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Abstract

Objective: GCN2 is a kinase which detects amino acid deficiency and is involved in the control of protein synthesis and energy metabolism. However, the role of hepatic GCN2 in the metabolic adaptations in response to the modulation of dietary protein has been seldom studied.

Methods: Wild-type (WT) and liver GCN2-deficient (KO) mice were fed either a normo-protein diet, a low-protein diet or a high-protein diet for 3 weeks. During this period, body weight, food intake and metabolic parameters were followed.

Results: In mice fed normo- and high protein diets, GCN2 pathway in the liver is not activated in WT mice leading to a similar metabolic profile with the one of KO mice. On the contrary, a low protein diet activates GCN2 in WT mice inducing FGF21 secretion. In turn, FGF21 maintains a high level of lipid oxidation leading to a different postprandial oxidation profile compared with KO mice.

Conclusions: Hepatic GCN2 controls FGF21 secretion under a low protein diet, and modulates a whole-body postprandial oxidation profile.

Keywords: GCN2; liver; protein; FGF21

Highlights

- Hepatic GCN2 controls FGF21 secretion under a low-protein diet.
- Hepatic GCN2 deletion's metabolic consequences depend on dietary protein content.
- Hepatic GCN2 modulates whole-body postprandial oxidation profile through FGF21.

1. Introduction

General control non-derepressible 2 (GCN2) is a serine/threonine kinase which was first identified to play a major role in sensing amino acid deprivation. When the availability of one or several amino acid decreases, GCN2 phosphorylates the eukaryotic initiation factor 2α (eIF2α) which leads to the blockade of translation initiation and protein synthesis (6). Simultaneously GCN2/eIF2alpha signaling activates a gene expression program mediated by the translational upregulation of the transcription factor ATF4 (2). GCN2 is also a sensor of amino acid availability, indeed, GCN2 phosphorylation in the liver is repressed in rats fed a high-protein diet for 14 days (4).

GCN2 is also involved in the control of carbohydrates and lipid metabolism. GCN2-KO mice have a less efficient neoglucogenesis in the fasting state and are not able to repress phosphoenolpyruvate carboxykinase (PEPCK) expression in the fed state (19). In response to a leucine-deficient diet GCN2-KO mice also have an increase in fatty acid synthase expression in the liver and develop steatosis (10). Moreover, in response to a low protein diet, GCN2-KO mice are not able to induce the secretion of Fibroblast Growth Factor 21 (FGF21), involved in the regulation of β-oxidation, ketogenesis, neoglucogenesis and lipogenesis via ATF4 (5, 12). When fed a normo-protein diet, no difference in term of body composition, food intake and energy expenditure are observed between WT and GCN2-KO mice whereas when fed a low-protein diet, some of the features observed in WT mice such as the increase in food intake and in energy expenditure and the decrease in body weight and lean body mass are blunted in GCN2-KO mice during the first weeks after the introduction of the diet (13).

However, to which extent GCN2, especially hepatic GCN2, is involved in the regulation of energy metabolism in response to changes in dietary protein intake is not clear at this time. In order to assess the contribution of hepatic GCN2 on the adaptation to dietary protein level, wild-type mice and genetically modified mice in which the expression of GCN2 in the liver is

84 deleted, were fed diets differing in their relative protein to carbohydrate content and
85 parameters related to energy metabolic pathways were examined.
86

2. Materials and methods

2.1 Animals

The study was approved by the French National Animal Care Committee (number 14-15) and conformed to the European legislation on the use of laboratory animals.

GCN2 knock-out liver specific *C57BL/6* mice were generated by crossing albumin-Cre transgenic mice with floxed GCN2 mice. The deletion was confirmed by PCR genotyping as previously described (3).

Fifty-two males, 27 wild-type mice (WT) and 25 GCN2-KO (KO) liver specific mice were produced and housed in the light and temperature-controlled animal facility of AgroParisTech (12:12 h reversed light/dark cycle, lights on at 21:00, 24 °C). Spawners were fed a 20% protein (P20) diet throughout the test. Young mice were weaned at 25 days, and fed with a normo-protein (14%) diet (NP) during three weeks (run-in period) before being switched to their experimental diet (test period). Diet compositions are detailed in table 1.

