TITLE PAGE

PREGNANE X RECEPTOR AGONISTS IMPAIR POSTPRANDIAL GLUCOSE TOLERANCE

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References 44

Figures 4

Tables 3

Key words: Diabetes, glucose, glucose transporter 2, insulin, oral glucose tolerance test, pregnane X receptor, pregnenolone 16α-carbonitrile, rifampin.

ABSTRACT

We conducted a randomized, open, placebo-controlled crossover trial to investigate the effects of pregnane X receptor (PXR) agonist rifampin on oral glucose tolerance test (OGTT) in 12 healthy volunteers. The subjects were administered 600 mg rifampin or placebo once daily for seven days and OGTT was performed on the eighth day. The mean incremental glucose and insulin areas under the plasma concentration–time curves (AUC_{incr}) increased by 192% ($P = 0.008$) and 45% ($P = 0.031$), respectively. The fasting glucose, insulin and C-peptide and the homeostasis model assessment for insulin resistance were not affected. The glucose AUC_{incr} during OGTT was significantly increased in rats after 4-day treatment with pregnenolone 16α-carbonitrile (PCN), an agonist of the rat PXR. The hepatic level of glucose transporter 2 (Glut2) mRNA was down-regulated by PCN. In conclusion, both human and rat PXR agonists elicited postprandial hyperglycemia suggesting detrimental role of PXR activation on glucose tolerance.

INTRODUCTION

Pregnane X receptor (PXR; NR1I2), mainly expressed in the human liver and intestine, is a ligand activated transcription factor belonging to the nuclear hormone receptor superfamily.(1) PXR is one of the main regulators of drug metabolism as it induces the expression of many major drug-metabolizing enzymes including cytochrome P450 (CYP) enzymes such as CYP2B6, CYP2C8, CYP2C9, CYP2C19, CYP3A4,(2) and drug-transporters such as P-glycoprotein (encoded by *ABCB1*).(3) Furthermore, PXR is of importance as a regulator of bile acid metabolism inducing bile acid-catabolizing CYP3A4 enzyme and the biliary efflux transporter MRP2 (multidrug resistance associated protein 2; encoded by *ABCC2*), and suppressing bile acid-synthesizing CYP7A1 enzyme, with bile acids acting as endogenous PXR agonists.(3)

In addition to these well-established functions, both *in vitro* observations and studies with experimental animals have shown that PXR activation affects glucose metabolism.(4) PXR was shown to crosstalk with various hormone responsive transcription factors such as forkhead box O1 (FOXO1), cAMP-responsive element binding protein (CREB), and peroxisome proliferator-activated receptor γ coactivator 1α (PGC1α) leading to down-regulation of gluconeogenesis and glycogenolysis. PXR agonist pregnenolone 16α-carbonitrile (PCN) treatment decreased serum glucose levels in fasting $Pxr^{+/+}$ but not in $Pxr^{-/-}$ mice.(5) Also, PCN treatment decreased blood glucose levels during intraperitoneal glucose tolerance test and the homeostasis model assessment for insulin resistance (HOMA-IR), an index of fasting insulin resistance, in mice fed with high-fat diet but did not affect glucose levels in mice fed with normal chow.(6) Thus, previous rodent studies suggest that PXR activation leads to lower plasma fasting glucose and amelioration of insulin resistance.

There are only few clinical studies on the effects of PXR agonism on glucose homeostasis; antiepileptic phenobarbital, which activates both constitutive androstane receptor (CAR) and PXR,(7) lowers plasma fasting glucose and increases insulin sensitivity in type II diabetics and lowers fasting insulin in healthy volunteers.(8-9) Rifampin, a tuberculosis antibiotic, is widely used in experimental and clinical studies as a PXR agonist due to its efficient and rapid inducing capacity and good specificity for PXR.(10) In patients with tuberculosis, rifampin was shown to increase glucose and insulin levels as studied with the oral glucose tolerance test (OGTT) during the rifampin treatment in comparison to the same tuberculosis patients before and after rifampin treatment, a separate group of tuberculosis patients with other antituberculous drugs without rifampin, and healthy control subjects.(11) Rifampin-containing regimen did not affect intravenous glucose tolerance when compared to the same tuberculosis subjects without rifampin medication and healthy controls.(11) However, in two other studies with tuberculosis patients,(12-13) rifampin was not associated with statistically significant changes in glucose levels during OGTT performed 7 and 30 days after the start of a rifampin-containing drug regimen when compared to patients on drug-regimen without rifampin. Fasting glucose was reported to be lower in tuberculosis patients treated with the rifampin-containing drug regimen in one study when compared to ciprofloxacin-based regimen,(14) whereas in other studies fasting glucose was not affected by rifampin when compared to antituberculous drug-regimen without rifampin.(11-13)

Oral dosing of rifampin reduces glucose-lowering effects of oral hypoglycemic agents glyburide, gliclazide and repaglinide, but enhances glucose-lowering effects of metformin.(15-19) A single intravenous dose of rifampin increased the AUC of glyburide and lowered glucose levels.(20) These findings are presumably explained by rifampin-elicited changes in the pharmacokinetics of the oral antidiabetic drugs as rifampin is not only an inducer of several CYPs (2) and transporters but also an inhibitor of organic anion-transporting polypeptide 1B1 (OATP1B1; encoded by *SLCO1B1*).(21-

22) We set out to elucidate the effects of PXR activation on human and rat glucose homeostasis without the interfering effects of diseases and other medications. No previous study has reported the effects of PCN and rifampin, the prototypical rat and human PXR agonists, on OGTT in rodents or healthy volunteers.

RESULTS

Twelve healthy subjects (three women, nine men) participated in the study exploring the effects of rifampin on glucose tolerance. The mean age was 24 years (SD ± 5.2, range 19–38 years), the mean weight 73 kg (SD ± 10.8, range 57–98 kg) and BMI 24.0 (SD ± 2.8, range 20.6–28.9 kg/m^2). All the participants were Caucasians. There were no dropouts.

The volunteers were administered 600 mg rifampin or placebo daily for a week and OGTT was performed on the morning of the eighth day. Rifampin increased glucose levels in OGTT compared with placebo treatment (Figure 1 and Table 1); plasma glucose concentrations were elevated at 30, 60 and 90 min time points (P = 0.028-0.033) and the incremental area under the plasma glucose concentration–time curve (AUC_{incr}) was noticeably higher (173 vs. 59 min*mmol/l; P = 0.008) by 192%. Serum insulin concentration was elevated in 90 min time point (P = 0.045) and insulin AUC_{incr} was increased significantly (8801 vs. 6071 min*mU/l; P = 0.031) by 45%. In addition, the total AUCs for insulin and glucose were significantly elevated by 40% and 16%, respectively (Table 1). The peak plasma glucose and peak serum insulin concentrations (C_{max}) were increased significantly. The time to peak serum insulin concentration (T_{max}) was slightly delayed by rifampin (50 vs. 43 min, statistically not significant) while the glucose T_{max} stayed the same for all the subjects (30 minutes). Fasting glucose, fasting insulin and C-peptide and HOMA-IR were not affected by rifampin dosing. The quantitative insulin sensitivity check index (QUICKI) and HOMA for β-cell function (HOMA-β) were not changed significantly.

