# The Effect of Pregnane X Receptor Agonists on Postprandial Incretin Hormone Secretion in Rats and Humans

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**Summary**

We recently showed that pregnane X receptor (PXR) agonists cause hyperglycaemia during oral glucose tolerance test (OGTT) in rats and healthy volunteers (Rifa-1 study). We now aimed to determine if the secretion of incretin hormones, especially glucagon-like peptide-1 (GLP-1) and gastric inhibitory polypeptide (GIP), are affected by PXR agonists since these gut-secreted hormones are major regulators of postprandial glucose metabolism. The Rifa-2 study had a one-phase, open-label design. Twelve subjects were given 600 mg of rifampicin a day for a week. OGTT with glucose, insulin, and incretin hormone measurements was performed before and after the rifampicin dosing. Incretins and insulin were analysed in previously collected rat OGTT samples after pregnenolone 16α-carbonitrile (PCN) or control treatment for 4 days. Rifampicin treatment did not affect glucose, insulin, GLP-1, GIP, glucagon, and peptide YY levels statistically significantly. Incremental AUCs ($AUC_{incr}$) of glucose and insulin tended to increase (41% increase in glucose $AUC_{incr}$, p = 0.21, 95% confidence interval (CI) of the difference -47, 187; 24% increase in insulin $AUC_{incr}$, p = 0.084, CI of the difference -110, 1493). Glucagon AUC was increased in women (53% increase, p = 0.028) and decreased in men (19% decrease, p < 0.001) after rifampicin dosing. In combined analysis of human Rifa-1 and Rifa-2 studies, glucose $AUC_{incr}$ was elevated by 63% (p = 0.010) and insulin $AUC_{incr}$ by 37% (p = 0.011). PCN increased rat insulin level at 60 min time point but did not affect incretin and insulin AUCs statistically significantly. In conclusion, PXR agonists do not affect the secretion of incretin hormones. The regulation of glucagon secretion by PXR may be sexually dimorphic in humans. The mechanism of disrupted glucose metabolism induced by PXR activation requires further study.

**Keywords**: Gastric inhibitory polypeptide, glucagon, glucagon-like peptide-1, hyperglycaemia, insulin, peptide YY, pregnane X receptor, pregnenolone 16α-carbonitrile, rifampicin

**Introduction**

Pregnane X receptor (PXR; NR1I2) belongs to the nuclear receptor superfamily (1). PXR is one of the main regulators of drug metabolism inducing the expression of several drug-metabolizing enzymes, including many cytochrome P450 (CYP) enzymes, and drug transporters (2-3). PXR is of importance also as a regulator of bile acid metabolism (3). In addition, both *in vitro* observations and studies with experimental animals have shown that PXR regulates hepatic glucose, lipid, and hormone metabolism, and certain aspects of immune response (4-6).

We recently showed that rifampicin, an efficient and specific human PXR agonist, caused postprandial hyperglycaemia and hyperinsulinemia in healthy volunteers during oral glucose tolerance test (OGTT) when compared with a placebo arm (Rifa-1 study)(7). We also demonstrated that pregnenolone 16α-carbonitrile (PCN), a prototypical rodent PXR agonist, caused a similar hyperglycaemic effect in rats during OGTT. A previous study in tuberculosis patients indicates that rifampicin increases glucose and insulin levels during OGTT (8), although in two other studies involving tuberculosis patients rifampicin was not associated with statistically significant changes in glucose tolerance (9-10). Studies with two different $Pxr^{-/-}$ mice strains on high-fat diet (HFD) show discrepant results with better (11) or impaired (12) glucose tolerance in comparison with $Pxr^{+/+}$ mice on HFD. Studies with humanized PXR (hPXR) mice are consistently showing that HFD-fed hPXR mice are more prone to obesity and glucose intolerance when compared to wild type mice on HFD (12-13). Thus, PXR appears to be a novel pathway for the regulation of glucose homeostasis.

