in kidney GLUT2 expression (increase of 105%, $p<0.0001$) compared to control rats. However, mRNA expression of GLUT1, SLGT1 and SGLT2 in the kidney remained stable. Both GLUT1 and GLUT4 mRNA expression in muscle were decreased by CKD (decrease of 31% and 29%, $p<0.05$ and

---

**Fig 1. Correlation between serum fructosamine and creatinine in CTL and CKD rats.** Fructosamine concentration expressed as a ratio of fructosamine reported on total protein content in serum, versus creatininemia of (A) $n=26$ SD rats and (B) $n=37$ Zucker Lepr$^{fa/ fa}$ rats. Measurements at Day 21.

https://doi.org/10.1371/journal.pone.0176650.g001
Table 2. Biochemical parameters and body weight of CTL and CKD SD rats at Day 21.

|                          | CTL (n = 17) | CKD (n = 25) | p value |
|--------------------------|-------------|-------------|---------|
| Body weight (g)          | 321.1 ± 10.6| 300.7 ± 28.6| < 0.01  |
| Creatininemia (μmol/L)   | 40.1 ± 3.8  | 152.6 ± 66.2| < 0.0001|
| Uremia (mmol/L)          | 3.2 ± 0.8   | 23.0 ± 11.4 | < 0.0001|
| Creatinine clearance (μl/100g b.wt./min) | 527.8 ± 96.9 | 111.8 ± 50.7 | < 0.0001 |
| Glycemia (mmol/L)        | 6.0 ± 1.0   | 6.5 ± 1.2   | N.S.    |
| Insulinemia (ng/mL)      | 1.05 ± 0.51 | 0.91 ± 0.54 | N.S.    |
| Fructosamine (μmol/g of protein) | 2.09 ± 0.09 | 2.58 ± 0.16 | < 0.0001 |

https://doi.org/10.1371/journal.pone.0176650.t002

**Fig 2.** Glucose and insulin responses to an intraperitoneal glucose tolerance test. (A) Glucose and (B) Insulin responses to an intraperitoneal glucose tolerance test (2g/kg) performed on SD rats at Day 28 following a 16 hours fast. (C) Glucose and (D) Insulin responses to an intraperitoneal glucose tolerance test (1.5g/kg) performed on Zucker Lepr<sup>b/f</sup> rats at Day 28 following a 16 hour fast. Values are mean ± S.D. n = 10 for each group. *, p<0.05; **, p<0.01; ***, p<0.001; ****, p<0.0001 as compared to CTL rats. Area under the curve is presented as percentage of controls, on the right corner of each graph.

https://doi.org/10.1371/journal.pone.0176650.g002
Fig 3. Glucose transporters mRNA expression in selected organs of CKD rats. mRNA encoding glucose transporters in CTL and CKD rats in the indicated tissues were measured by quantitative Real-Time PCR. mRNA levels are expressed in relative quantities and calculated using the ΔΔCT method [33] with their respective housekeeping gene (Villin-1 for kidneys, β-actin for liver and GAPDH for muscles and adipose tissues). Data was normalized to the mean relative quantity of each gene in CTL rats. The graph shows the mean expression in CKD rats expressed as a percentage of controls ± S.D. of at least 10 rats in each group.

(A) Glucose transporters mRNA expression in the kidney and liver of SD rats.

(B) GLUT1 and GLUT4 mRNA expression in skeletal muscle tissues.

(C) GLUT1 and GLUT4 mRNA expression in adipose tissue.

* p<0.05; ** p<0.01; *** p<0.001; **** p<0.0001 as compared to CTL rats. (A) Glucose transporters mRNA expression in the kidney and liver of SD rats. (B) GLUT1 and GLUT4 mRNA expression in skeletal muscle tissues. (C) GLUT1 and GLUT4 mRNA expression in adipose tissue.
GLUT1 and GLUT4 mRNA expression levels in the adipose tissue were increased (increase of 89% and 42%, \( p < 0.01 \) and \( p < 0.05 \) respectively).

### Protein levels of GLUT and SGLT glucose transporters in SD rats

Fig 4 presents the relative protein levels of the principal glucose transporters in various tissues involved in carbohydrate metabolism. Renal impairment caused a down-regulation of hepatic GLUT1 and GLUT2 (decrease of 24% and 54%, \( p < 0.01 \) and \( p < 0.0001 \) respectively) as compared to control rats. Conversely, kidney GLUT2 and SGLT2 levels were increased in the presence of CKD (increase of 49% and 73%, \( p < 0.01 \) and \( p < 0.01 \) respectively). Kidney levels of