Nine rats per group were studied in the experiments investigating the effect of PCN on glucose tolerance. The rats were given a daily intraperitoneal injection of PCN or vehicle for 4 days and OGTT was performed on the morning of the fifth day. In rats, PCN caused significant increase in

glucose levels during OGTT compared with vehicle control (Figure 2 and Table 2). Blood glucose concentrations were elevated at 60, 90 and 120 min (P = 0.0084-0.019) and the glucose AUC_{incr} was increased by 43% (740 vs. 539 min*mmol/l; P = 0.0029). The total glucose AUC increased by 21% (P = 0.0035). The glucose C_{max} increased significantly while the glucose T_{max} was delayed (57 vs. 37 min, statistically not significant) by PCN in comparison to vehicle control (Table 2).

We screened the expression of genes with an mRNA expression array in cultured mouse primary hepatocytes after 12-h exposure to PCN vs. vehicle control. Interestingly, the seven most down-regulated genes included three novel PXR regulated genes involved in glucose metabolism; glucose transporter 2 (Glut2; encoded by *Slc2a2*; -2.7 fold), pyruvate dehydrogenase kinase isoenzyme 2 (Pdk2; -2.4 fold) and glucokinase (Gck; -2.4 fold). In addition, the expression of phosphoenolpyruvate carboxykinase 1 (Pck1; -1.8 fold) was down-regulated as previously reported.(23) The repressed *in vitro* expression of these transcripts was confirmed with quantitative RT-PCR (qPCR) (Figure 3).

Next we studied if the hepatic expression of the newly identified PXR-regulated genes is affected by 1, 3, or 6 days of intraperitoneal 40 mg/kg PCN treatment vs. vehicle control in rats *in vivo.* Glut2 mRNA was repressed on day 3 (P = 0.011) and day 6 (P = 0.032)(Figure 4). Also Pdk2 was repressed on days 3 (P = 0.02) and 6 (P = 0.04). Glucokinase levels had a tendency towards repression by PCN on days 3 and 6 but this did not reach statistical significance. Furthermore, the expression of Pck1 and glucose-6-phosphatase (G6p), two key enzymes in gluconeogenesis, were significantly repressed. Expression of Pgc1α, a transcriptional coactivator regulating energy metabolism, was not affected by PCN.

DISCUSSION

This study demonstrates that the administration of prototypical PXR agonists PCN and rifampin leads to elevated glucose levels during OGTT in rats and humans. In healthy young adults, one-week rifampin administration led to elevated glucose and insulin levels in OGTT without affecting fasting glucose levels. This state resembles impaired glucose tolerance (IGT), a prediabetic condition, although none of the participants reached the clinical criterion of IGT (2 hour glucose ≥ 7.8 mmol/l). Our finding is supported by one early study by Takasu *et al*. with rifampin dosing in tuberculosis patients showing that both glucose and insulin levels were elevated during OGTT,(11) but contrasted by two other early studies with tuberculosis patients where no statistically significant differences were seen.(12-13) In the study by Takasu *et al*. the study groups were the tuberculosis patients before, during, and after rifampin treatment, a separate group of tuberculosis patients with other antituberculous drugs without rifampin, and healthy control subjects without medication.(11) In the other two studies without statistically significant differences, tuberculosis patients with rifampin-containing drug-regimen were compared to a separate group of tuberculosis patients on drug-regimen without rifampin.(12-13) In addition, some tuberculosis case reports have associated rifampin dosing with impaired glucose metabolism in insulin-dependent diabetics.(24-25) Infections such as tuberculosis affect glucose homeostasis(26) complicating the interpretation and encumbering the generalization of the results to subjects without infection. Thus, the previous human studies on the effects of PXR on glucose metabolism *in vivo* are contradictory, and possibly confounded by concurrent tuberculosis. The subjects in our study were healthy and non-obese generating more reliable and generalizable results suggesting that rifampin markedly disturbs glucose metabolism even in the absence of other glucose homeostasis-disrupting factors. One recent study exploring the effects of rifampin on glucose-lowering action of metformin detected no rifampin effect on OGTT although no numerical data on this were presented in the paper.(16) The

design of that study, however, did not include rifampin-only or placebo arms whereas our study had a more robust crossover design and no interfering medications.

We detected no rifampin effect on fasting indices of glucose metabolism such as HOMA-IR, a measure of insulin resistance. Fasting glucose was reported to be lower in patients with tuberculosis treated with rifampin-containing drug regimen in one study,(14) whereas in other studies fasting glucose was not affected by rifampin.(11-12) Phenobarbital, which activates both CAR and PXR,(7) lowers plasma fasting glucose and increases insulin sensitivity as studied with the euglycemic clamp technique in type II diabetics and lowers fasting insulin in healthy volunteers.(8-9) In the light of the results of our study, it could be speculated that the improved insulin sensitivity caused by phenobarbital is due to CAR activation and not PXR agonism. In agreement with this hypothesis, the activation of CAR ameliorated hyperglycemia and improved insulin sensitivity in ob/ob mice and high-fat diet fed wild-type mice.(27-29)

In agreement with the effect of rifampin in humans, we detected elevated glucose levels during OGTT in PCN-treated rats without a change in fasting glucose. Rifampin and PCN are widely used specific agonists of human and rat PXR, respectively. Similar effects of these two species-specific, structurally unrelated compounds strongly suggest that their hyperglycemic action is mediated by PXR. Rifampin is also an inhibitor of OATP1B1.(12-13) The last rifampin dose was taken more than 12 hours before OGTT and the half-life of rifampin in induced state is about 2-3 hours suggesting that there were no significant amounts of rifampin present during OGTT rendering any inhibitory effects unlikely. Furthermore, PCN is not known to have transporter-inhibiting properties suggesting that the role of OATP1B1 in the observed hyperglycemic effect is highly unlikely. Other putative explanations might include unspecific repressive effects on hepatic glucose transporters or activation of intestinal transporters. It should be noted that OGTT with prolonged blood sampling

(up to 4 to 6 hours) might have captured delayed effects not observable in the standard 2-hour OGTT utilized in this study, and perhaps offered more information on the role of intestinal glucose transporters. Antibiotic effects on the intestinal microflora might explain hyperglycemic effects of rifampin but not the effects of non-antibiotic PCN, and there is no clear evidence on hyperglycemic effects of other antibiotics although some fluoroquinolones have been associated with dysglycemia (hyperglycemia and hypoglycemia).(30) Altogether, PXR agonism remains the most likely explanation for hyperglycemic effects of PCN and rifampin.

