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Chronic administration of hydrolysed pine nut oil to mice improves insulin sensitivity and glucose tolerance and increases energy expenditure via a free fatty acid receptor 4-dependent mechanism

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Short title: hPNO improves insulin sensitivity via FFAR4

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Abstract

A healthy diet is at the forefront of measures to prevent type 2 diabetes. Certain vegetable and fish oils, such as pine nut oil (PNO), have been demonstrated to ameliorate the adverse metabolic effects of a high-fat diet. The present study study investigates the involvement of the free fatty acid receptors 1 (FFAR1) and 4 (FFAR4) in the chronic activity of hydrolysed PNO (hPNO) on high-fat diet-induced obesity and insulin resistance. Male C57BL/6J wild-type, FFAR1 knockout (-/-) and FFAR4-/- mice were placed on 60% high-fat diet for 3 months. Mice were then dosed hPNO for 24 days, during which time body composition, energy intake and expenditure, glucose tolerance and fasting plasma insulin, leptin and adiponectin were measured. hPNO improved glucose tolerance and decreased plasma insulin in the wild-type and FFAR1-/- mice, but not the FFAR4-/- mice. hPNO also decreased high-fat diet-induced bodyweight gain and fat mass, whilst increasing energy expenditure and plasma adiponectin. None of these effects on energy balance were statistically significant in FFAR4-/- mice but it was not shown that they were significantly less than in wild-type mice. In conclusion, chronic hPNO supplementation reduces the metabolically detrimental effects of high-fat diet on obesity and insulin resistance in a manner that is dependent on the presence of FFAR4.

Keywords: pine nut oil, FFAR1, FFAR4, high-fat diet, insulin resistance, glucose tolerance, energy expenditure

Introduction

In 2017, there were 462 million people with type 2 diabetes (T2D), corresponding to 6.3% of the global population, and this is estimated to increase to over 7% by 2030 [1]. Whilst genetic factors are strongly involved in susceptibility to this disease [2], a healthy diet and regular physical activity are important in preventing the disease [3]. The same is true of obesity [3], which is a major cause of T2D.

Some dietary oils, such as marine fish oils [4, 5] and olive oil-based diets [6], have been associated with protection against metabolic disorders [7]. Non-esterified fatty acids (NEFAs) are known to exert biological effects by acting as precursors of various oxidised messenger molecules and by acting directly on both intracellular and cell surface receptors. Their established biological activities suggest that NEFAs may be the active ingredients responsible for dietary health benefits [8].

The free fatty acid receptors FFAR1 (GPR40) and FFAR4 (GPR120) are G protein-coupled 7-transmembrane receptors that are activated by medium- to long-chain NEFAs and have been proposed as therapeutic targets for the treatment of T2D and obesity [9, 10]. FFAR1 is highly expressed in pancreatic β-cells and enhances glucose-stimulated insulin secretion in response to various medium- and long-chain NEFAs [11, 12, 13]. The receptor has been clinically validated as a target for treatment of T2D by a phase 2 and 3 clinical study that investigated the synthetic agonist TAK-875 [14]. FFAR1 expression in enteroendocrine cells has been associated with release of glucose- and the appetite-regulating hormones glucagon-like peptide-1 (GLP-1), glucose-dependent insulinotropic polypeptide and cholecystokinin (CCK) [15, 16, 17].

FFAR4 is expressed in intestinal enteroendocrine cells, where activation is reported to increase secretion of GLP-1, although this is controversial [18], and to inhibit secretion of the orexigenic hormone ghrelin [19, 20, 21, 22]. FFAR4 is also expressed in the pancreas, adipose tissue, macrophages, and the brain, and has been associated with the protection of islets, improvement of insulin sensitivity and the mediation of anti-inflammatory and appetite-lowering effects [23, 24, 25, 26, 27, 28].

Pine nut oil (PNO) supplementation has been found to alleviate the obesity caused by a high-fat diet in rats [29]. When delivered to the small intestine by delayed-release capsules, hydrolysed PNO (hPNO) enhances insulin sensitivity and acutely improves glucose tolerance in humans [30, 31]. Delayed-release PNO and pinolenic acid also reduce appetite, possibly by augmenting GLP-1 release and attenuating ghrelin secretion in the late postprandial state [32, 33]. In addition, pine nut oil and pinolenic acid increase plasma levels of the appetite-suppressing gut hormones GLP-1 and CCK in obese, post-menopausal women [32, 33].

Pinolenic acid, a major component (~20%) of PNO, acutely improves glucose tolerance via agonism of both free fatty acid receptors FFAR1 and FFAR4 [34]. A lack of FFAR4 in mice or dysfunctional FFAR4 in humans has been linked to an increased risk of obesity [35], whilst chronic dosing of a nonacidic sulphonamide FFAR4 agonist to high-fat diet-induced obese mice resulted in a mild improvement in obesity and a substantial improvement in insulin sensitivity [36].

To investigate the involvement of these receptors in the activity of PNO and pinolenic acid, the present study examined the effect of chronically administered hPNO on high-fat diet-induced obesity and insulin resistance in wild-type and FFAR1 and FFAR4 knockout mice.

Materials and Methods

All procedures were conducted in accordance with the UK Government Animals (Scientific Procedures) Act 1986 and approved by the University of Buckingham Ethical Review Board (Bu16004). Male wild-type mice were obtained from Charles Rivers (Maidstone, Kent, UK). Mice were received at five weeks of age. FFAR1-/- and FFAR4-/- mice on a C57BL/6J background (Taconic Biosciences, New York, USA) were maintained in-house and were crossed to the Bl6 background over more than eight generations.

The mice were housed in cages of three such that there were seven cages for each genotype and treatment, except that there were only enough mice for two cages of control FFAR4-/- mice (see Supplementary Table 1 for number of animals per group. These numbers did not change throughout the dosing period. Mice were housed at 22^{o}C with lights on at 08.00h, lights off at 20.00h and fed on standard laboratory chow (Beekay Feed; B&K Universal Ltd., Hull, UK) until 6 weeks of age, then transferred to a high-fat diet (60% by metabolizable

energy; D12492, Research Diets, New Brunswick, NJ, USA) for 3 months. The diets conform with AIN93 regarding vitamin, mineral and protein content.