---

**Fig 4. Glucose transporters protein expression in selected organs of CKD rats.** Protein levels are expressed in densitometry units. The densitometry units measured for glucose transporters were normalized to their respective loading control (\( \beta \)-actin: liver; GAPDH: muscles and adipose tissues; villin-1: kidneys) values. The normalized densitometry units of control rats were arbitrarily defined as 100%. The graph shows the mean expression in CKD rats expressed as percentage of CTL ± S.D of at least 5 rats in each group. *, \( p < 0.05 \); **, \( p < 0.01 \); ***, \( p < 0.001 \); ****, \( p < 0.0001 \) as compared to CTL rats. Protein levels of glucose transporters in the (A) Kidneys and liver of SD rats, (B) Skeletal muscle and white adipose tissue of SD rats, (C) Skeletal muscle and white adipose tissue in Zucker Lepr \( ^{fa/fa} \) rats, (D) Representative Western blots for each glucose transporter. Each blot contains examples of three CKD (left) and three CTL (right) SD rats. Measurements at Day 42.

https://doi.org/10.1371/journal.pone.0176650.g004
GLUT1 and SGLT1 remained unchanged. In muscle, both GLUT1 and GLUT4 were considerably diminished (decrease of 27% and 31%, p<0.05 and p<0.01 respectively) by kidney disease, and GLUT1 and GLUT4 were inversely affected by CKD in adipose tissue as compared to the muscle (increase of 27% and 106%, N.S. and p<0.01 respectively).

**Ex Vivo** uptake of radiolabeled 2-deoxyglucose in the *epitrochlearis* and the *soleus* muscles of SD rats

Analysis of insulin stimulated glucose uptake (measured by fold increase in glucose uptake) revealed a lower glucose uptake in the CKD cohort for both *epitrochlearis* (decrease of 34% vs controls, p<0.0001) and *soleus* (decrease of 39% vs controls, p<0.0001) muscles (Fig 5A).

---

Fig 5. **Ex vivo** accumulation of radio-labeled 2-deoxyglucose in muscles. Uptake of radio-labeled 2-deoxyglucose in CTL and CKD rat muscles expressed as insulin response vs. basal condition. Basal condition of each muscle was arbitrarily defined as 1.0. Data are expressed as fold increase (mean) ± S.D for at least 5 rats per group. ***, p<0.001; ****, p<0.0001 as compared to CTL rats. (A) Sprague-Dawley rats and (B) Zucker Lep^fa/la^ rats. Measurements at Day 42.

https://doi.org/10.1371/journal.pone.0176650.g005
Interestingly, as shown in supporting information S3A Fig, we observe that insulin induced a greater glucose uptake in the soleus than in the epitrochlearis. These findings are in accordance with the results of Henriksen et al. [34] pointing out the importance of fiber type selective differences in glucose uptake [35].

**Insulin resistance in Zucker rats is affected by CKD**

Similar to SD rats, CKD Zucker rats showed an increase in serum fructosamine that correlated with serum creatinine levels (Fig 1B) \((r_{spearman} = 0.78, p < 0.0001)\). Unlike SD rats, Zucker rats with CKD had an elevated fasting glycemia (increase of 50% vs controls, \(p < 0.0001\)) without higher insulin levels (Table 3).

Insulin resistant Zucker rats showed an even greater response to glucose load under CKD conditions (Fig 2C and 2D) as compared to SD rats, and demonstrated a similar impairment in insulin levels. In addition, changes in glucose transporter mRNA expression (Fig 3C) in CTL and CKD Zucker rats were similar to that observed in SD rats in the muscle (GLUT1 decrease of 26%, \(p < 0.05\); GLUT4 decrease of 25%, \(p < 0.01\)) and adipose tissue (GLUT1 increase of 70%, \(p < 0.05\); GLUT4 increase of 23%, N.S.). This was also true of glucose transporter protein levels of Zucker CTL versus CKD rats (Fig 4C) with a reduction of glucose transporters in muscles (GLUT1 decrease of 41%, \(p < 0.01\); GLUT4 decrease of 39%, \(p < 0.01\)) and higher protein levels in adipose tissues (GLUT1 increase of 56%, \(p < 0.05\); GLUT4 increase of 47%, N.S.).

Differences in insulin stimulated glucose uptake in muscles from Zucker CTL and CKD rats were also observed. Since 2mU/mL of insulin did not significantly increase glucose uptake by muscles from Zucker rats with CKD (data not shown), a higher dose of insulin (10mU/mL) was necessary to induce insulin-stimulated glucose uptake in the muscles of these rats (Fig 5B). As compared to controls, Zucker rats affected by CKD had a reduced insulin stimulated glucose uptake in both epitrochlearis (decrease of 22% vs controls, \(p > 0.0001\)) and soleus (decrease of 20% vs controls, \(p > 0.0001\)) muscles. As observed in supporting information S3B Fig, in Zucker rats, insulin induced a greater glucose uptake in the soleus than in the epitrochlearis. This difference was slightly less than in SD rats.