The previous studies have indicated that PXR activation down-regulates gluconeogenesis and glycogenolysis by interfering with the transcription factors FOXO1, CREB and PGC1α leading to the repression of Pck1 and G6p;(6, 23, 31-33) these findings are corroborated by our study as Pck1 and G6p were down-regulated by PCN *in vivo*. Interestingly, we detected for the first time the down-regulation of hepatic Glut2 mRNA in cultured mouse primary hepatocytes *in vitro* and rat liver *in vivo* by PCN. As GLUT2 is the major hepatic glucose transporter facilitating the glucose influx (when plasma glucose is high) and efflux (in fasting condition),(34) its repression may be involved in the postprandial hyperglycemia caused by PCN and rifampin.

Pyruvate dehydrogenase kinase isoenzyme 2 (Pdk2) was repressed in mouse primary hepatocytes and rat liver *in vivo* by PCN. Mitochondrial pyruvate dehydrogenase complex plays a major role in the regulation of glucose homeostasis by catalyzing the conversion of pyruvate (a product of glycolysis) to acetyl-CoA.(35) Pyruvate dehydrogenase activity is inhibited by PDK (isoenzymes PDK1, PDK2, PDK3, and PDK4 are known with PDK2 most abundantly expressed in liver) and thus, the repression of PDK2 leads to the activation of pyruvate dehydrogenase and increased acetyl-CoA supply to Krebs cycle. In addition, glucokinase was repressed in mouse primary hepatocytes and similar but not statistically significant trend was seen in rat liver *in vivo* by PCN.

As phosphorylation of glucose to glucose-6-phosphate by glucokinase is the first step of both glycogen synthesis and glycolysis, and glucokinase gene mutations leading to reduced enzyme activity are the cause of the maturity-onset diabetes of the young 2 (MODY 2), the down-regulation of glucokinase would be of consequence to glucose metabolism.

In a previous study one-day 20 mg/kg PCN treatment decreased serum glucose levels *in vivo* in fasting $Pxr^{+/+}$ but not in $Pxr^{-/-}$ mice,(5) and seven-week PCN treatment (twice weekly injections of 50 mg/kg PCN) decreased blood glucose levels during intraperitoneal glucose tolerance test and decreased fasting HOMA-IR (but did not affect fasting glucose) in mice fed with high-fat diet.(6) Fasting glucose was not affected in our study with four-day 40 mg/kg PCN dosing. The discrepant *in vivo* findings could be due to species-specific factors or differences in the dose and the length of the administration. Additionally, to our knowledge, in no experimental animal study has OGTT or meal test been utilized to explore the effects of PXR agonists on postprandial glucose homeostasis and thus, the substantial incretin effect of intestine has previously been left unstudied. The current view in the literature regards PXR as a down-regulator of gluconeogenesis and, therefore, an antihyperglycemic pathway (see reviews (1, 4, 36)). Our findings suggest that other actions of PXR, perhaps Glut2 repression, override the antihyperglycemic effects of PXR in the regulation of whole body glucose homeostasis *in vivo*.

Several PXR-activating pharmaceuticals such as endothelin-1 receptor antagonist bosentan, antiepileptics carbamazepine and phenytoin, antiretrovirals etravirine and ritonavir, and many others, are currently in the market. In addition, many herbal remedies including St John's wort and Ginkgo biloba have ingredients with PXR-activating properties.(2) Although the hyperglycemic effect of rifampin was modest in absolute terms, the 40% increase in postprandial insulin exposure (total insulin AUC) in healthy young adults raises the possibility of major hyperglycemic effects in

overweight and elderly patients with existing insulin resistance. Thus, the widespread chronic use of PXR agonists puts a sizeable proportion of the population at risk of postprandial hyperglycemia which might, speculatively, predispose to diabetes if the insulin-producing capacity of the pancreas is exhausted over time. However, as rifampin is one of the most efficient PXR agonists, the hyperglycemic and hyperinsulinemic actions of other, less effective, PXR activators are probably less than the impact of rifampin, mitigating the potential for harm. As statins and antiretrovirals are associated with incident diabetes(37-38) and both drug classes include several PXR agonists,(2, 39) it would be tempting but premature to speculate on the role of PXR on their diabetic effects.

Our results should be taken into consideration when designing studies with rifampin, especially in interaction studies with diabetes medications,(15-19) so that the study design would include OGTT or meal test to allow for the postprandial effects of rifampin to be discerned with and without the test medication. In addition, the other PXR agonists in clinical use should be tested for postprandial glucose effects.

While our manuscript was at late stage of review process, He *et al.* published a paper showing that $Pxr^{-/-}$ mice on high-fat diet had reduced insulin resistance, lower fasting glucose, and better glucose tolerance (studied with intraperitoneal glucose tolerance test) in comparison to $Pxr^{+/+}$ mice while knockout and wildtype mice on regular chow did not show differences in glucose metabolism.(40) Although He *et al.* did not study human volunteers or rats, and did not perform OGTT in mice, their results and our findings support the conclusion that PXR is a prohyperglycemic regulator of glucose metabolism.

In conclusion, prototypical PXR agonists PCN and rifampin elevated postprandial glucose levels as studied with OGTT in rats and healthy volunteers without affecting fasting glucose. The repression

of hepatic Glut2 transporter may play a role in the hyperglycemic effect. These novel findings suggest that PXR-mediated pathway defines a new regulatory mechanism for postprandial glucose homeostasis which may have implications for the etiology of impaired glucose tolerance and diabetes.

METHODS

Subjects. Healthy volunteers, aged 18 – 40 years, were recruited for the study. Exclusion criteria included major medical or psychiatric conditions including any liver disease (as judged by the study physician based on history, physical examination and basic laboratory values), any continuous medication including oral contraceptives, insensitivity to rifampin, pregnancy and breast feeding, continuous use of soft contact lenses (rifampin may color), history of difficult venipuncture, drug or alcohol abuse, and participation in any other trial within one month.