Mice were then dosed 250 mg.kg^{-1} hPNO or vehicle (10% DMSO, 10% Cremophor®, 80% mannitol solution (5% mannitol$_{aq}$) by oral gavage twice a day (one hour after lights on, and one hour before lights out for 25 days). hPNO was produced by treatment of PNO (The Siberian Pines Company, Stara Zagora, Bulgaria) with aqueous NaOH as described previously [31]. The fatty acid composition of hPNO was 20.2% pinolenic acid, 46.7% linoleic acid, 23.0% oleic acid, 4.1% palmitic acid, 2.3% stearic acid, 1.1% eicosenoic acid, 1.0% eicosatrienoic acid, 0.6% eicosadienoic acid, and 0.5% α-linolenic acid, as determined by methyl ester formation and analysis by gas chromatography [34]. 250 mg hPNO was initially dissolved in 1 ml DMSO, followed by 1 ml Cremophor®, and finally 8 ml of 5% mannitol$_{aq}$. The hPNO solution or vehicle was made fresh before each dose and used within 30 min. The dose volume was 10 ml.kg^{-1}. Bodyweight was measured on days 0 (before the first dose), 7, 14, 21 and 24 (day of termination).

Day 0 bodyweights were not significantly different between genotypes and treatment groups (Supplementary Table 1). Energy expenditure was measured on day 7 by open-circuit indirect calorimetry with mice in their home cages [37, 38, 39]. An oral glucose tolerance test was performed on day 21. After fasting for five hours, mice were dosed with glucose (3 g.kg^{-1}, body weight PO by gavage). Blood samples were collected from the tail at -30, 0, +30, +60, +120 and +180 minutes, relative to glucose dosing. Blood glucose was measured using a glucose oxidase reagent kit (Gluc-PAP, GL2623; Randox, Crumlin, UK). Plasma insulin was measured by ELISA (Ultra-Sensitive Mouse Insulin ELISA kit, Catalog #: 90080; Crystal Chem, Downers Grove, IL, USA). Body fat and lean content was measured on day 23 using a Minispec LF90II Nuclear Magnetic Resonance (Bruker Corporation, Germany). Mice were culled by concussion followed by cervical dislocation 5 hours after the morning dose on day 24 and a terminal blood sample was collected for plasma leptin (Catalog #: 90030; Chrystal Chem) and adiponectin (Catalog # MRP300; R&D Systems, Minneapolis, MN, USA) ELISA measurements.

Body fat and lean content were measured at termination using a Minispec LF90II Nuclear Magnetic Resonance (Bruker Corporation, Germany).

Only differences between hPNO- and vehicle-treated mice were tested for significance to avoid the complications of interpreting multiple comparisons [40]. The statistical significance of any differences between vehicle-treated animals and drug-treated animals was determined using Prism 10.0 (GraphPad Software Inc., San Diego, California, USA) by 2-way ANOVA (genotype; treatment with hPNO) followed by Sidak's post-tests. Statistical significance is shown as: *p <0.05, **p <0.01; ***p <0.001; ****p <0.0001.

Results

Energy balance

Two-way ANOVA followed by Sidak's multiple comparison test showed that hPNO significantly reduced bodyweight change in wild-type (p<0.05) and FFAR1-/- (p<0.05), but not FFAR4-/- mice over the 24 days dosing regimen (Figure 1). However, energy intake was not affected by hPNO in any of the genotypes (Figure 2). Likewise, hPNO significantly reduced fat mass in wild-type (p<0.05) and FFAR1-/- mice (p<0.01) but not FFAR4 knockout mice, whereas no difference in lean mass was observed between the groups (Figure 3). hPNO also caused a significant increase in energy expenditure in the wild-type (p<0.05) and FFAR1-/- mice (p<0.05) but did not have a significant effect in FFAR4 knockout mice (Figure 4).

Consistent with the effect on body fat content, hPNO significantly reduced plasma leptin levels in wild-type (p<0.05) and FFAR1 knockout mice (p<0.001), though not in FFAR4-/- mice (Figure 5A). In addition, hPNO significantly increased plasma adiponectin in wild-type (p<0.01) and FFAR1 knockout (p<0.05), but not FFAR4 knockout mice (Figure 5B). Also, in concordance with the whole-body fat measurement, hPNO significantly decreased interscapular fat pad mass in wild-type (p<0.05) and FFAR1-/- mice (p<0.001), but did not have a significant effect in FFAR4-/- mice (Figure 6). However, neither the epididymal nor the inguinal fat pad masses were significantly affected.

Glucose homeostasis

hPNO improved glucose tolerance overall in wild-type ($P < 0.05$, figure 7A) and FFAR1-/- ($P < 0.01$, Figure 7B) and specifically at 30- and 60-minutes post glucose load. hPNO did not affect glucose tolerance in FFAR4-/- mice either overall or at any time point (Figure 7C). Fasting plasma insulin was significantly lowered by hPNO in wild type ($p<0.01$) and FFAR1-/- ($p<0.05$) mice (Figure 7D and E). There was no significant effect of hPNO in FFAR4-/- mice ($p=0.24$, Figure 7F).

Discussion

Several studies, primarily in rodents and cells, suggest that PNO and pinolenic acid reduce appetite and have potential benefits in human health [32, 33]. Recent clinical studies support this suggestion in finding that hPNO (3 or 6 g) acutely promotes GLP-1 release and reduces appetite in humans [30, 31], although no effect on glucose tolerance or insulin sensitivity was found in these studies. Pinolenic acid, a major component (~20%) of PNO, is a dual agonist of the free fatty acid receptors FFAR1 and FFAR4 that improves glucose tolerance acutely [41]. FFAR1 activation improves glucose tolerance by increasing insulin secretion by the pancreatic β-cells [42]). FFAR4 signalling occurs through the Gαq/11 and Gαi/o pathways and the noncanonical β-arrestin pathway [43, 44], with the activation of Gαq/11 found to increase the translocation of glucose transporter type-4 to cell membranes in adipocytes and increases glucose uptake, whereas β-arrrestin2 mediates anti-inflammatory effects [23]. A lack of FFAR4 in mice or dysfunctional FFAR4 in humans has been linked to an increased risk of obesity [35]. To investigate the involvement of these receptors in the activity of pinolenic acid and PNO, this study examined the activity of hPNO on high-fat diet-induced obesity and insulin resistance in wild-type, FFAR1-/- and FFAR4-/- mice.