**Presence of glucose in urine (Glycosuria)**

The presence of glycosuria is due to the inability of the kidney to reabsorb filtered glucose or spillage due to abnormally high levels of blood glucose. Spillage does not generally occur until blood glucose exceeds \(~10\text{mmol/L}\) [36]. None of the SD rats had detectable urine glucose levels. In contrast, \~30% of the Zucker rats with severe CKD (creatininemia \(\geq 200\mu\text{mol/L}\)) presented glycosuria whereas none in the control Zucker rats had detectable urine glucose (Fig 6).

### Table 3. Biochemical parameters and body weight of CTL and CKD Zucker Leprfa/faq rats (Day 21).

| Parameter                          | CTL (\(n = 19\)) | CKD (\(n = 19\)) | \(p\) value |
|------------------------------------|------------------|------------------|-------------|
| Body weight (g)                    | 345.6 ± 13.3     | 340.8 ± 13.4     | N.S.        |
| Creatininemia (\(\mu\text{mol/L}\)) | 51.8 ± 1.7       | 136.2 ± 23.8     | \(< 0.0001\) |
| Uremia (mmol/L)                    | 5.5 ± 0.3        | 29.6 ± 5.4       | \(< 0.0001\) |
| Creatinine clearance (\(\mu\text{l}/100g b.wt./min\)) | 146.3 ± 7.1     | 83.2 ± 9.2       | \(< 0.01\)  |
| Glycemia (mmol/L)                  | 6.0 ± 0.2        | 9.1 ± 0.6        | \(< 0.0001\) |
| Insulinemia (ng/mL)               | 5.76 ± 0.92      | 6.65 ± 0.85      | N.S.        |
| Fructosamine (\(\mu\text{mol/g of protein}\)) | 1.85 ± 0.02     | 2.20 ± 0.08      | \(< 0.0001\) |

https://doi.org/10.1371/journal.pone.0176650.t003
Discussion

Despite extensive study of glucose transporters, there is little and conflicting information regarding their role in CKD. Our results suggest that in a 5/6th nephrectomised Sprague-Dawley rat model, there is a correlation between the severity of chronic kidney disease and hyperglycemia as evaluated by serum fructosamine levels. Glucose tolerance tests also indicate an increase of 25% in glycemia in chronic kidney disease rats as compared to controls, whereas insulin levels remained unchanged. We also observed modulation of glucose transporters expression in several tissues such as the liver and muscles. There was a significant reduction in insulin-dependent glucose uptake in the muscles of chronic kidney disease rats but the development of type 2 diabetes was not observed. Furthermore, in a rat model of metabolic syndrome (Zucker Lepr^fa/fa), chronic kidney disease caused a significant increase in fasting glucose and an exacerbated glycemic response during glucose challenge. Similar modulations of glucose transporters expression and glucose uptake were observed in the two models. However, 30% of chronic kidney disease Zucker rats developed characteristics of type 2 diabetes.

In the present study, we provided evidence that CKD rats have chronic hyperglycemia as indicated by a significant increase in serum fructosamine, although fasting glycemia was not significantly elevated. To further characterize the insulin sensitivity and secretion in our models of CKD, we performed glucose tolerance tests (GTT). Our results confirmed impaired glucose tolerance in the nephrectomised cohort as revealed by their inability to restore basal blood glucose levels (Fig 2A) despite increased insulin levels (Fig 2B). The increased insulin concentration was insufficient to counteract hyperglycemia as higher blood glucose levels were measured in rats with renal impairment, thus providing evidence of an insulin resistance evolution.

Fig 6. Time of glycosuria apparition in Zucker Lepr^fa/fa rats. The graph shows the proportion of rats with glycosuria as confirmed by a positive Diastix™ (Bayer) test on two consecutive days for 15 Zucker Lepr^fafa rats per group.

https://doi.org/10.1371/journal.pone.0176650.g006
Having established that CKD may lead to insulin resistance, the expression of glucose transporters was measured in several key organs involved in glucose metabolism, in order to determine the mechanisms of this metabolic condition when kidney function is compromised. We primarily focused on fat and muscle glucose transporters, as impaired insulin sensitivity in CKD is generally ascribed in peripheral tissues [37, 38]. Furthermore, we focused on GLUT4, as any abnormality affecting its expression, its translocation and/or its activity has been strongly linked to insulin resistance [39, 40]. Interestingly, our results show a significant decrease in GLUT4 protein levels in skeletal muscle from rats with impaired renal function (Fig 4).