2.2 Experimental Design

During an additional three-week period, corresponding to the test period, mice were either kept on the NP diet (N=18, 9 KO / 9 WT), switched on a low protein (LP) diet (N=18, 9 KO / 9 WT) or switched on a high protein (HP) diet (N=16, 7 KO / 9 WT). Feed intake was measured daily except on weekends.

Each mouse was submitted to 2 meal-tolerance tests during the study. To this purpose they were fasted overnight, and refed a standardized test-meal (1-gram pellet of their usual diet) in the morning. The first meal-tolerance test was performed during the second week while the mice were housed in an indirect calorimeter during which respiratory exchanges and spontaneous motor activity were continuously recorded at 2 s interval in order to compute the evolution of glucose and lipid oxidation during the transition from the fasting to the fed state as previously described (8). At the termination of the study, the meal tolerance test was

repeated in order to standardize the energy intake for all mice. Thus, only the macronutrients composition of the diet could impact the metabolic orientation. Mice were fasted overnight. At 7:00, 50µL of blood were collected from the tail vein to measure plasma parameters in the fasting state (see below). At 8:00 the mice were fed with 1-gram pellet of their test-diet and two hours after the meal they were killed with a pentobarbital injection (50 mg/kg). Blood was taken from the vena cava (~200µl), collected on EDTA, centrifuged (4°C, 3000rpm, 10min) and plasma was stored at -80°C until analysis.

Afterwards, body composition was analyzed by dissection and weighing of organs and tissues. Samples of liver, gastrocnemius muscle, epididymal adipose tissue, brown adipose tissue were placed in TRIzol (Invitrogen) and frozen at -80°C for further measurement of mRNA abundance. Additional liver samples were frozen to assay glycogen and triglyceride content.

2.3 Glucose and lipid oxidation in response to ingestion of the test-meal

Each mouse was housed individually from 17:00 to 16:00 the next day in an indirect-calorimeter in which temperature was maintained at 30°C, and oxygen consumption (VO_2), carbon dioxide production (VCO_2) and motor activity (assessed by piezoelectric cells) were continuously recorded at 2sec interval.

After an overnight fast (~17:00 to 09:00 the next day), a 1-gram pellet (16kJ) of their test-diet was introduced in the calorimetry cage and it was controlled that the food was eaten within 15 min.

Glucose (Gox) and lipid (Lox) and protein oxidation (Pox) were computed according to the Weir formula (8):

$$\text{Gox (Watts)} = ((4.57*VCO_2)-(3.23*VO_2)-(2.87*N)) * (0.279),$$

$$\text{Lox (Watts)} = ((1.69*VO_2)-(1.69*VCO_2)-(1.92*N) * (0.628),$$

with VO_2, VCO_2 in mL/min and N in mg/min. 0.279 and 0.628 are conversion factor of Gox and Lox from mg/min to W respectively (8). N was estimated assuming that Pox (Pox=N*6.25) usually contributes to resting metabolic rate in proportion to the protein content in each diet, i.e. 5%; 14 %, 55% respectively for LP, NP and HP diets. However, it was taken into account that in response to ingestion of a HP meal, only half of the deaminated amino acids are oxidized four hours after meal onset (9, 18) and therefore, protein oxidation was limited to 25% of resting metabolic after ingestion of the HP test-meal. To validate these calculations and to compare protein oxidation between WT and KO mice, the floor of the calorimetry cage was covered with blotting paper to collect excreted urea. The blotting paper was changed when the meal was given to separate collection during the pre- and post-meal periods. Urea was recovered by soaking the blotting paper into sterile water overnight. Urea was measured using a kit (Urea kit, Biomérieux) and real protein oxidation calculated assuming (8):