The daily dose of hPNO used in the present study was 250 $mg.kg^{-1}$ orally twice daily. This is equivalent to a total dose 2.8 g daily in a human weighing 70 kg if doses are comparable on a body surface area [45]. This study shows that daily dosing with hPNO for 21 days (without the acute dose prior to the glucose tolerance test) improved insulin resistance and glucose tolerance in a high-fat diet-induced model of obesity and diabetes. hPNO has a high energy content, but the present study shows that the beneficial effects on insulin sensitivity, glucose

tolerance and energy expenditure are obtained with dose levels that do not add significantly to overall energy intake or adiposity.

The effect of hPNO on glucose tolerance and insulin sensitivity was dependent on the presence of the FFAR4 receptor. This is consistent with previous publications which show that whilst chronic FFAR4 activation improves glucose tolerance by enhancing insulin sensitivity [36, 46], FFAR1 activation instead improves glucose tolerance by enhancing glucose-induced insulin secretion [34, 47]. FFAR1 activation retains insulin secretagogue activity even after chronic high-fat feeding [48] or chronic dosing with a specific FFAR1 agonist [41], so FFAR1-mediated effects cannot be excluded in the present study. However, hPNO was not given immediately prior to glucose tolerance tests or plasma insulin measurements and the effects of hPNO on glucose tolerance and insulin sensitivity were the same in FFAR1-/- and wild-type mice. Moreover, others have shown that the combined deletion of FFAR1 and FFAR4 minimally impacts glucose homeostasis in mice compared to deletion of FFAR4 alone [49].

In this study, administration of hPNO for 24 days reduced bodyweight gain, whole body fat content and interscapular fat pad mass of mice on a high-fat diet via FFAR4 without affecting energy intake. Energy expenditure was also increased by hPNO in wild-type but not FFAR4-/- mice, suggesting that FFAR4 plays a major role. Other receptors may contribute to the effects of PNO on insulin sensitivity and glucose tolerance, but the present study suggests that FFAR4 plays a major role.

Adiponectin increases energy expenditure [50] and, as the effect of hPNO on plasma adiponectin was similar to that on energy expenditure in this study, increased adiponectin levels may have been the causative factor. However, it has been shown that omega-3 polyunsaturated fatty acids can increase circulating adiponectin in mice independently of FFAR4 although these effects were not shown to be directly associated with an effect on energy expenditure [51]. In contrast, the main effect of hPNO in this study was found to depend on FFAR4.

Conclusions

In conclusion, hPNO is effective in reducing high-fat diet-induced obesity, insulin resistance and glucose intolerance. These effects are dependent on the presence of FFAR4. PNO or pinolenic acid could have a place in a dietary or nutraceutical approach directed at impeding the development of T2D.

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Declaration of Interests

The authors declare none.

Authorship

ETW was responsible for data curation (lead), formal analysis (lead), investigation (lead), methodology (lead) and writing the original draft (lead)

MK was responsible for formal analysis (supporting), investigation (supporting) and writing review and editing (supporting)

MHK was responsible for methodology (supporting) and resources (supporting)

ERU was responsible for methodology (supporting), resources (supporting) and writing review and editing (equal)

JA was responsible for resources (supporting), writing the original draft (supporting) and writing review and editing (equal)

TU was responsible for conceptualization (equal), funding acquisition (lead), project administration (equal) and writing review and editing (equal)

CJS was responsible for data curation (supporting), funding acquisition (supporting), methodology (supporting), project administration (equal), resources (equal), supervision (lead), validation (equal), visualization (equal) and writing review and editing (equal)

Abbreviations:

CCK: cholecystokinin

GLP-1: glucagon-like peptide-1 (GLP-1)

FFAR1: free fatty acid receptor 1

FFAR4: free fatty acid receptor 4

hPNO: hydrolysed pine nut oil

GPR: G protein-coupled receptor

NEFA: non-esterified fatty acid

PNO: pine nut oil

T2D: type 2 diabetes mellitus

References

1. Khan MAB, Hashim MJ, King JK *et al*. (2020) Epidemiology of Type 2 Diabetes – Global Burden of Disease and Forecasted Trends. *J Epidemiol Glob Health* **10**, 107–111.

2. Prasad RB, Groop L (2015) Genetics of type 2 diabetes-pitfalls and possibilities. *Genes (Basel).* **6**, 87-123.

3. World Health Organisation (2021) Diabetes. https://www.who.int/news-room/fact-sheets/detail/diabetes (accessed August 2023).

4. Wu JH, Micha R, Imamura F *et al*. (2012) Omega-3 fatty acids and incident type 2 diabetes: a systematic review and meta-analysis. *Br J Nutr* **107**, S214–S227.

5. Yanai H, Hamasaki H, Katsuyama H *et al*. (2015) Effects of intake of fish or fish oils on the development of diabetes. *J Clin Med Res* **7**, 8–12.

6. Pérez-Martínez P, García-Ríos A, Delgado-Lista J *et al*. (2011) Mediterranean diet rich in olive oil and obesity, metabolic syndrome and diabetes mellitus. *Curr Pharm Des* **17**, 769–777.

7. Forouhi NG, Krauss RM, Taubes G *et al*. (2018) Dietary fat and cardiometabolic health: evidence, controversies, and consensus for guidance. *BMJ* **361**: k2139.

8. Ulven T, Christiansen E (2015) Dietary Fatty Acids and Their Potential for Controlling Metabolic Diseases Through Activation of FFA4/GPR120. *Annu Rev Nutr* **35**, 239-263.

9. Watterson KR, Hudson BD, Ulven T *et al*. (2014) Treatment of type 2 diabetes by free Fatty Acid receptor agonists. *Front Endocrinol (Lausanne)* **5**, 137.