The study was approved by the Ethics Committee of The Northern Ostrobothnia Hospital District (Oulu, Finland) and the Finnish Medicines Agency. Written, informed consent was obtained from each subject. The study procedures performed were in accordance with the ethical standards of the Helsinki Declaration. The participants were financially compensated for participation. The number of subjects (n = 12) was based on a power analysis to detect an effect size of 18% in HOMA-IR assuming a coefficient of variation of 111% of the effect size with $\alpha = 0.05$ and $\beta = 0.2$. This trial was registered at clinicaltrials.gov as NCT00985270.

Experimental protocol of the volunteer study. This was a randomized, open, placebo-controlled crossover trial. Twelve healthy individuals were administered 600 mg rifampin (RIMAPEN; Orion Corporation, Espoo, Finland) daily for a week compared with a placebo control arm with at least a four-week washout period. The treatments were not blinded since there were no subjective measurements, the endpoints were based on laboratory assays, and rifampin colors urine red rendering blinding impossible. The sequence (i.e. rifampin or placebo period first) of the two arms was randomized. The participants were asked to abstain from the use of alcohol, over-the-counter-medications, and dietary and herbal supplements for 5 days before and during the study arms. The

study was conducted on an outpatient basis and each subject visited the Internal Medicine Research Laboratory of Oulu University Hospital two times in each arm. At the start of each arm, the first rifampin or placebo tablet was administered under the supervision of a study nurse while the subsequent daily doses were taken by the subjects at home between 4 – 8 p.m. at least one hour before meal or 2 hours after meal at subjects' convenience. To monitor the compliance to drug regimen, the volunteers wrote the date and time of each dose taken on a medication diary and the participants were required to return the used medication containers. The subjects consumed their regular diets during the study arms.

At the end of each 7-day-arm, blood and urine samples were collected and a 2-hour 75-gram OGTT was performed on the morning of the eighth day after a 10-hour fast. An intravenous catheter was inserted in the forearm for blood drawings. Blood samples were taken 20 minutes and 5 minutes before OGTT and at 0, 30, 60, 90 and 120 min time points.

Experimental protocol of the rat OGTT. Male 2-month old Sprague-Dawley rats (weighing 240-300 g) from the colony of the Centre of Experimental Animals at the University of Oulu were used. All rats were kept in plastic cages with free access to tap water and regular rat chow in a room with a controlled 40 % humidity and a temperature of 22 °C. A controlled 12-hour environmental light cycle was maintained. The experimental designs were approved by the national Animal Experiment Board in Finland. The investigation conforms to the Guide for the Care and Use of Laboratory Animals published by the US National Institutes of Health.

The rats were given a single daily intraperitoneal injection of PCN (40 mg/kg) in corn oil plus 30% DMSO or vehicle (corn oil plus 30% DMSO) for 4 days. All rats (9 rats per group) were fasted overnight (at least 12h) prior to the test. At time 0 (prior to the glucose load) blood sample (á 300

μl) was collected from the tail vein. Each rat was given an oral glucose load, 2 g/kg body weight, by oral gavage. Then the rats were anesthetized with 0.26 mg/kg fentanyl citrate and 8.25 mg/kg fluanisone (HYPNORM; VetaPharma Limited, Leeds, UK), and 4.1 mg/kg midazolam (DORMICUM; Roche AG, Basel, Switzerland) subcutaneously. A PE-60 catheter was inserted into the left femoral artery for collection of blood samples, filled with heparinized (15 IU/ml) saline, and plugged with a three-way stopcock. Blood samples were collected 30, 60, 90 and 120 minutes after the glucose load. Blood glucose concentrations were measured using Precision Xtra Blood Glucose & Ketone Monitoring System (Abbott Laboratories, Abbott Park, IL).

Additionally, Sprague-Dawley rats were dosed with 40 mg/kg PCN or vehicle control for 1, 3, or 6 days (5 rats per group). At the end of the experiment, the rats were decapitated, the liver was removed, and its weight recorded, immersed in liquid nitrogen, and stored at -70°C for further analysis.

Mouse primary hepatocytes. Primary hepatocytes were isolated from a male 9-week-old C57BL/6 mouse and cultured as described previously.(41) The cultures were maintained for 24 hours before treatment with vehicle (DMSO) or 10 μM PCN for 12 hours, after which cells were collected and RNA isolated.

RNA extraction and qPCR. Total RNA was isolated using TRI-Reagent (Sigma-Aldrich, St. Louis, MO) according to the manufacturer's protocol and treated with DNase (Promega, Fitchburg, WI). 1 μg of RNA was reverse transcribed to produce cDNA using p(dN)6 random primers (Roche Diagnostics, Mannheim, Germany) and M-MLV reverse transcriptase (Promega). The qPCR reactions were performed using FastStart Universal SYBRGreen Master Mix (Roche AG). Sequences of mice primers were obtained from Primer Bank database.(42) The sequences of mice

and rat primers are presented in Table 3. Fluorescence values of the qPCR products were corrected with the fluorescence signals of the passive reference dye (ROX). The RNA levels of target genes were normalized against the 18S control levels using the comparative C_T ($\Delta\Delta C_T$) method.

Microarray experiment. Three parallel RNA samples from mouse primary hepatocytes were pooled. RNA quality was measured on an Agilent 2100 Bioanalyzer (Agilent Technologies, Amsterdam, The Netherlands) using 6000 Nano Chips. Five micrograms of RNA were used for one cycle cRNA synthesis (Affymetrix, Santa Clara, CA). Affymetrix Mouse Genome 430 2.0 Arrays were used according to instructions from the manufacturer. Probesets were redefined according to Dai *et al*. (43) using remapped CDF v9, based on Entrez Gene database (build 36,v2). Expression estimates were done as described previously.(44)

Analytic methods and data analyses. The clinical laboratory analyses were performed by the Clinical Laboratory of Oulu University Hospital and were validated for clinical use. The enzymatic hexokinase (plasma glucose) and chemiluminometric (serum insulin, serum C-peptide) methods were used. The following equations were calculated; HOMA-IR, [fasting glucose (mmol/L) × fasting insulin (mU/L)]/22.5; HOMA-β, 20 × fasting insulin (mU/l)/[fasting glucose (mmol/L) - 3.5]; and QUICKI, 1 / [log fasting insulin (mU/l) + log fasting glucose (mg/dl)].

Prism 5 software (GraphPad Software Inc., La Jolla, CA) was used to compute AUC and incremental AUC with the trapezoidal method. The calculations of incremental AUCs had fasting glucose and insulin as a baseline. If the subtraction of the baseline from the subsequent OGTT time point resulted in a negative value leading to a negative peak in incremental AUC calculation, the resulting negative peak area was subtracted from the positive peak area (net area calculation). In the human study, fasting glucose and insulin were calculated as a mean of two and three measurements,

respectively, to increase the precision of indices based on fasting measures (intraindividual coefficient of variation 2.9% for glucose and 22% for insulin). The parameters were compared across treatments by two-tailed Student's t-test (paired test for the human study; unpaired for rodent studies). $P < 0.05$ was considered statistically significant.