$$Pox_r \text{ (Watts)} = 6.25*\text{Excreted urea (mg/min)}*2*(15.6/60).$$

2.4 Analytical procedures

Glucose was measured immediately from a drop of blood using automatic analyzer (Life-scan, One touch Vita). Metabolites profile (Non-esterified fatty acid, urea, cholesterol, HDL cholesterol, β-hydroxybutyrate, triglycerides (TG)) was measured using an automated chemistry analyzer, Olympus AU400. Hepatic TG concentration was assayed on homogenized samples of liver using a TG assay kit (Randox Triglycerides). Glycogen was extracted from liver samples using KOH 30%, digested into glucose using amyloglucosidase (Sigma) and assayed using a glucose assay kit (Randox Gluc). Plasma insulin and FGF21 were assayed using an enzyme linked immunoassay (Mercodia Mouse Insulin/.FGF21 ELISA kit, Symansis).

2.5 RNA preparation and gene expression measurement

We investigated lipid and carbohydrate metabolism and energy expenditure assessing mRNA abundance of key genes involved in lipogenesis, lipolysis, glycolysis, neoglucogenesis and glycogen synthesis in the liver, muscle and white adipose tissue. We also studied the expression of gene involved in the GCN2/ATF4 pathway specifically in the liver.

Total RNA from samples of liver, gastrocnemius muscle, epididymal adipose tissue, brown adipose tissue, hypothalamus, nucleus accumbens and epithelial cells from ileum and duodenum, was extracted using TRIzol reagent (Invitrogen). RNA concentration was estimated using a nanodrop spectrophotometer at 260 nm and RNA integrity was confirmed by electrophoresis on agarose gel. To synthetize cDNA using High Capacity cDNA Archive Kit (Applied Biosystems, retrotranscription was accomplished on 0.4 µg of RNA. Real Time PCR was performed to measure gene expression on the Step One (Applied Biosystems) using Power SYBR GREEN PCR MIX (Applied Biosystems) on 5 ng (2.5 µl) of cDNA. Gene expression was calculated as $2^{-\Delta CT}$, where Δ_{CT}= CT $_{Gene}$ - CT $_{18S}$. Data were expressed as a percentage of the values of the WT mice fed the NP diet. Negative controls were used to detect potential contamination (control without Retrotranscriptase or RNA). For each run, a melt curve was performed to analyze the products generated and controlled for possible contamination resulting from residual genomic DNA amplification (using control without reverse transcriptase), and/or from primer-dimer formation (controls with no cDNA template and no reverse transcriptase) and/or primer specificity. PCR efficiency was determined for each gene using a serial dilution of reverse transcribed RNA. PCR primers were designed using Primer Express and are available under request. The sequences of primers used is detailed in supplemental Table S1 (publically available DOI for Figshare data: https://doi.org/10.6084/m9.figshare.8187989.v2).

183 *2.6 Statistical analysis*

184 Data are presented as means ± SEM. The effect of the diets and genotype was tested by two-
185 way ANOVA with interaction using R©. Pairwise comparisons were performed with Post hoc
186 Bonferonni tests for multiple comparisons. Differences were considered significant at P
187 <0.05.

188

3. Results

3.1 Food intake, body weight, body composition and hepatic triglycerides and glycogen concentrations.

To investigate how hepatic GCN2 is involved in the regulation of energy metabolism in response to changes in dietary protein intake, wild-type mice and liver specific GCN2 KO mice were fed during three weeks with low, normo- and high protein diets. Results showed that in comparison with the NP diet, food intake was larger with the LP diet and lower with the HP one (Table 2). Thus, mice fed the LP diet had a food intake 1.3-fold and 1.7-fold higher than mice fed the NP and HP diet respectively. No difference was observed between body weights but weights of total fat and in particular of mesenteric, epididymal, retroperitoneal and subcutaneous fat pads were reduced in HP fed mice compared to LP and NP fed mice, whereas there was no significant difference between NP et LP fed mice. Hepatic triglycerides and glycogen contents decreased with the increase in the protein content of the diet. However, no differences were observed between WT and KO mice on all of these parameters.