10. Kimura I, Ichimura A, Ohue-Kitano R *et al*. (2020) Free Fatty Acid Receptors in Health and Disease. *Physiol Rev* **100**, 171-210.

11. Itoh Y, Kawamata Y, Harada M *et al*. (2003) Free fatty acids regulate insulin secretion from pancreatic b cells through GPR40. *Nature* **422**, 173–176.

12. Briscoe CP, Peat AJ, McKeown SC *et al*. (2006) Pharmacological regulation of insulin secretion in MIN6 cells through the fatty acid receptor GPR40: identification of agonist and antagonist small molecules. *Br J Pharmacol* **148**, 619–628.

13. Del Guerra S, Bugliani M, D'Aleo V *et al*. (2010) G-protein coupled receptor 40 (GPR40) expression and its regulation in human pancreatic islets: the role of type 2 diabetes and fatty acids. *Nutr Metab Cardiovasc Dis* **20**, 22–25.

14. Burant CF, Viswanathan P, Marcinak J *et al*. (2012) TAK-875 versus placebo or glimepiride in type 2 diabetes mellitus: a phase 2, randomised, double-blind, placebo-controlled trial. *Lancet* **379**, 1403–1411.

15. Edfalk S, Steneberg P, Edlund H. (2008) Gpr40 is expressed in enteroendocrine cells and mediates free fatty acid stimulation of incretin secretion. *Diabetes* **57**, 2280–2287.

16. Liou AP, Lu X, Sei Y *et al*. (2011) The G-protein-coupled receptor GPR40 directly mediates long-chain fatty acid induced secretion of cholecystokinin. *Gastroenterology* **140**, 903–912.

17. Luo J, Swaminath G, Brown SP *et al*. (2012) A potent class of GPR40 full agonists engages the enteroinsular axis to promote glucose control in rodents. *PLOS ONE* **7**, e46300.

18. Secor JD, Fligor SC, Tsikis ST *et al*. (2021) Free Fatty Acid Receptors as Mediators and Therapeutic Targets in Liver Disease. *Front Physiol* **12**, 656441b.

19. Hirasawa A, Tsumaya K, Awaji T *et al*. (2005) Free fatty acids regulate gut incretin glucagon-like peptide-1 secretion through GPR120. *Nat Med* **11**, 90–94.

20. Engelstoft MS, Park WM, Sakata I *et al*. (2013) Seven transmembrane G protein-coupled receptor repertoire of gastric ghrelin cells. *Mol Metab* **2**, 376–392.

21. Gong Z, Yoshimura M, Aizawa S *et al*. (2014) G protein coupled receptor 120 signalling regulates ghrelin secretion in vivo and in vitro. *Am J Physiol Endocrinol Metab* **306**, E28–E35.

22. Paulsen SJ, Larsen LK, Hansen G *et al*. (2014) Expression of the fatty acid receptor GPR120 in the gut of diet induced-obese rats and its role in GLP-1 secretion. *PLOS ONE* **9**, e88227.

23. Oh DY, Talukdar S, Bae EJ *et al.* (2010) GPR120 is an omega-3 fatty acid receptor mediating potent anti-inflammatory and insulin-sensitizing effects. *Cell* **142**, 687–698.

24. Cintra DE, Ropelle ER, Moraes JC *et al*. (2012) Unsaturated fatty acids revert diet-induced hypothalamic inflammation in obesity. *PLOS ONE* **7**, e30571.

25. Li X, Yu Y, Funk CD. (2013). Cyclooxygenase-2 induction in macrophages is modulated by docosahexaenoic acid via interactions with free fatty acid receptor 4 (FFA4). *FASEB J* **27**, 4987-4997.

26. Stone VM, Dhayal S, Brocklehurst KJ *et al*. (2014) GPR120(FFAR4) is preferentially expressed in pancreatic delta cells and regulates somatostatin secretion from murine islets of Langerhans. *Diabetologia* **57**, 1182 –1191.

27. Wellhauser L, Belsham DD. (2014) Activation of the omega-3 fatty acid receptor GPR120 mediates anti-inflammatory actions in immortalized hypothalamic neurons. *J Neuroinflammation* **11**, 60.

28. Dragano NRV, Solon C, Ramalho AF *et al*. (2017) Polyunsaturated fatty acid receptors, GPR40 and GPR120, are expressed in the hypothalamus and control energy homeostasis and inflammation. *J Neuroinflammation* **14**, 91.

29. Bhandari C, Agnihotr N. (2022) Pine nut oil supplementation alleviates the obesogenic effects in high-fat diet induced obese rats: A comparative study between epididymal and retroperitoneal adipose tissue. *Nutr Res* **106**, 85-100.

30. Sørensen KV, Kaspersen MH, Ekberg JH *et al*. (2021) Effects of Delayed-Release Olive Oil and Hydrolyzed pine nut oil on Glucose Tolerance, Incretin Secretion and Appetite in Humans. *Nutrients* **13**, 3407.

31. Sørensen KV, Korfitzen SS, Kaspersen MH *et al*. (2021) Acute effects of delayed-release hydrolyzed pine nut oil on glucose tolerance, incretins, ghrelin and appetite in healthy humans. *Clin Nutr* **40**, 2169-2179.

32. Baker EJ, Miles EA, Calder PC. (2021) A review of the functional effects of pine nut oil, pinolenic acid and its derivative eicosatrienoic acid and their potential health benefits. *Prog Lipid Res* **82**, 101097.

33. Pasman WJ, Heimerikx J, Rubingh CM *et al*. (2008) The effect of knockoutrean pine nut oil on in vitro CCK release, on appetite sensations and on gut hormones in post-menopausal overweight women. *Lipids Health Dis* **7**, 10.

34. Christiansen E, Watterson KR, Stocker CJ *et al*. (2015) Activity of dietary fatty acids on FFA1 and FFA4 and characterisation of pinolenic acid as a dual FFA1/FFA4 agonist with potential effect against metabolic diseases. *Br J Nutr* **113**, 1677-1688.

35. Ichimura A, Hirasawa A, Poulain-Godefroy O *et al*. (2012) Dysfunction of lipid sensor GPR120 leads to obesity in both mouse and human. *Nature* **483**, 350–354.