Glucose uptake studies were performed on skeletal muscle since this tissue represents the main site of insulin resistance in CKD [37, 41]. As skeletal muscle glucose transport can be stimulated by both insulin and contractile activity [34], we used sedentary rats to measure the glucose transport mediated principally by insulin. Experiments were done ex vivo to avoid any extra-muscular hormonal and metabolic factors that may influence glucose uptake [42, 43]. Our results demonstrated a clear reduction of insulin-stimulated glucose transport in CKD muscles (Fig 5) which is likely attributable, at least in part, by the down-regulation of muscle GLUT4 glucose transporter expression in kidney failure. Friedman and colleagues have already reported lower glucose uptake by skeletal muscle preparations obtained from uremic patients while the expression of GLUT4 remained unchanged [17], whereas Aksentijevic and colleagues noted diminished GLUT4 protein expression in the skeletal muscles of uremic animals [21]. Furthermore, insulin acts on microvascular perfusion to promote glucose uptake in peripheral tissues. The impact of kidney disease on insulin-mediated microvascular responses has not been studied and may contribute to a reduction in glucose uptake in the muscles under in vivo conditions [44, 45]. Thus, the down-regulation of GLUT4 expression in skeletal muscle of nephrectomised rats may explain the insulin resistance in kidney disease. That said, other organs are also involved in carbohydrate metabolism and may contribute to the insulin resistance.

The presence of defective hepatic glucose metabolism in CKD is often disregarded, and no study has focused on the modulation of liver glucose transporters in CKD. Notably, our results demonstrate a substantial decrease in the protein levels of GLUT1 and GLUT2 (Fig 4A). As the liver extracts approximately 1/3 of oral glucose loads, a decrease of glucose transporters in this tissue could have major repercussions on blood glucose regulation [46]. Basu et al. have suggested that impaired glucose uptake in the liver and peripheral tissues could contribute to hyperglycemia in people with T2D [47]. However, few studies have suggested impaired hepatic glucose disposal and production as a cause of insulin resistance in CKD. According to Schmitz et al., uremia seems to clearly impair glucose-induced glucose uptake by the liver [48].

The kidneys play an important role in glucose homeostasis as they ensure its reabsorption through glucose transporters. The modulation of glucose transporters in renal impairment has rarely been mentioned in the literature. Our data suggest upregulation of kidney GLUT2 and SGLT2 levels in CKD (Fig 4A). Since GLUT2 and SGLT2 transporters are responsible for ~90% of glucose reabsorption, this implies that the kidneys try to reabsorb more glucose from the glomerular filtrate. In a rodent model of T2D with long-term hyperglycemia, kidney GLUT2 and SGLT2 levels are increased. Thus, it seems plausible that dysregulation of GLUT2 and SGLT2 could contribute to the hyperglycemia in CKD [49].

We have not observed any case of T2D among SD rats with kidney disease. Since CKD commonly arises alongside other metabolic imbalances such as metabolic syndrome, kidney disease could worsen glucose homeostasis in these rats. The Zucker Lepr<sup>fa/fa</sup> is a rat model affected by insulin resistance and metabolic syndrome. Inducing kidney disease in Zucker rats exacerbated the hyperglycemia state (Fig 1B) and insulin resistance (Fig 2B and 2D) as compared to SD rats. The modulation of glucose transporters were similar (Figs 3C and 4C) to...
those observed in SD rats, but the reduction in glucose uptake by the muscle was more severe (Fig 5B). Kidney disease and metabolic syndrome in Zucker rats acts synergistically on glucose homeostasis and reduced insulin sensitivity. Moreover, glycosuria appeared in severely affected CKD Zucker rats with a fasting glycemia near 11mmol/L. Glycemia rose to a critical point at which the kidneys can no longer entirely reabsorb the filtered glucose. Thus, these rats displayed a chronic hyperglycemic state, which can be associated with T2D as insulinemia did not rise to normalize blood glucose [50].

Some limitations of this study should be mentioned. First, although we measured circulating insulin levels, the insulin secretion of CKD rats was not directly measured in vitro. Indeed, Koppe et al. [10] recently showed that urea caused impaired insulin secretion in pancreatic β-cells. Whether this mechanism could partly explain our results remains to be confirmed. Second, since reduced translocation to the plasma membrane can only be presumed from total GLUT4 protein levels, this issue will have to be further investigated. Third, it remains to be demonstrated if our results apply to CKD patients. A recent study by de Boer et al. [51] has showed that in moderate CKD patients (mean eGRF 37 mL/min per 1.73 m²), 65% of them have impaired glucose tolerance and there was no association of kidney function with insulin resistance parameters. Others parameters such as lifestyles factors or body composition have been proposed to partially explain the insulin resistance in these patients. However, in the current study, these factors have been controlled. Furthermore, it does not exclude the hypothesis that as renal failure worsens (in our CKD rats, the GFR was reduced by 80%, which means stage 4 and 5 in humans), CKD plays a direct role in insulin resistance. More importantly, our results emphasize that in predialysis patients, insulin resistance, and possibly T2D development, should be followed more closely.