Incretin hormones, most notably glucagon-like peptide-1 (GLP-1), are major regulators of postprandial glucose levels as they stimulate insulin secretion during hyperglycaemia and suppress glucagon secretion (14). Several GLP-1 receptor agonists are currently used in the treatment of

type 2 diabetes and liraglutide recently gained marketing authorisation as a treatment of obesity (15-16). Although PXR agonism affects mostly postprandial glucose tolerance and incretins are major regulators of postprandial insulin and glucagon release, there are no previous human or animal studies on the effect of PXR on incretin hormone secretion. As rifampicin is known to lower the levels of secondary bile acids lithocholic acid (LCA) and deoxycholic acid (DCA) in human bile and serum via induction of cytochrome P450 3A4 (CYP3A4) -mediated bile acid hydroxylation (17-18), and LCA and DCA are known to promote the GLP-1 secretion via activation of G protein-coupled bile acid receptor (TGR5)(19-20), we hypothesised that PXR activation could attenuate incretin hormone secretion. Thus, we set out to elucidate the effects of PXR activation on postprandial incretin hormone secretion in rats and healthy volunteers.

**Materials and methods**

*Subjects*. Healthy volunteers, aged 18–45 years, with body mass index between 19 – 28 kg $m^{-2}$ were recruited for the study. Exclusion criteria included major medical or psychiatric conditions including any liver disease (as judged by the study physician on the basis of history, physical examination, and basic laboratory values); any continuous medication, including oral contraceptives; insensitivity to rifampicin; pregnancy; breast feeding; continuous use of soft contact lenses (rifampicin may colour); history of difficult venipuncture; drug or alcohol abuse; and participation in any other trial within 1 month. The study was approved (number 73/2010) 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 Declaration of Helsinki and guidelines on Good Clinical Practice. The participants were financially compensated for participation. The number of subjects was based on our previous study Rifa-1 (7) where sample

size of 12 was sufficient to detect a statistically significant difference in glucose tolerance during OGTT after one-week dosing of rifampicin 600 mg per day. This trial was registered at ClinicalTrials.gov as NCT01293422.

*Study design and experimental protocols*. The Rifa-2 study had a one-phase, open-label design. Twelve subjects were given 600 mg of rifampicin (Rimapen; Orion Corporation, Espoo, Finland) a day for a week. The daily dose of 600 mg is the maximum clinically used dose as well as the most commonly used dose in experimental human studies. 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 arm. 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. On the morning of the first day, a 2 h 75 g OGTT was performed after a 10 h fast. An i.v. catheter was inserted in the forearm for blood drawings. Blood samples were taken 5 min before the OGTT and at 0-, 30-, 60-, 90-, and 120-min time points. The first rifampicin tablet was administered under the supervision of a study nurse after the OGTT; the subsequent daily doses were taken by the subjects at home between 4–8 p.m. at least 1 h before a meal or 2 h after a meal, at the subjects' convenience. To monitor the compliance to drug regimen, the volunteers wrote the date and time of each dose taken in 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 the study arm, OGTT was performed on the morning of the eighth day. A 3 ml blood sample was drawn for incretin and other hormone measurements and mixed immediately with 30 µl of dipeptidyl peptidase-4 (DPP4) inhibitor (Merck Millipore; Darmstadt, Germany) followed by 30 min incubation in room temperature, 10 min centrifugation (2000 x g) at +4 °C, and storage at -70 °C.

The experimental protocol of the rat OGTT has been described in detail in our previous publication, including rat OGTT glucose results (7). Briefly, male Sprague Dawley rats were given a daily i.p. injection of PCN (40 mg/kg) in corn oil plus 30% dimethyl sulfoxide or vehicle (corn oil plus 30% dimethyl sulfoxide) for 4 days. The daily 40 mg/kg dose is in the middle of the dose-range used in the previous Sprague Dawley studies (21-24). All rats (nine per group) were fasted overnight. At time 0, a blood sample 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 anesthetised with fentanyl citrate and fluanisone (Hypnorm; VetaPharma, Leeds, UK), and midazolam (Dormicum; Roche, Basel, Switzerland) subcutaneously. A PE-60 catheter was inserted into the left femoral artery for collection of blood samples. Blood samples were collected 30, 60, 90, and 120 min after the glucose load. A 100 µl blood sample was drawn for incretin and other hormone measurements and mixed immediately with 1 µl of DPP4 inhibitor followed by 30 min incubation in room temperature, 10 min centrifugation (2000 x g) at room temperature, and storage at -70 °C. Additionally, rats were dosed with 40 mg/kg PCN or vehicle for 1, 3, or 6 days. The liver was immersed in liquid nitrogen and stored at −70 °C for further analysis. RNA extraction and quantitative reverse-transcriptase PCR was performed as described previously (7). CYP3A2 mRNA was measured with a predesigned Taqman gene expression assay Rn00756461_m1 (ThermoFisher Scientific, Waltham, MA USA), and 18S was used as an internal control as described (7). The experimental design was 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.