36. Azevedo CM, Watterson KR, Wargent ET *et al*. (2016). Non-Acidic Free Fatty Acid Receptor 4 Agonists with Antidiabetic Activity. *J Med Chem* **59**, 8868-8878.

37. Arch JRS, Hislop D, Wang SJY *et al*. (2006) Some mathematical and technical issues in the measurement and interpretation of open-circuit indirect calorimetry in small animals. *Int J Obes* **30**, 1322-1331.

38. Stocker CJ, Wargent E, O'Dowd J *et al*. (2007) Prevention of diet-induced obesity and impaired glucose tolerance in rats following administration of leptin to their mothers. *Am J Physiol Regul Integr Comp Physiol* **292**, R1810-R1818.

39. Wargent ET, Ahmad SJS, Lu QR *et al*. (2021). Leanness and Low Plasma Leptin in GPR17 Knockout Mice Are Dependent on Strain and Associated With Increased Energy Intake That Is Not Suppressed by Exogenous Leptin. *Front Endocrinol* **12**, 698115.

40. West CP, Dupras DM. (2013). 5 ways statistics can fool you- tips for practicing clinicians. *Vaccine*. **31**, 1550-1552.

41. Christiansen E, Hansen SV, Urban C *et al*. (2013) Discovery of TUG-770: A Highly Potent Free Fatty Acid Receptor 1 (FFA1/GPR40) Agonist for Treatment of Type 2 Diabetes. *ACS Med Chem Lett* **4**, 441-445.

42. Nolan CJ, Madiraju MSR, Delghingaro-Augusto V *et al*. (2006) Fatty Acid Signaling in the β-Cell and Insulin Secretion. *Diabetes* **55**, S16–S23.

43. Im DS. (2018) FFA4 (GPR120) as a fatty acid sensor involved in appetite control, insulin sensitivity and inflammation regulation. *Mol Aspects Med* **64**, 92-108.

44. Hilgendorf KI, Johnson CT, Mezger A *et al*. (2019) Omega-3 fatty acids activate ciliary FFAR4 to control adipogenesis. *Cell* **179**, 1289-1305.

45. Nair AB, Jacob S. (2016) A simple practice guide for dose conversion between animals and human. *J Basic Clin Pharm* **7**, 27-31.

46. Satapati S, Qian Y, Wu MS *et al*. (2017) GPR120 suppresses adipose tissue lipolysis and synergizes with GPR40 in antidiabetic efficacy. *J Lipid Res* **58**, 1561-1578.

47. Hamid YH, Vissing H, Holst B *et al*. (2005) Studies of relationships between variation of the human G protein-coupled receptor 40 Gene and Type 2 diabetes and insulin release. *Diabet Med* **22**, 74-80.

48. Kebede M, Alquier T, Latour MG *et al*. (2008). The fatty acid receptor GPR40 plays a role in insulin secretion in vivo after high-fat feeding. *Diabetes* **57**, 2432-2437.

49. Croze ML, Guillaume A, Ethier M *et al*. (2021) Combined Deletion of Free Fatty-Acid Receptors 1 and 4 Minimally Impacts Glucose Homeostasis in Mice. *Endocrinology* **162**, bqab002.

50. Vasseur F, Leprêtre F, Lacquemant C *et al*. (2003) The genetics of adiponectin. *Current Diabetes Reports* **3**, 151–158.

51. Pærregaard SI, Agerholm M, Serup AK *et al*. (2016) FFAR4 (GPR120) Signaling Is Not Required for Anti-Inflammatory and Insulin-Sensitizing Effects of Omega-3 Fatty Acids. *Mediators Inflamm*. 2016, 1536047.

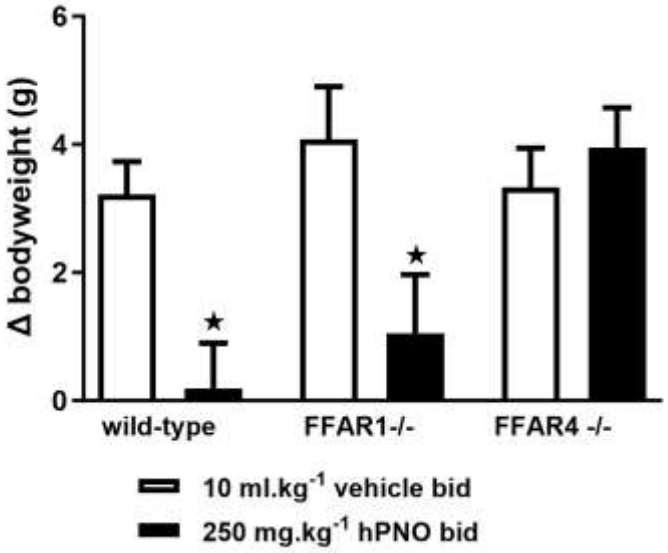

Figure 1. Bodyweight change of wild-type, FFAR1-/- and FFAR4-/- mice on high-fat diet during 24 days of treatment with 250 mg.kg^{-1} hPNO bid. Two-way ANOVA followed by Sidak's multiple comparison test showed no statistically significant effect of hPNO or genotype, or interaction between treatment and genotype. Results are means of 21 values (19 for FFAR4-/- control dose) ± SEM. ★ $P < 0.05$ for differences between mice given vehicle and PNO.