In conclusion, GTT experiments showed an altered glucose transporter expression that could be associated with an impairment of glucose metabolism in CKD. Indeed, a significant reduction of GLUT4 in skeletal muscle was likely important for the measured insulin resistance. This modulation may also explain the reduced glucose uptake by muscles reported in CKD. Moreover, the increased protein expression of the majority of kidney glucose transporters in CKD may allow greater glucose reabsorption while hyperglycemia further contributes to the exacerbation of insulin resistance. Reduced levels of hepatic GLUT1 and GLUT2 proteins may reflect dysregulated glucose metabolism by the liver in CKD and may suggest the presence of hepatic glucose intolerance that would subsequently contribute to the impaired glucose metabolism in CKD. Thus, the insulin resistance seen in CKD could be mediated, in part, by the modulation of the expression of various key transporters involved in glucose homeostasis. However, CKD by itself does not lead to T2D. Rather, CKD seems to worsen the insulin resistance in Zucker rats in which insulin resistance is already present before nephrectomy.

Supporting information
S1 Fig. Timeline of experimentation. (TIF)
S2 Fig. Western blots. (ZIP)
S3 Fig. Ex vivo accumulation of radio-labeled 2-deoxyglucose in muscles. Uptake of radio-labeled 2-deoxyglucose in CTL and CKD rat muscles. Data are expressed as 2-DG uptake (mean) ± S.D. for (A) 2 representative experiments in Sprague-Dawley rats and (B) 3 representative experiments in Zucker Lep<sup>fa/fa</sup> rats. (TIF)
Acknowledgments

François Dion and Christopher Dumayne contributed equally to this work. This work was presented in part at the 76th Scientific Sessions of the American Diabetes Association (June 10th to June 14th 2016). The authors would like to thank Naoual Elftouh and Jean-Philippe Lafrance for statistical analysis, Julie Dubeau and Marie-Laure Durand for skillful technical work, Marie-Ève Gingras for helpful discussions and Heather Melichar for revising the manuscript.

Author Contributions

Conceptualization: VP CD FD FAL.
Data curation: FD CD FAL.
Formal analysis: FD CD FAL.
Funding acquisition: VP FD FAL.
Investigation: FD CD NH FAL SB.
Methodology: VP FAL NH FD CD GDC EBA.
Project administration: VP FAL.
Resources: FAL.
Supervision: VP FAL GDC.
Validation: FD CD NH.
Visualization: FD CD.
Writing – original draft: CD FD.
Writing – review & editing: FD NH VP CD FAL GDC S. Lefrançois S. Lesage SB EBA.

References

1. Abboud H, Henrich WL. Clinical practice. Stage IV chronic kidney disease. N Engl J Med. 2010; 362 (1):56–65. https://doi.org/10.1056/NEJMcp0906797 PMID: 20054047
2. Slee AD. Exploring metabolic dysfunction in chronic kidney disease. Nutrition & metabolism. 2012; 9 (1):36.
3. Cibulka R, Racek J. Metabolic disorders in patients with chronic kidney failure. Physiol Res. 2007; 56 (6):697–705. PMID: 17298212
4. KDOQI. KDOQI Clinical Practice Guidelines and Clinical Practice Recommendations for Diabetes and Chronic Kidney Disease. Am J Kidney Dis. 2007; 49(2 Suppl 2):S12–154. https://doi.org/10.1053/ajkd.2006.12.005 PMID: 17276798
5. Fox CS, Golden SH, Anderson C, Bray GA, Burke LE, de Boer IH, et al. Update on Prevention of Cardiovascular Disease in Adults With Type 2 Diabetes Mellitus in Light of Recent Evidence: A Scientific Statement From the American Heart Association and the American Diabetes Association. Circulation. 2015; 132(8):691–718. https://doi.org/10.1161/CIR.0000000000000230 PMID: 26246173
6. Niewczas MA, Sirich TL, Mathew AV, Skupien J, Mohney RP, Warram JH, et al. Uremic solutes and risk of end-stage renal disease in type 2 diabetes: metabolomic study. Kidney Int. 2014; 85(5):1214–24. https://doi.org/10.1038/ki.2013.497 PMID: 24429397
7. Sit D, Kadiroglu AK, Kayabay H, Yilmaz ME. The prevalence of insulin resistance in nondiabetic nonobese patients with chronic kidney disease. Adv Ther. 2006; 23(6):988–98. PMID: 17276966
8. Shipman KE, Jawad M, Sullivan KM, Ford C, Gama R. Effect of chronic kidney disease on A1C in individuals being screened for diabetes. Primary care diabetes. 2015; 9(2):142–6. https://doi.org/10.1016/j.pcd.2014.05.001 PMID: 24893965
22. Jacobs DB, Hayes GR, Truglia JA, Lockwood DH. Alterations of glucose transporter systems in insulin-resistant uremic humans: glucose transport, glucose transporters, and insulin receptors. The American journal of physiology. 1991; 261(1 Pt 1):E87–94. PMID: 1858777