*Analytic methods and data analyses*. The plasma glucose analysis was performed by the Clinical Laboratory (Nordlab) of Oulu University Hospital by an enzymatic hexokinase method validated for

clinical use. The following equations were calculated: the homeostasis model assessment for insulin resistance (HOMA-IR), (fasting glucose × fasting insulin)/22.5; Matsuda index, 10,000/square root of [(fasting insulin × fasting glucose) × (mean glucose × mean insulin during OGTT)]; insulinogenic index, the change in insulin during the first 30 min/the change in glucose during the first 30 min; and oral disposition index, the product of Matsuda index and insulinogenic index. HOMA-IR is an index of insulin resistance (25) based on fasting measurements, Matsuda index is a measure of insulin sensitivity based on fasting and OGTT measurements (26), the insulinogenic index is a measure of insulin secretion at the beginning of the OGTT (27), and the oral disposition index estimates β-cell function (28). For the conversion of insulin SI units to international units required in the equations, the following conversion factor was used: of 1 mU/l insulin = 6 pmol/l (29).

Human plasma gastric inhibitory polypeptide (GIP;total), GLP-1 (active), glucagon, insulin and peptide YY (PYY) levels were quantified using a Human Metabolic Hormone Magnetic Bead Panel Milliplex® MAP Kit (EMD Millipore, Billerica, MA, USA) and rat plasma GLP-1 (active), GIP (total), C-peptide, insulin and glucagon levels were quantified using a Rat Metabolic Magnetic Bead Panel Milliplex® MAP Kit (EMD Millipore). The kits are based on the Luminex® xMAP® technology where each bead is coated with reagents that specifically recognize the analyte to be measured from the sample. The panels were read with Bio-Plex multiplex system (Bio-Plex 200, Bio-Rad Laboratories, Incorporation, CA, USA) as described previously (30-31). The intra-assay CV %:s for the human analytes were 3% (GIP), 7% (GLP-1), 3% (glucagon), 3% (insulin) and 2% (PYY). The inter-assay CV %:s for analytes were 5% (GIP), 10% (GLP-1), 7% (glucagon), 6% (insulin) and 11% (PYY). The intra-assay CV %:s for the rat analytes were 3% (GLP-1), 2% (GIP), 2% (C-peptide 2), 2% (insulin) and 8% (glucagon). The inter-assay CV %:s for analytes were 9% (GLP-1), 13% (GIP), 9% (C-peptide 2), 9% (insulin) and 29% (glucagon).

Prism 5 software (GraphPad Software, La Jolla, CA, USA) was used to compute AUC and incremental AUC with the trapezoidal method. The calculations of incremental AUCs had measurements performed in fasting state 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 was calculated as a mean of two measurements (-5 min and 0 min). The parameters were compared across treatments by two-tailed Student's t-test (paired test for the human study; unpaired for rodent studies) and interindividual comparisons between sexes in healthy volunteers were analysed with independent-samples Mann-Whitney U test. $P < 0.05$ was considered statistically significant.

**Results**

Six women and six men participated in the study; there were no dropouts. The mean age was 23 years (SD ±3.5; range 19 – 31), the mean weight 65 kg (SD ±7.6; range 55 – 78) and the BMI 22.5 (SD ±2.2; range 19.5 – 26.3). All the subjects were Caucasian.