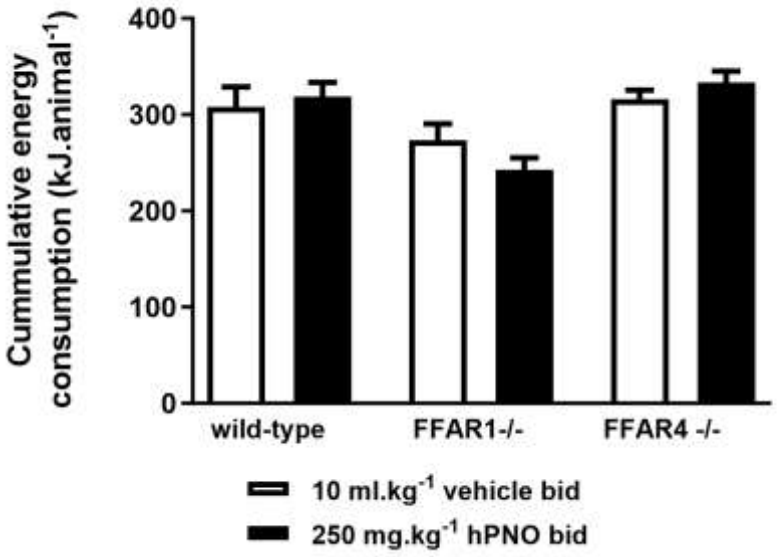

Figure 2. Cumulative energy intake of wild-type, FFAR1-/- and FFAR4-/- mice on high-fat diet during 24 days of treatment with 250 mg.kg^{-1} hPNO bid. Two-way ANOVA followed by Sidak's multiple comparison test showed no significant effect of hPNO. Results are means of 7 values ± SEM.

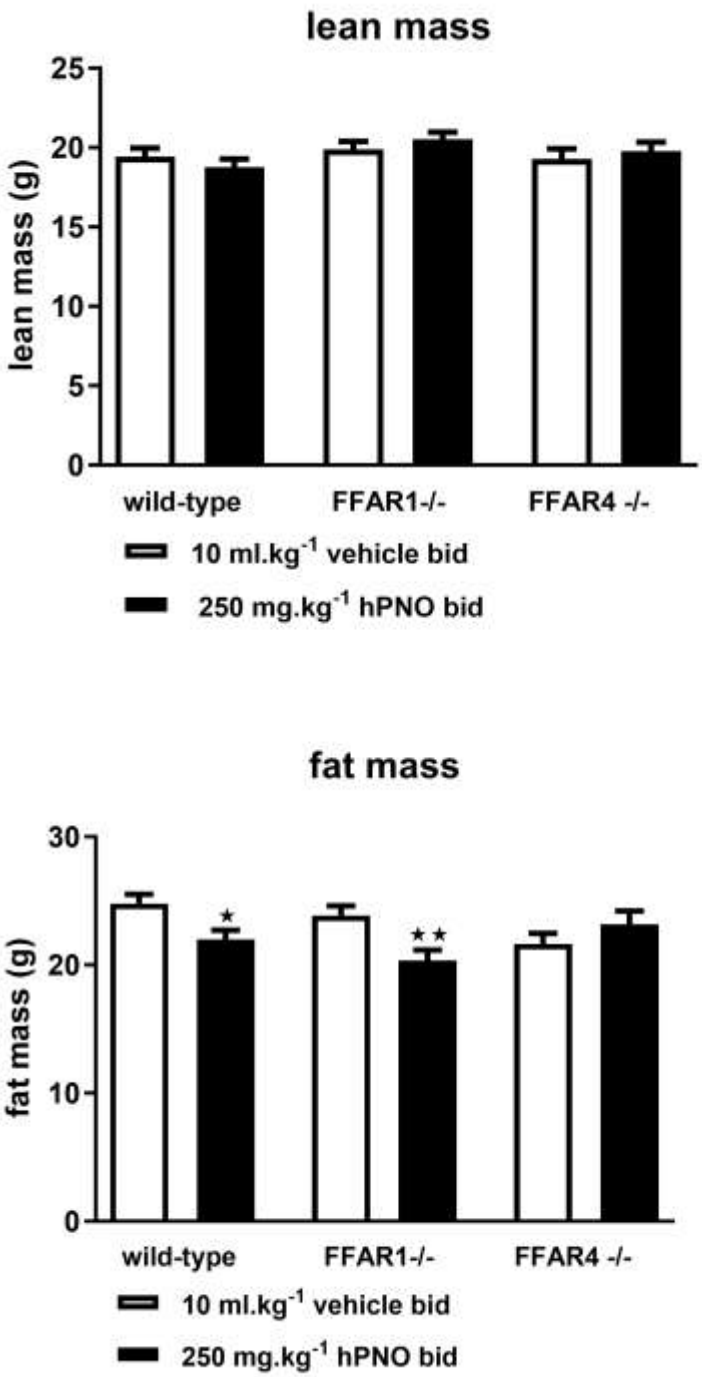

Figure 3. Body composition (A, lean mass and B, fat mass) in wild-type, FFAR1-/- and FFAR4-/- mice on high-fat diet after 23 days of treatment with 250 mg.kg^{-1} hPNO bid. Results are means of 21 values (19 for FFAR4 knockout control dose) ± SEM. ★ $P < 0.05$, ★★ $P < 0.01$ for differences between mice given vehicle and PNO.

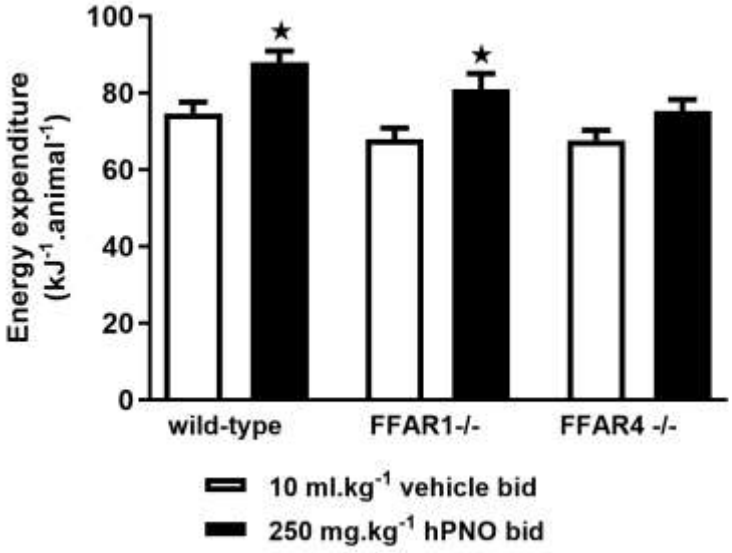

Figure 4. Total 24-hour energy expenditure on day 7. Results are means of 7 values ± SEM. ★ $P < 0.05$ for differences between mice given vehicle and PNO.