STUDY HIGHLIGHTS

What is the current knowledge on the topic?

PXR is a major regulator of drug and bile acid metabolism. In addition, the current view regards PXR as a down-regulator of gluconeogenesis based on rodent studies.

What question this study addressed?

We investigated the effects of PXR activation on glucose homeostasis without interfering effects of diseases and other medications. No previous study has reported the effects of PCN and rifampin, the prototypical rat and human PXR agonists, on oral glucose tolerance test in rodents or healthy volunteers.

What this study adds to our knowledge?

PXR agonists elicit postprandial hyperglycemia in oral glucose tolerance test without affecting fasting glucose. The postprandial hyperglycemia may be explained by the down-regulation of hepatic Glut2 glucose transporter.

How this might change clinical pharmacology and therapeutics?

This novel finding suggests that PXR-mediated pathways define a new regulatory mechanism for postprandial glucose homeostasis which may have implications for the etiology of impaired glucose tolerance and diabetes. The hyperglycemic effects of PCN and rifampin should be taken into account when designing experiments and studies with these compounds.

ACKNOWLEDGEMENTS

We thank Asta Hietala, Anneli Kangas-Kerkelä and Marketta Niiranen for assistance in conducting the clinical study, Sirpa Rutanen and Tuula Stranius for assistance in conducting the *in vivo* animal

studies, and Päivi Tyni for expert technical assistance. This work has been supported by the Academy of Finland (Center of Excellence), the Sigrid Jusélius Foundation, the Finnish Diabetes Research Foundation and the Finnish Foundation for Cardiovascular Research.

CONFLICT OF INTEREST/DISCLOSURE

The authors declare no conflict of interests.

AUTHOR CONTRIBUTIONS

H.R., J.Ha., J.Hu., J.R., M.B. and M.J.S. wrote the manuscript, J.Ha., J.Hu., J.R., and M.J.S. designed the research, J.Hu., J.R., and M.B. performed the research, J.Ha., J.Hu., J.R., and M.B. analyzed the data.

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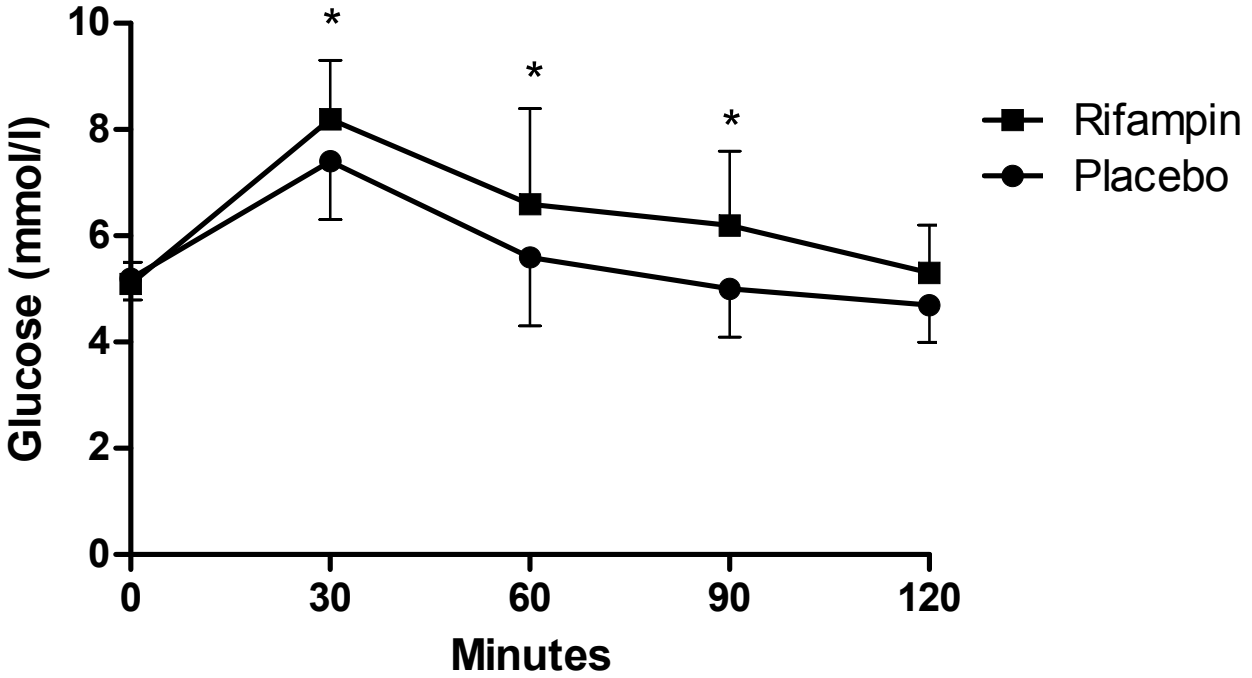
FIGURE LEGENDS

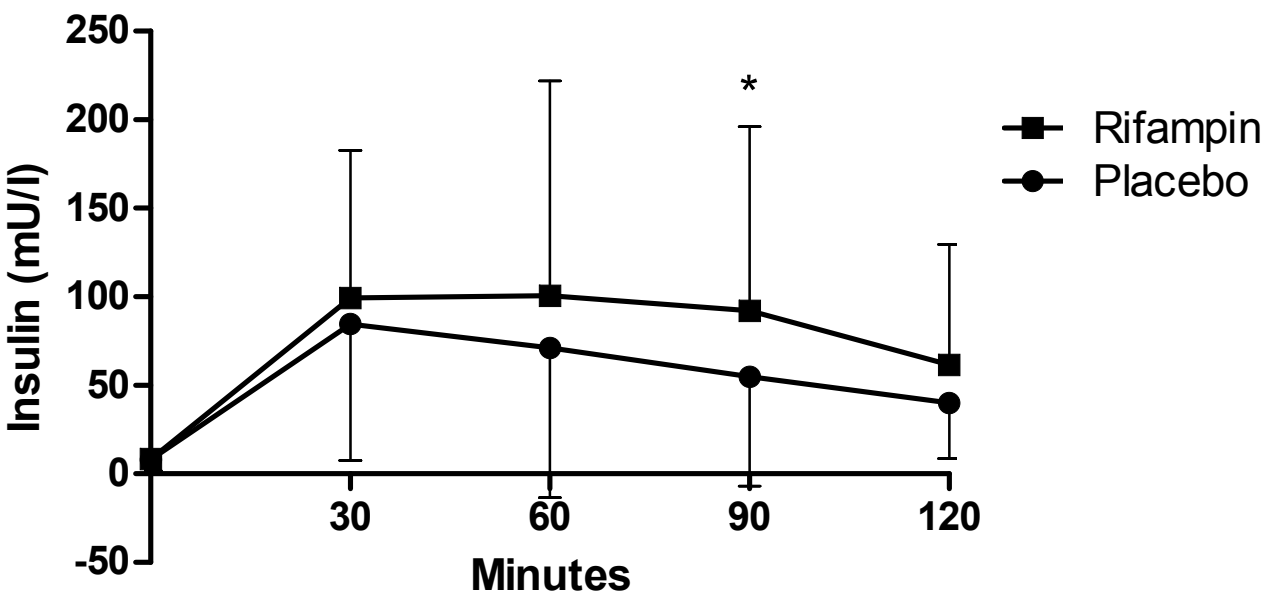
Figure 1 Effect of the treatment with 600 mg rifampin or placebo once daily for seven days on glucose and insulin responses during oral glucose tolerance test in volunteers. Values are represented as means ± SD. * $P < 0.05$ rifampin versus placebo.