The volunteers were administered 600 mg rifampicin daily for a week, and the OGTT was performed on the first and the eight study day. Rifampicin did not affect glucose, insulin, GLP-1, GIP, glucagon, and PYY levels during OGTT statistically significantly (*Table 1*, *Fig. 1*). Also the incremental areas under the concentration–time curve ($AUC_{incr}$) were not significantly different. However, incremental AUCs of glucose and insulin tended to increase (41% increase in glucose $AUC_{incr}$, $p = 0.21$; 24% increase in insulin $AUC_{incr}$, $p = 0.084$). Also total insulin AUC tended to increase (14% increase, $p = 0.10$). Fasting measurements including HOMA-IR, and Matsuda, insulinogenic, and disposition indices were not affected by rifampicin dosing (*Table 1*). Liver transaminases, alkaline phosphatase, and bilirubin were measured before and after the study

week to ensure subject safety; no significant changes were detected with no elevations above the upper limit of normal (data not shown).

Glucagon AUC was statistically significantly increased in women (2794 min pg $ml^{-1}$ ± 1411 to 4275 min pg $ml^{-1}$ ± 1746; 53% increase, p = 0.028; 95% CI 245, 2719) and decreased in men (11269 min pg $ml^{-1}$ ± 14285 to 9136 min pg $ml^{-1}$ ± 14888; 19% decrease, p < 0.001; 95% CI -2855, -1411) by rifampicin treatment (*Fig. 2*). Before the rifampicin treatment, there was a significant difference in glucagon AUC between men and women (11269 min pg $ml^{-1}$ ± 14285 vs. 2794 min pg $ml^{-1}$ ± 1411; p = 0.026) and the fasting glucagon tended to be lower in women (men 125 pg $ml^{-1}$ ± 115, women 44.2 pg $ml^{-1}$ ± 28.1; p = 0.093). When comparing the percent changes between men and women, glucagon and GLP-1 secretion were differently affected by rifampicin; increased in women and decreased in men (glucagon AUC: 66% ± 45 increase in women, 42% ± 26 decrease in men, p = 0.0043; GLP-1 AUC: 37% ± 62 increase in women, 30% ± 32 decrease in men, p = 0.026).

Fasting GIP concentration was higher in men than women (35.5 pg $ml^{-1}$ ± 15.2 vs. 21.3 pg $ml^{-1}$ ± 7.5; p = 0.041) before rifampicin treatment but the difference was no longer statistically significant after rifampicin treatment (men 32.8 pg $ml^{-1}$ ± 16.9, women 25.8 pg $ml^{-1}$ ± 10.8; p = 0.6). Fasting PYY was higher in men than women after rifampicin treatment (240 pg $ml^{-1}$ ± 278 vs. 58.2 pg $ml^{-1}$ ± 51.1; p = 0.015). Also before the treatment, fasting PYY tended to be higher in men than women (men 246 pg $ml^{-1}$ ± 301, women 42.6 pg $ml^{-1}$ ± 68.1; p = 0.093). PYY AUC was significantly higher in men when compared with women before the treatment (men 29397 min pg $ml^{-1}$ ± 35516, women 7868 min pg $ml^{-1}$ ± 4309; p = 0.041). After the treatment PYY AUC was not significantly different between sexes (men 29089 min pg $ml^{-1}$ ± 31235, women 11923 min pg $ml^{-1}$ ± 6200; p = 0.24). There were no significant sex differences in the insulin and glucose fasting measures and AUCs (data not shown).

The effect of PXR activation was also studied in rats. Treatment of rats with PXR agonist PCN with the daily dose of 40 mg/kg for 1, 3 and 6 days resulted in induction of PXR target gene CYP3A2 in liver by 2.7, 3.2 and 2.3-fold ($p < 0.002$), respectively (data not shown). Furthermore, the rats were given a daily i.p. injection of PCN or vehicle for 4 days (nine rats per group), and the OGTT was performed on the morning of the fifth day. We have previously reported the glucose results (7); PCN caused a significant increase in glucose levels during the OGTT as compared with vehicle control (*Table 2*). Furthermore, insulin level was significantly elevated by PCN at 60 min time point but the insulin AUC and $AUC_{incr}$ were not significantly affected (*Fig. 3*). The C-peptide, GLP-1, GIP, and glucagon levels were not affected by PCN. Some of the rat GLP-1 OGTT time series had several values below the detection limit of the assay used. The time series of a particular rat were discarded from the AUC analyses if there were two or more values missing (2 PCN and 4 control rats excluded). Correspondingly, one GIP time series was discarded (a control rat). There were no significant differences in GLP-1 or GIP AUCs between the treatment arms even if these time series were included.