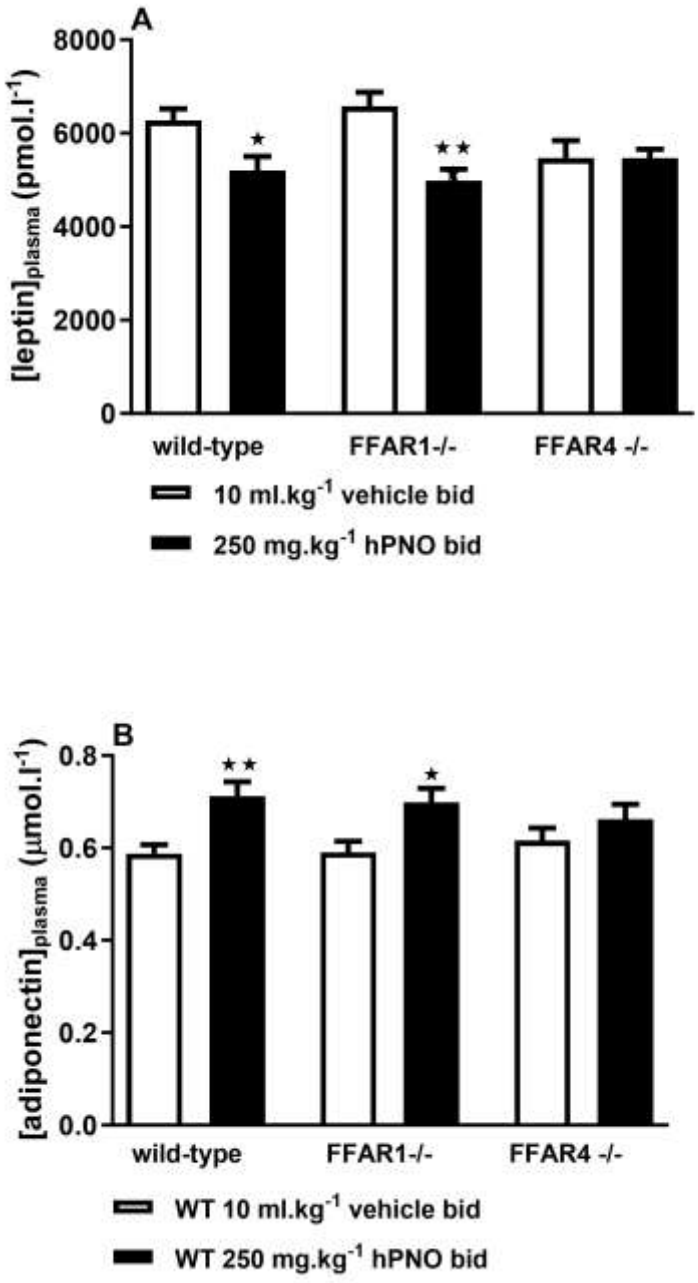

Figure 5. Plasma leptin (A) and adiponectin (B) in wild-type, FFAR1 knockout and FFAR4 knockout mice on high-fat diet after 24 days of treatment with 250 mg.kg^{-1} hPNO bid. Results are means of 21 values (19 for FFAR4-/- control dose) ± SEM. ★ $P < 0.05$, ★★ $P<0.01$, ★★★ $P < 0.001$ for differences between mice given vehicle and PNO.