3.2 Glucose and lipid oxidations in response to ingestion of the test-meal

Basal glucose and lipid oxidation measured in overnight fasted mice was similar in all groups also indicating that basal metabolic rate (sum of Glucose, lipid and protein oxidation) was similar in all groups. Meal-induced changes in glucose and lipid oxidation were larger in response to the LP test-meal and lower in response to the HP one which fitted with the largest carbohydrate content in the LP meal and the lowest in the HP meal. No difference in glucose and lipid oxidations were observed between KO and WT mice fed the NP and HP meals whereas when fed the LP meal, from 0.25 h to 2.5 h after ingestion of the test-meal, KO mice exhibited a small but significantly larger increase in glucose oxidation and decrease in lipid oxidation than WT mice (Figure 1). In all groups, since changes in glucose oxidation were

quite exactly compensated by similar and opposite changes in lipid oxidation, the thermogenic response to feeding was not different (data not shown).

3.3 Plasma metabolites and hormonal concentration

Fasting (but not fed) blood glucose was lower in KO mice compared with WT mice and lower in LP fed mice compared with HP fed mice (Table 2).

Triglycerides, non-esterified fatty acids, total cholesterol and HDL cholesterol as well as insulin were not affected neither by the protein content of the diet nor by the deletion of hepatic GCN2 (Supplemental Table S2, publically available DOI for Figshare data: https://doi.org/10.6084/m9.figshare.8187989.v2). However, there was a trend to a trend to a higher insulinemia for HP fed mice diet effect P=0.82).

Plasma urea was higher in HP fed mice both in the fasting and fed state in comparison with both NP and LP fed mice. Interestingly, fasting plasma β-hydroxybutyrate was higher in LP fed mice whereas in the fed state it was higher in HP fed ones. No effect of the deletion was observed on these two parameters (Supplemental Table S2, publically available DOI for Figshare data: https://doi.org/10.6084/m9.figshare.8187989.v2).

Fasting plasma FGF21 concentration was higher in LP fed mice than in NP and HP fed ones. Ingestion of the test-meal significantly increased plasma FGF21 only in WT mice fed the LP diet and not in KO mice (Figure 2).

3.4 Gene expression measurement

In liver, but not in muscle (Table 3), as expected, GCN2 mRNA abundance was much reduced in KO mice. Residual expression is due to the abundance of Kupffer cells, blood cells in the liver [13]. Expressions of genes involved in lipid metabolism were affected only by the

protein content of the diet: mRNA encoding fatty acid synthase (FAS), acetyl-CoA carboxylase a (ACCa) and Elongation of very long chain fatty acids protein 6 (ELOVL6) were lower in mice fed the HP diet compared to NP or LP diets and mRNA encoding diglyceride acyltransferase (DGAT) and glycerol-3- phosphate acyltransferase (GPAT) were higher in mice fed the LP diet compared to mice fed the NP diet. These changes suggest that the decrease in protein and the parallel increase in carbohydrates in the diet induced an increase in hepatic fatty acid synthesis. Moreover, the expression of lipoprotein lipase (LPL) and low density lipoprotein receptor (LDLr) was higher in mice fed the LP diet suggesting that these higher expressions could help coping with the higher triglyceride synthesis.

Regarding carbohydrate metabolism, mRNA encoding phosphoenolpyruvate carboxykinase (PEPCK) was higher in mice fed the HP diet whereas the expression of glucose-6-phosphatase (G6PC1) was lower in mice fed the LP-test meal. Taken together, these observations suggest that neoglucogenesis is stimulated when the diet is high in protein and low in carbohydrate and inhibited when the diet is low in protein and high in carbohydrates.

Interestingly, the expressions of ATF4 and target genes, *Ddit3* (encoding CHOP) and *Trib3* (encoding TRB3), were decreased in KO mice under the LP diet compared to WT mice. Similarly, liver mRNA encoding *FGF21* was lower in KO mice fed the LP test-meal (Figure 3A).