When the results of our previous human Rifa-1 study (7) and the current Rifa-2 study were combined, hyperglycaemic and hyperinsulinemic effects of rifampicin were evident (*Table 3*, *Fig. 4*). Glucose AUC was elevated by 10% ($p = 0.038$) and insulin AUC by 30% ($p = 0.012$). Incremental AUCs were increased by 63% (glucose; $p = 0.010$) and 37% (insulin; $p = 0.011$). There were no significant differences between women and men (data not shown).

**Discussion**

This study demonstrates that the administration of the prototypical PXR agonists PCN and rifampicin does not affect the secretion of incretin hormones during the OGTT in rats and humans. Although the metabolism of bile acids is modulated by rifampicin (17-18), the presumably lower

gut levels of secondary bile acids LCA and DCA are not reflected as attenuated secretion of incretins. In healthy young adults, 1-week rifampicin administration in comparison to a placebo arm in crossover design led to elevated glucose and insulin levels in the OGTT in our previous Rifa-1 study (7) while in the current Rifa-2 study with one-arm design no statistically significant effect was seen although glucose and insulin levels, especially the incremental AUCs, tended to increase by rifampicin dosing. When Rifa-1 and Rifa-2 results were combined, rifampicin increased glucose and insulin concentrations significantly during OGTT. In rats, glucose levels were increased by PCN as previously reported (7) and insulin concentration at 60 min was increased during OGTT while AUC and incremental AUC of insulin were not increased significantly. Regardless of this clear hyperglycaemic action in rats, no attenuating effect of PXR activation on incretin secretion was detected.

The combined results (Rifa-1 and Rifa-2) demonstrate that the PXR agonism-elicited hyperglycaemia and hyperinsulinemia are apparent when sample size increases although the Rifa-2 study in itself had statistically non-significant results. Rifa-1 study had a crossover study design with rifampicin and placebo arms, and Rifa-2 had a one-arm design. The subjects were young healthy adults with exactly the same amount and length of rifampicin dosing in both studies. Insulin was analysed in serum in Rifa-1 and in plasma in Rifa-2; this should not lead to obvious systematic error when combining the results as the reference ranges for plasma and serum insulin are the same. The method of insulin quantitation differed between the studies. As the statistical test is paired (intraindividual), a possible systematic error in insulin measurements between Rifa-1 and Rifa-2 should not affect the interpretation of the results. The absence of statistically significant glucose and insulin effect in the current study in contrast to the previous randomised Rifa-1 study is probably explained by, in addition to a chance afforded by a small sample size, a less robust one-arm study design. It could be speculated that glucose-elevating stress hormones such as cortisol

and catecholamines are elevated more pronouncedly during the OGTT in the first study visit in comparison to the OGTT in the second visit. In Rifa-1 study, this stress hormone-related effect should be equalised between the arms due to randomisation. To explore this hypothesis, we reanalysed Rifa-1 results according to the randomisation allocation (rifampicin or placebo arm first). While the rifampicin effect on AUC and incremental AUC of glucose remained statistically significant in 6 subjects with the rifampicin arm first, the rifampicin effect on OGTT glucose levels lost the statistical significance (but not the direction of the effect) in 6 subjects who had the placebo arm first (data not shown). This supports the notion that stress hormones may mask the glucose-elevating effects of drugs with a modest effect size, and emphasises the importance of a randomised study design in future studies.

Glucagon AUC was increased in women and decreased in men after rifampicin dosing. Also the percent changes in glucagon and GLP-1 secretion were significantly different when compared across sexes interindividually; both increased in women and decreased in men. It should be noted that the comparison of interindivivual sex differences (unpaired) is easily affected by the small number of women and men in the study. In contrast, the analysis in a paired fashion (intraindividually) is inherently more robust statistical analysis; this method was applied throughout this study, including the analysis of rifampicin effect on glucagon AUC in men and women separately.