Figure 2 Effect of 4-day intraperitoneal 40 mg/kg pregnenolone 16α-carbonitrile (PCN) vs. vehicle control on glucose response during oral glucose tolerance test in rats. Values are represented as means ± SD. * $P < 0.05$ PCN versus vehicle control.

Figure 3 Effect of 12 hour pregnenolone 16α-carbonitrile (PCN) treatment on the mRNA levels of selected genes involved in glucose metabolism in cultured mouse primary hepatocytes. Gck, glucokinase; Glut2, glucose transporter 2; Pck1, phosphoenolpyruvate carboxykinase 1; Pdk2, pyruvate dehydrogenase kinase isoenzyme 2. Values are represented as means + SD. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$ PCN versus vehicle control.

Figure 4 Effect of 1, 3, or 6 days of pregnenolone 16α-carbonitrile (PCN) vs. vehicle control treatment on mRNA expression of selected genes involved in glucose metabolism in rat liver *in vivo*. G6p, glucose-6-phosphatase; Gck, glucokinase; Glut2, glucose transporter 2; Pck1, phosphoenolpyruvate carboxykinase 1; Pdk2, pyruvate dehydrogenase kinase isoenzyme 2; Pgc1α, peroxisome proliferator activated receptor gamma coactivator 1α. Values are represented as means + SD. * $P < 0.05$, ** $P < 0.01$ PCN versus vehicle control.


Glucose (mmol/l)
10
8
6
4
2
0
*
*
*
0
30
60
90
120
Minutes
Rifampin
Placebo


Insulin (mU/l)
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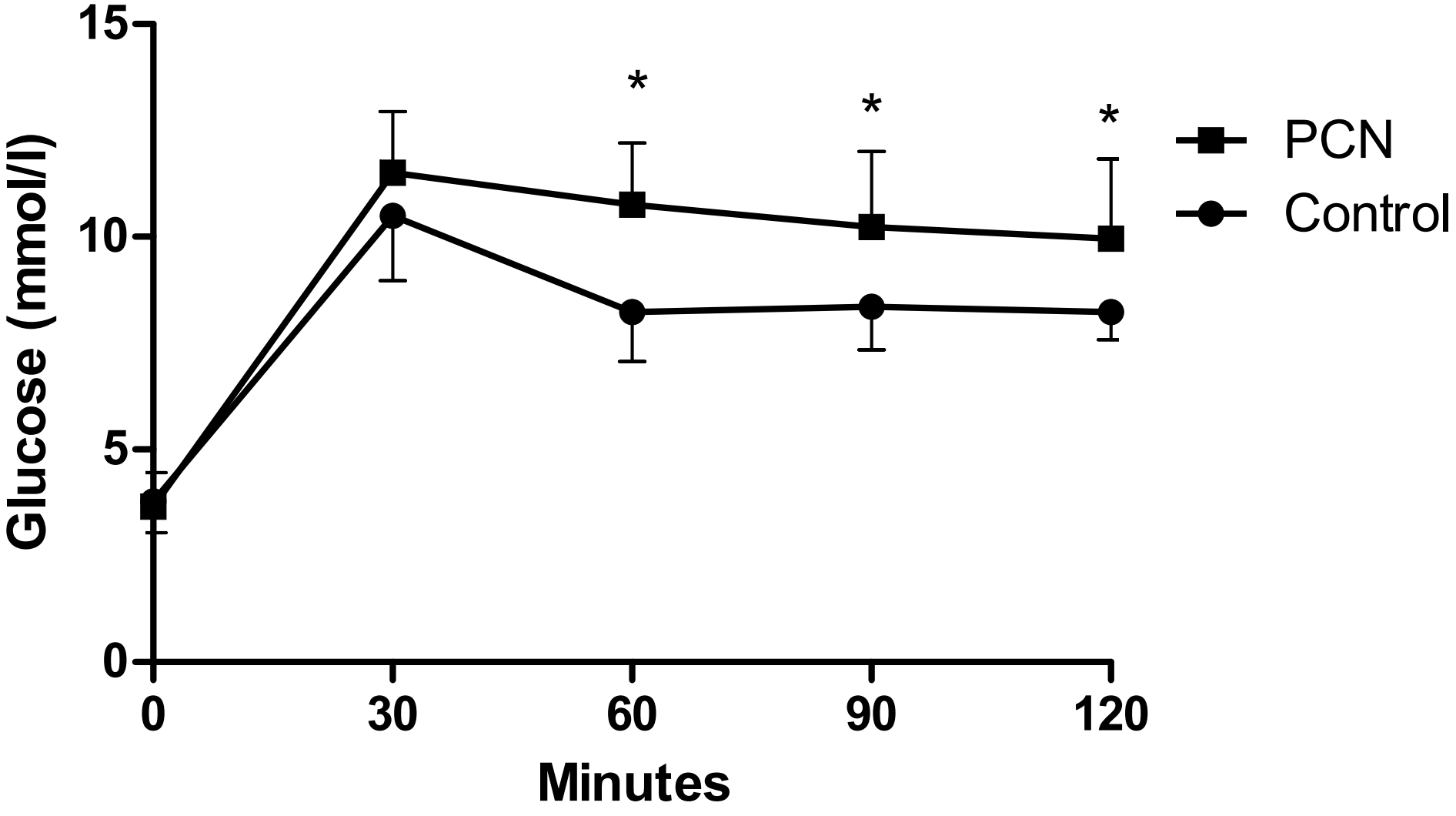
200
150
100
50
0
-50
*
30
60
90
120
Minutes
Rifampin
Placebo