19. Naud J, Michaud J, Leblond FA, Lefrancois S, Bonnardeaux A, Pichette V. Effects of chronic renal failure on liver drug transporters. Drug metabolism and disposition: the biological fate of chemicals. 2011; 39(8):1363–9.

11. Liao MT, Sung CC, Hung KC, Wu CC, Lo L, Lu KC. Insulin resistance in patients with chronic kidney disease. Journal of biomedicine & biotechnology. 2012; 2012:691369.

27. Sun H, Frassetto L, Benet LZ. Effects of renal failure on drug transport and metabolism. Pharmacology & therapeutics. 2006; 109(1–2):1–11.

20. Leblond F, Guevin C, Demeurs C, Pellerin I, Gascon-Barre M, Pichette V. Downregulation of hepatic cytochrome P450 in chronic renal failure. J Am Soc Nephrol. 2001; 12(2):326–32. PMID: 11158222

25. Castorena CM, Mackrell JG, Bogan JS, Kanzaki M, Cartee GD. Clustering of GLUT4, TUG, and RUVBL2 protein levels correlate with myosin heavy chain isoform pattern in skeletal muscles, but AS160 and TBC1D1 levels do not. Journal of applied physiology (Bethesda, Md: 1985). 2011; 111(4):1106–17.

28. Sharma N, Arias EB, Bhat AD, Sequa DA, Ho S, Croff KK, et al. Mechanisms for increased insulin-stimulated Akt phosphorylation and glucose uptake in fast- and slow-twitch skeletal muscles of calorie-restricted rats. American journal of physiology Endocrinology and metabolism. 2011; 300(6):E966–78. https://doi.org/10.1152/ajpendo.00659.2010 PMID: 21386065

9. Jia T, Riserus U, Xu H, Lindholm B, Arnlow J, Sjogren P, et al. Kidney function, beta-cell function and glucose tolerance in older men. J Clin Endocrinol Metab. 2015; 100(2):587–93. https://doi.org/10.1210/jc.2014-3313 PMID: 25429626

10. Koppe L, Nyam E, Vivot K, Manning Fox JE, Dai XQ, Nguyen BN, et al. Urea impairs beta cell glycolysis and insulin secretion in chronic kidney disease. The Journal of clinical investigation. 2016.

11. Liao MT, Sung CC, Hung KC, Wu CC, Lo L, Lu KC. Insulin resistance in patients with chronic kidney disease. Journal of biomedicine & biotechnology. 2012; 2012:691369.

12. Koppe L, Pelletier CC, Alix PM, Kalbacher E, Fouque D, Soulage CO, et al. Insulin resistance in chronic kidney disease: new lessons from experimental models. Nephrol Dial Transplant. 2014; 29(9):1666–74. https://doi.org/10.1093/ndt/gft435 PMID: 24286973

13. Massry SG. Sequence of cellular events in pancreatic islets leading to impaired insulin secretion in chronic kidney disease. J Ren Nutr. 2011; 21(1):92–9. https://doi.org/10.1053/j.jrn.2010.11.001 PMID: 21195928

14. Hung AM, Ikizler TA. Factors determining insulin resistance in chronic hemodialysis patients. Contributions to nephrology, 2011; 171:127–34. https://doi.org/10.1159/000327177 PMID: 21625101

15. Sandsmark DK, Messe SR, Zhang X, Roy J, Nessel L, Lee Hamm L, et al. Proteinuria, but Not eGFR, Predicts Stroke Risk in Chronic Kidney Disease: Chronic Renal Insufficiency Cohort Study. Stroke; a journal of cerebral circulation. 2015; 46(8):2075–80.