Glucagon levels are known to be lower in women than men in fasting state, postprandially, and during hypoglycaemia (32-34), indicating that glucagon secretion is regulated in a sexually dimorphic manner in humans. Furthermore, glucagon induces PXR expression in mice primary hepatocytes (35) and PXR represses glucagon-regulated transcription of genes involved in gluconeogenesis in mice but induces gluconeogenic genes in human hepatic cells (36). Interestingly, female mice are more resistant to high-fat diet-induced (HFD) obesity than their

male counterparts while PXR-humanized transgenic female mice become rapidly obese and diabetic when exposed to HFD (13). These findings suggest that glucagon and PXR may be components of a network regulating glucose metabolism, possibly in species- and sex-specific fashion. As there were no significant sex differences in the insulin and glucose AUCs in the Rifa-2 and the combined Rifa-1 and Rifa-2 data, the significance of these findings is unknown and require confirmation in future studies.

Gut microbiota is known to affect incretin secretion in animal models and humans (37). Thus, it is possible that the antibiotic effect of rifampicin on the intestinal microflora might explain the hyperglycaemic effect. However, the effects of nonantibiotic PCN are unlikely to be mediated by changes in intestinal microbes. Furthermore, there is no clear evidence on the hyperglycaemic effects of antibiotics, although some fluoroquinolones have been associated with dysglycemia (hyperglycemia and hypoglycemia)(38). PXR agonism remains the most likely explanation for the hyperglycaemic effect shared by PCN and rifampicin. It is of note that PXR agonism has been linked with amelioration of the inflammatory bowel disease in animal models (39-41). The suppression of intestinal inflammation may have effects on glucose metabolism that require further study.

Although the difference in glucose concentration is modest in absolute terms (up to 1 mmol $l^{-1}$ during OGTT), the about 60% increase in glucose $AUC_{incr}$ and the about 40% increase in insulin $AUC_{incr}$ by rifampicin (combined Rifa-1 and Rifa-2 analysis) show that there is a noticeable disturbance in postprandial glucose metabolism. This may precipitate type II diabetes in patients with existing impaired glucose tolerance treated for longer periods of time with rifampicin or other efficient PXR agonists such as antiepileptics (carbamazepine, phenytoin, barbiturates), some antiretrovirals, bosentan, and St. John's wort (2). Interestingly, epidemiological links between exposure to chemicals and disorders of glucose homeostasis have been established (42-43). It is possible that long-term exposure to environmental toxicants with PXR-activating properties

including some pesticides, plasticizers such as bisphenol A, and certain polychlorinated biphenyls could contribute to increased risk of type II diabetes (44). These hypotheses should be explored in future studies.

We conclude that the activation of PXR does not affect the secretion of incretin hormones. However, the regulation of glucagon secretion by PXR may be sexually dimorphic in humans. The exact mechanism of disrupted glucose metabolism induced by PXR activation requires further study.

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**Declaration of Interest**

No conflict of interest.

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*Fig. 1*. Effect of the treatment with 600 mg rifampicin once daily for seven days on glucose, insulin, glucagon-like peptide-1 (GLP-1), gastric inhibitory polypeptide (GIP), glucagon, and peptide YY (PYY) levels of oral glucose tolerance test in healthy volunteers (Rifa-2 study). Values are represented as means ± SD.

*Fig. 2*. Effect of the treatment with 600 mg rifampicin once daily for seven days on glucagon, glucagon-like peptide-1 (GLP-1), gastric inhibitory polypeptide (GIP), and peptide YY (PYY) levels of oral glucose tolerance test in healthy volunteers (Rifa-2 study) in women and men. Values are represented as means ± SD. * $P < 0.05$ rifampicin vs. control.

*Fig. 3*. Effect of 4-day i.p. 40 mg/kg pregnenolone 16α-carbonitrile (PCN) vs. vehicle control on insulin, C-peptide, glucagon-like peptide-1 (GLP-1), gastric inhibitory polypeptide (GIP), and glucagon levels of oral glucose tolerance test in rats. Values are represented as means ± SD. *$P < 0.05$ PCN vs. control.

*Fig. 4*. Effect of the treatment with 600 mg rifampicin once daily for seven days on glucose metabolism in healthy volunteers (combined Rifa-1 and Rifa-2 study results). Values are represented as means ± SD. *$P < 0.05$ rifampicin vs. control.