15
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5
0
Glucose (mmol/l)
*
*
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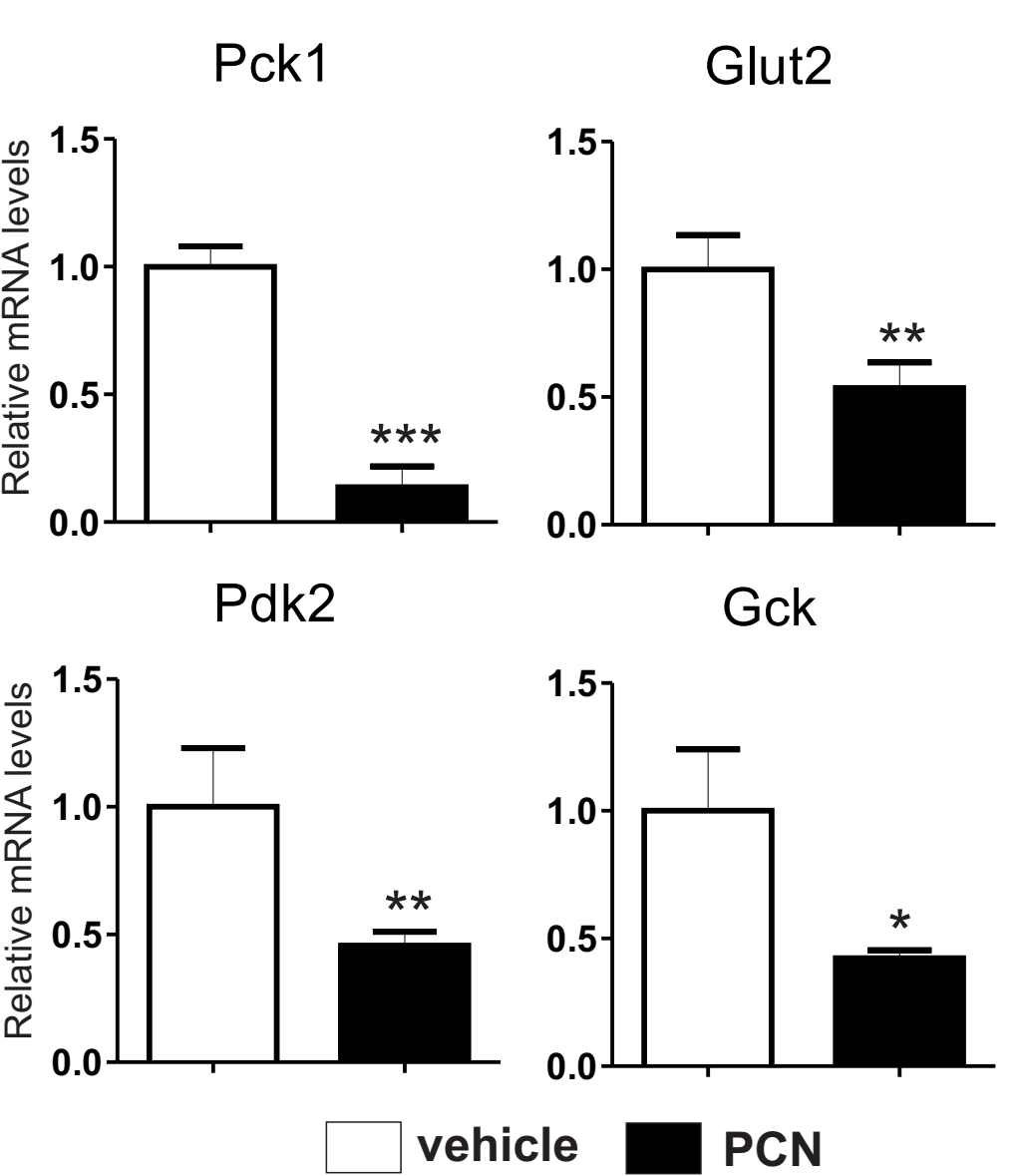
0
30
60
90
120
Minutes
PCN
Control


Pck1
Glut2
Pdk2
Gck
Relative mRNA levels
Relative mRNA levels
1.5
1.0
0.5
0.0
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*
vehicle
PCN

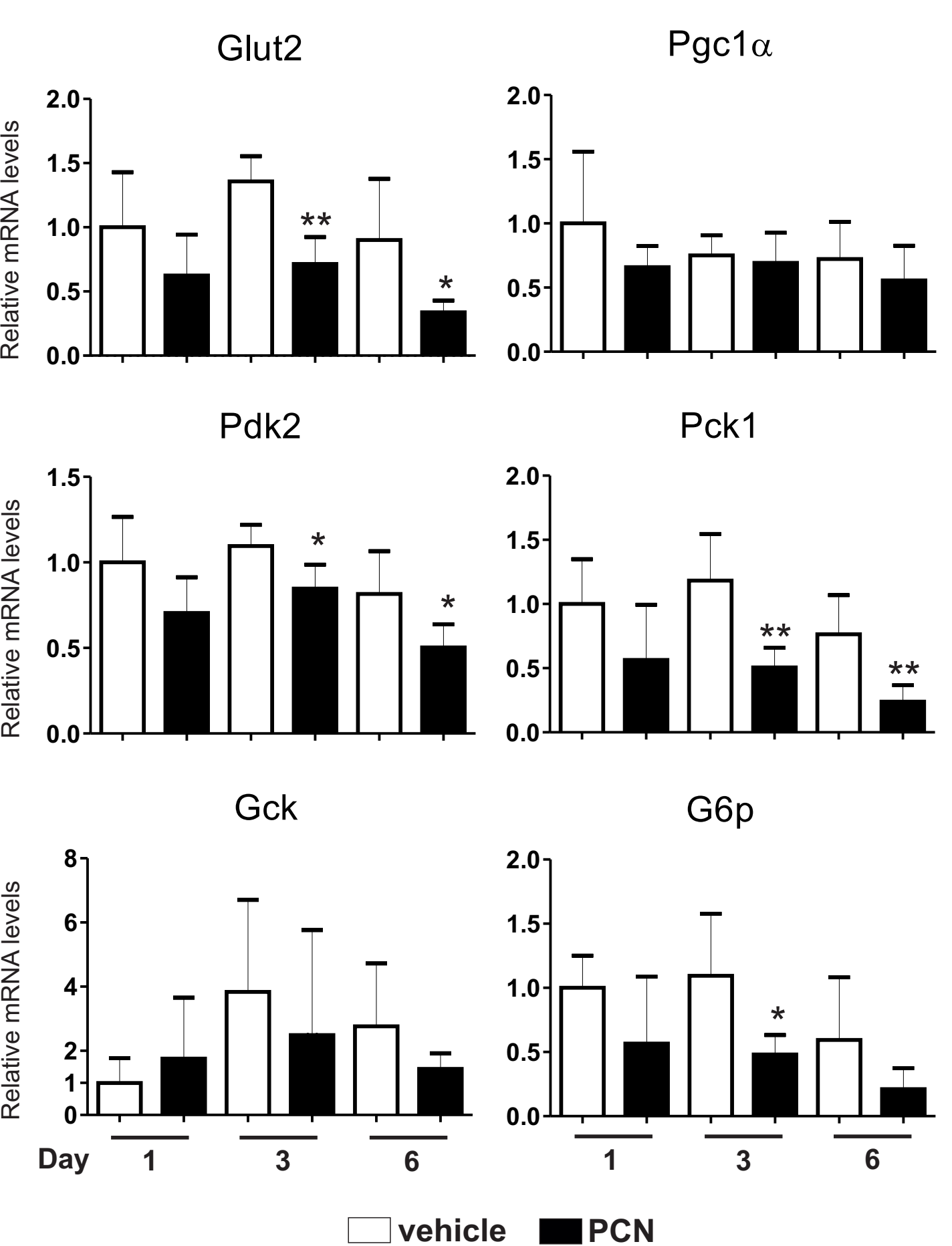
Glut2
Pgc1α
Pdk2
Pck1
Gck
G6p
Relative mRNA levels
Day
1
3
6
vehicle
PCN