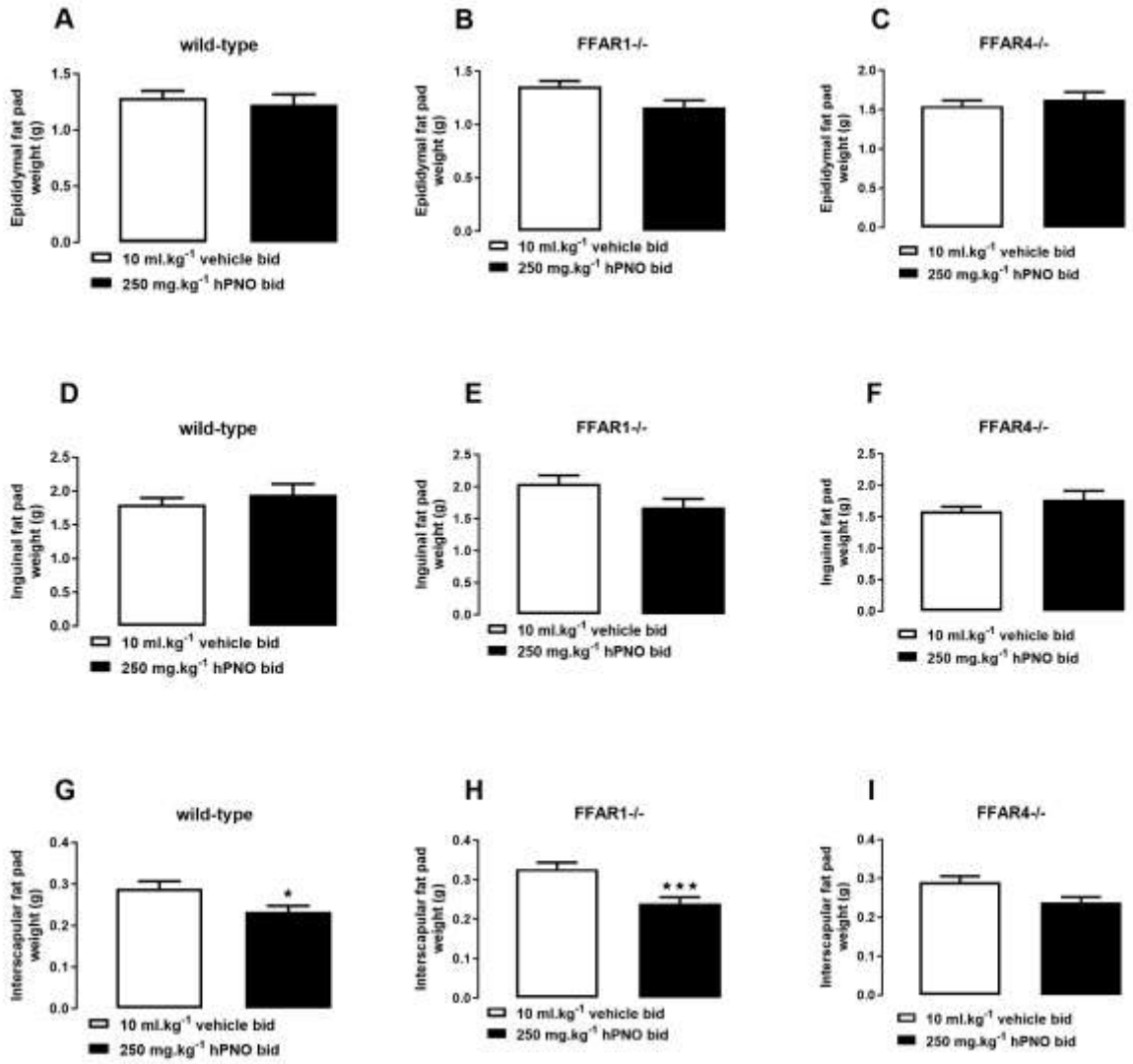

Figure 6. Epididymal (A-C), inguinal (D-F) and interscapular (G-I) fat pad weights in wild-type, FFAR1-/- and FFAR4-/- mice on high-fat diet after 24 days of treatment with 250 mg.kg^{-1} hPNO bid. Results are means of 21 values (19 for FFAR4-/- control dose) ± SEM. ★ $P < 0.05$, ★★ $P<0.01$, ★★★ $P < 0.001$ for differences between mice given vehicle and PNO.

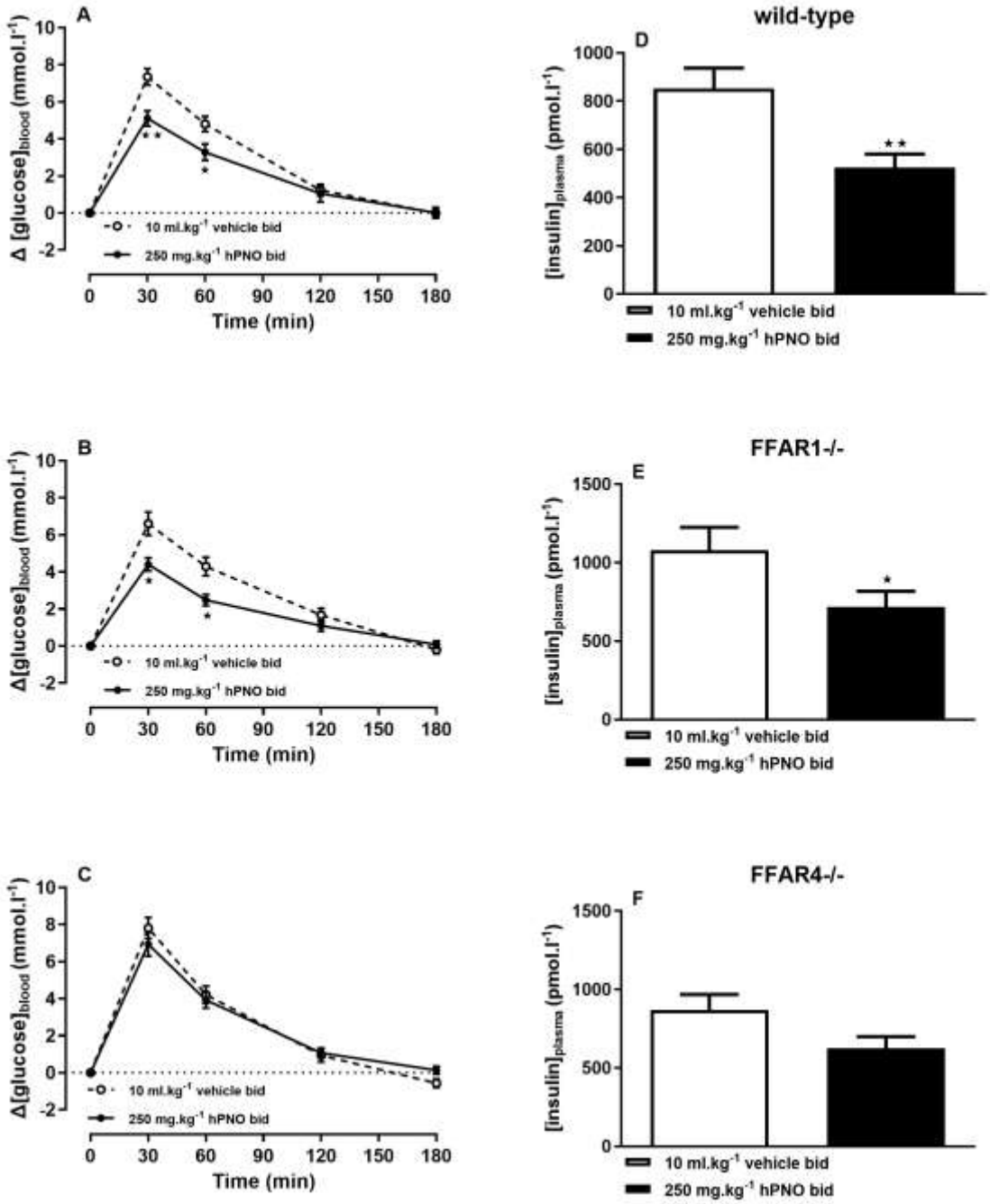

Figure 7. Change in blood glucose levels during an oral glucose tolerance test in wild-type (A), FFAR1-/- (B) and FFAR4-/- (C) mice on high-fat diet after 21 days of treatment with 250 mg.kg^{-1} hPNO bid. Fasting plasma insulin levels (after 5 hours fast) in wild-type (D), FFAR1-/- (E) and FFAR4-/- (F) mice on high-fat diet after 21 days of treatment with 250 mg.kg^{-1} hPNO bid. Results are means of 21 values (19 for FFAR4-/- control dose) ± SEM. ★ $P < 0.05$, ★★ $P < 0.01$ for differences between mice given vehicle and PNO.