16. Sud M, Tangri N, Pintilie M, Levey AS, Naimark D. Risk of end-stage renal disease and death after cardiovascular events in chronic kidney disease. Circulation. 2014; 130(6):458–65. https://doi.org/10.1161/CIRCULATIONAHA.113.007106 PMID: 24899688

17. Friedman JE, Dohm GL, Elton CW, Rovira A, Chen JJ, Leggett-Frazier N, et al. Muscle insulin resistance in uremic humans: glucose transport, glucose transporters, and insulin receptors. The American journal of physiology. 1991; 261(1 Pt 1):E87–94. PMID: 1858777

18. Naud J, Michaud J, Beauchemin S, Hebert MJ, Roger M, Lefrancois S, et al. Effects of chronic renal failure on kidney drug transporters and cytochrome P450 in rats: Drug metabolism and disposition: the biological fate of chemicals. 2011; 39(8):1363–9.

19. Naud J, Michaud J, Leblond FA, Lefrancois S, Bonnardeaux A, Pichette V. Effects of chronic renal failure on liver drug transporters. Drug metabolism and disposition: the biological fate of chemicals. 2008; 36(1):124–8.

20. Sun H, Frassetto L, Benet LZ. Effects of renal failure on drug transport and metabolism. Pharmacology & therapeutics. 2006; 109(1–2):1–11.

21. Aksemtijevic D, Bhandari S, Seymour AM. Insulin resistance and altered glucose transporter 4 expression in experimental uremia. Kidney Int. 2009; 75(7):711–8. https://doi.org/10.1038/ki.2008.691 PMID: 19177156

22. Jacobs DB, Hayes GR, Truglia JA, Lockwood DH. Alterations of glucose transporter systems in insulin-resistant uremic rats. The American journal of physiology. 1989; 257(2 Pt 1):E193–7. PMID: 2669514

23. Szolkiewicz M, Sucajtys E, Wołyniec W, Rukiowski P, Stelmanska E, Korczynska J, et al. Mechanisms of enhanced carbohydrate and lipid metabolism in adipose tissue in uremia. J Ren Nutr. 2005; 15(1):166–72. PMID: 15648024

24. Leblond F, Guevin C, Demers C, Pellerin I, Gascon-Barre M, Pichette V. Downregulation of hepatic cytochrome P450 in chronic renal failure. J Am Soc Nephrol. 2001; 12(2):326–32. PMID: 11158222

26. Bowe JE, Franklin ZJ, Hauge-Evans AC, King AJ, Persaud SJ, Jones PM. Metabolic phenotyping guidelines: assessing glucose homeostasis in rodent models. The Journal of endocrinology. 2014; 222(3):G13–25. https://doi.org/10.1530/JOE-14-0182 PMID: 25056117

27. George C, Lochner A, Huisamen B. The efficacy of Prosopis glandulosa as anti-diabetic treatment in rat models of diabetes and insulin resistance. Journal of ethnopharmacology. 2011; 137(1):298–304. https://doi.org/10.1016/j.jep.2011.05.023 PMID: 21645608

28. Castorena CM, Mackrell JG, Bogan JS, Kanzaki M, Cartee GD. Clustering of GLUT4, TUG, and RUVBL2 protein levels correlate with myosin heavy chain isoform pattern in skeletal muscles, but AS160 and TBC1D1 levels do not. Journal of applied physiology (Bethesda, Md: 1985). 2011; 111(4):1106–17.

29. Sharma N, Arias EB, Bhat AD, Sequa DA, Ho S, Croff KK, et al. Mechanisms for increased insulin-stimulated Akt phosphorylation and glucose uptake in fast- and slow-twitch skeletal muscles of calorie-restricted rats. American journal of physiology Endocrinology and metabolism. 2011; 300(6):E966–78. https://doi.org/10.1152/ajpendo.00659.2010 PMID: 21386065
30. Wang H, Sharma N, Arias EB, Cartee GD. Insulin Signaling and Glucose Uptake in the Soleus Muscle of 30-Month-Old Rats After Calorie Restriction With or Without Acute Exercise. The journals of gerontology Series A, Biological sciences and medical sciences. 2015.

31. Byrdwell WC, Sato H, Schwarz AK, Borchman D, Yappert MC, Tang D. 31P NMR quantification and monophasic solvent purification of human and bovine lens phospholipid. Lipids. 2002; 37(11):1087–92. PMID: 12558059

32. Lowry OH, Rosebrough NJ, Farr AL, Randall RJ. Protein measurement with the Folin phenol reagent. J Biol Chem. 1951; 193(1):265–75. PMID: 14907713

33. Livak KJ, Schmittgen TD. Analysis of relative gene expression data using real-time quantitative PCR and the 2(-Delta Delta C(T)) Method. Methods. 2001; 25(4):402–8. https://doi.org/10.1006/meth.2001.1262 PMID: 11846609

34. Henrikson EJ, Bourey RE, Rodnick KJ, Koranyi L, Permutt MA, Holloszy JO. Glucose transporter protein content and glucose transport capacity in rat skeletal muscles. The American journal of physiology. 1990; 259(4 Pt 1):E593–8. PMID: 1699426