Table 1 Effect of the treatment with 600 mg rifampin or placebo once daily for seven days on indices of glucose metabolism

	Rifampin	Placebo	*P*-value	Ratio and 95% CI of difference
Plasma fasting glucose (mmol/l)	5.1 ± 0.4	5.2 ± 0.4	0.38	0.98 (-0.43, 0.18)
Serum fasting insulin (mU/l)	8.4 ± 5.7	8.0 ± 6.2	0.53	1.05 (-0.98, 1.79)
Serum fasting C-peptide (nmol/l)	0.49 ± 0.25	0.44 ± 0.20	0.37	1.12 (-0.07, 0.17)
HOMA-IR	2.0 ± 1.5	1.9 ± 1.7	0.82	1.02 (-0.33, 0.41)
HOMA-β	99.4 ± 46.0	87.6 ± 48.8	0.12	1.13 (-3.7, 27.3)
QUICKI	0.36 ± 0.04	0.36 ± 0.03	0.85	1.00 (-0.22, 0.02)
Glucose AUC_{incr} (min*mmol/l)	172.5 ± 108.9	59.1 ± 63.1	0.008	2.92 (37, 190)
Glucose AUC (min*mmol/l)	786 ± 130	688 ± 92	0.032	1.14 (10, 186)
Glucose C_{max} (mmol/l)	8.2 ± 1.1	7.4 ± 1.1	0.033	1.10 (0.07, 1.41)

Glucose T_{max} (min)	30 (30 - 30)	30 (30 - 30)	-	1.0
Insulin AUC_{incr} (min*mU/l)	8,801 ± 9342	6,071 ± 6385	0.031	1.45 (298, 5162)
Insulin AUC (min*mmol/l)	9,811 ± 9970	7,032 ± 7058	0.031	1.40 (308, 5249)
Insulin C_{max} (mU/l)	124.2 ± 123.5	91.0 ± 84.6	0.048	1.36 (0.40, 66)
Insulin T_{max} (min)	45 (30 - 90)	30 (30 - 60)	0.39	1.18 (-11, 26)

Data are presented as mean ± SD (median and range for T_{max} values), the ratio of arithmetic means and the 95% CI of differences between arms. Two-tailed paired Student's t-test was used as the statistical test. AUC, area under the plasma concentration–time curve 0 – 120 min; AUC_{incr}, incremental AUC 0 – 120 min; CI, confidence interval; C_{max}, peak concentration; HOMA-β, homeostasis model assessment for β-cell function; HOMA-IR, homeostasis model assessment for insulin resistance; QUICKI, quantitative insulin sensitivity check index; T_{max}, time to peak concentration.

Table 2 Effect of 4-day intraperitoneal 40 mg/kg pregnenolone 16α-carbonitrile (PCN) vs. vehicle control treatment on glucose metabolism in rats

	PCN	Control	*P*-value
Blood fasting glucose (mmol/l)	3.7 ± 0.8	3.8 ± 0.7	0.74
Glucose AUC_{incr} (min*mmol/l)	740.2 ± 135.1	539.2 ± 106.6	0.0029
Glucose AUC (min*mmol/l)	1179 ± 129	993 ± 100	0.0035
Glucose C_{max} (mmol/l)	11.9 ± 1.4	10.5 ± 1.5	0.049
Glucose T_{max} (min)			0.128
	60 (30 - 120)	30 (30 - 90)	

Data are presented as mean ± SD (median and range for T_{max}). Two-tailed unpaired Student's t-test was used as the statistical test.

Table 3 Sequences of the primers used in quantitative RT-PCR

Gene	Forward (5′to 3′)	Reverse (5′to 3′)
18S	CGCCGCTAGAGGTGAAATTC	CCAGTCGGCATCGTTTATGG
Slc2a2	TCAGAAGACAAGATCACCGGA	GCTGGTGTGACTGTAAGTGGG
Pgc1α	GCAGGTCGAACGAAACTGAC	CTCAGCCTGGGAACACGTTA
G6p	CATCAATCTCCTCTGGGTGG	TGCTGTAGTAGTCGGTG
Pck1	GGTGTTTACTGGGAAGGCATC	CAATAATGGGGCACTGGCTG
Pdk2 rat	AGGGGCGCCCAAGTACATC	TGCCGGAGGAAGGTGAATGA
Gck rat	ATGGCTATGGATACTACAAGG	TCAGGCCACGGTCCATCT
Pdk2 mouse	AGGGGCACCCAAGTACATC	TGCCGGAGGAAAGTGAATGAC
Gck mouse	ATGGCTGTGGATACTACAAGGA	TTCAGGCCACGGTCCATCT

18S, 18S ribosomal RNA; G6p, glucose-6-phosphatase; Gck, glucokinase; Pck1, phosphoenolpyruvate carboxykinase 1; Pdk2, pyruvate dehydrogenase kinase isoenzyme 2; Pgc1α, peroxisome proliferator activated receptor gamma coactivator 1α; Slc2a2, solute carrier family 2, facilitated glucose transporter member 2 (encodes glucose transporter 2)