35. Cartee GD, Arias EB, Yu CS, Pataky MW. Novel single skeletal muscle fiber analysis reveals a fiber type-selective effect of acute exercise on glucose uptake. American journal of physiology Endocrinology and metabolism. 2016; 311(5):E818–e24. https://doi.org/10.1152/ajpendo.00289.2016 PMID: 27600826

36. Marsenic O. Glucose control by the kidney: an emerging target in diabetes. Am J Kidney Dis. 2009; 53(5):875–83. https://doi.org/10.1053/j.ajkd.2008.12.031 PMID: 19324482

37. Shinohara K, Shoji T, Emoto M, Tahara H, Koyama H, Ishimura E, et al. Insulin resistance as an independent predictor of cardiovascular mortality in patients with end-stage renal disease. J Am Soc Nephrol. 2002; 13(7):1894–900. PMID: 12089386

38. DeFronzo RA, Alvestrand A, Smith D, Hendler R, Hendler E, Wahren J. Insulin resistance in uremia. Endocrinol Metab. 1982; 54(2):217–22. https://doi.org/10.1210/jcem-54-2-217 PMID: 7033272

39. Byrdwell WC, Sato H, Schwarz AK, Borchman D, Yappert MC, Tang D. 31P NMR quantification and monophasic solvent purification of human and bovine lens phospholipid. Lipids. 2002; 37(11):1087–92. PMID: 12558059

40. Nawano M, Oku A, Ueta K, Umebayashi I, Ishirahara T, Arakawa K, et al. Hyperglycemia contributes insulin resistance in hepatic and adipose tissue but not skeletal muscle of ZDF rats. American journal of physiology Endocrinology and metabolism. 2000; 278(3):E535–43. PMID: 10710509

41. Rutter MK, Meigs JB, Sullivan LM, D’Agostino RB Sr., Wilson PW. Insulin resistance, the metabolic syndrome, and incident cardiovascular events in the Framingham Offspring Study. Diabetes. 2005; 54(11):3252–7. PMID: 16249452

42. Rossetti L. Glucose toxicity: the implications of hyperglycemia in the pathophysiology of diabetes mellitus. Clinical and investigative medicine Medecine clinique et experimentale. 1995; 18(4):255–60. PMID: 8549010

43. Hidaka H, Nagulesparan M, Klimes I, Clark R, Sasaki H, Aronoff SL, et al. Improvement of insulin secretion but not insulin resistance after short term control of plasma glucose in obese type II diabetics. J Clin Endocrinol Metab. 1982; 54(2):217–22. https://doi.org/10.1210/jcem-54-2-217 PMID: 7033272

44. St-Pierre P, Genders AJ, Keske MA, Richards SM, Rattigan S. Loss of insulin-mediated microvascular perfusion in skeletal muscle is associated with the development of insulin resistance. Diabetes Obes Metab. 2010; 12(9):798–805. https://doi.org/10.1111/j.1463-1326.2010.01235.x PMID: 20649632

45. Youd JM, Rattigan S, Clark MG. Acute impairment of insulin-mediated capillary recruitment and glucose uptake in rat skeletal muscle in vivo by TNF-alpha. Diabetes. 2000; 49(11):1904–9. PMID: 11078458

46. Moore MC, Coate KC, Winnick JJ, An Z, Cherrington AD. Regulation of hepatic glucose uptake and storage in vivo. Advances in nutrition (Bethesda, Md). 2012; (3)(3):286–94.

47. Basu A, Basu R, Shah P, Vella A, Johnson CM, Nair KS, et al. Effects of type 2 diabetes on the ability of insulin and glucose to regulate splanchnic and muscle glucose metabolism: evidence for a defect in hepatic glucokinase activity. Diabetes. 2000; 49(2):272–83. PMID: 10868944

48. Schmitz O. Glucose metabolism in non-diabetic and insulin-dependent diabetic subjects with end-stage renal failure. Danish medical bulletin. 1991; 38(1):36–52. PMID: 20260051

49. Calado J, Santer R, Rueff J. Effect of kidney disease on glucose handling (including genetic defects). Kidney Int Suppl. 2011(120):S7–13. PMID: 21358700

50. Abdul-Ghani MA. Type 2 diabetes and the evolving paradigm in glucose regulation. The American journal of managed care. 2013; 19(3 Suppl):S43–50. PMID: 23448119

51. de Boer IH, Zelinski L, Afkarian M, Ayers E, Curtin L, Himelfarb J, et al. Impaired Glucose and Insulin Homeostasis in Moderate-Severe CKD. J Am Soc Nephrol. 2016; 27(9):2861–71. https://doi.org/10.1681/ASN.2015070756 PMID: 26823551