No effect was observed on the expression of gene encoding proteins involved in ketogenesis (ACAA, HMG lyase), in fatty acid oxidation (CPT1a) and glycolysis (L-PK) (Supplemental Table S3, publically available DOI for Figshare data: https://doi.org/10.6084/m9.figshare.8187989.v2).

In brown adipose tissue (Table 3), mRNA expression was affected only by the protein content of the diet. mRNA encoding for carnitine palmitoyltransferase a (CPT1a), CPT1b and uncoupling protein 2 (UCP2) were larger in LP fed mice and ACCa was lower in HP fed mice

264 whereas no significant changes were observed for UCP1. In line with these observations, in
265 white adipose tissue, mRNA expression was also affected only by the protein content of the
266 diet. LP diet intake was associated with higher levels of mRNA encoding ACCa and FAS
267 (Table 3). Taken together, these observations suggest a concomitant upregulation of
268 lipogenesis, fatty acid oxidation and energy expenditure in these two tissues. Interestingly,
269 UCP3 mRNA was lower in KO mice fed the LP diet compared with WT mice (Figure 3B). In
270 muscle, *U*CP2 mRNA was higher in mice fed the LP diet (Table 3) but no effect of the diet or
271 the genotype was observed on the expression of other genes (Supplemental Table S3,
272 publically available DOI for Figshare data: https://doi.org/10.6084/m9.figshare.8187989.v2).
273 No effect of genotype or diet was observed on gene encoding proteins involved in the control
274 of food intake in the hypothalamus, nucleus accumbens, epithelial intestinal cells
275 (Supplemental Table S3 publically available DOI for Figshare data:
276 https://doi.org/10.6084/m9.figshare.8187989.v2).

277 ## 4. Discussion

278 The purpose of this study was to get a better understanding of the role played by liver GCN2
279 in the metabolic adaptations to dietary protein content. To this end, wild-type mice and mice
280 in liver specific GCN2 KO mice were fed during three weeks with low, normo- and high
281 protein diets.
282 KO mice exhibited globally lower fasting blood glucose levels and, when fed the LP test-
283 meal, had a higher increase in glucose oxidation and a higher decrease in lipid oxidation, and
284 failed to induce FGF21, ATF4, CHOP, TRB3 mRNA as did the WT mice. The consumption
285 of a low-protein diet was associated an increase in food intake, and an increase in gene
286 expression involved in lipogenesis, fatty acid oxidation and energy expenditure as reported
287 previously (12, 1, 15). These processes probably compensated each other mice fed the LP diet

did not exhibit significant differences in term of body composition with NP fed mice. On the contrary, we observed that the HP diet induced a decrease in food intake associated with a decrease in adiposity which is consistent with the literature (15). Moreover, HP diet intake induced an increase in ketogenesis in the fed state and potentially an increase in gluconeogenesis or glyconeogenesis as suggested by the upregulation of PEPCK expression in the liver.

Laeger et al. have reported that general deletion of GCN2 in mice resulted in an inability to induce FGF21 secretion in response to the intake of a low-protein diet (11). Our results are in line this observation and suggest the central roles of hepatic GCN2 in the sensing of protein content and in the expressions of ATF4, CHOP and TRB3 in the liver. These results should be confirmed by measuring protein changes of GCN2 signaling pathway. In addition, it has been reported that FGF21 treatment in cardiomyocytes in culture induces the expression of genes encoding UCP3 (16). In our study, we observed that UCP3 mRNA was increased in white adipose tissue in WT, but not in KO mice, fed a LP diet. UCP3 is highly expressed in brown adipose tissue (11) and thus, the increase of its expression in white adipose tissue suggests a browning of white adipose tissue and could explain the maintenance of a slightly higher rate of LOX observed in LP fed WT mice.

Laeger et al. also reported that the higher food intake observed with LP diets was suppressed in GCN2-KO and FGF21-KO mice (13), suggesting that FGF21 is responsible for the higher intake observed with LP diets. In the present study, we observed that WT and KO mice fed the LP diet had the same food intake but that a LP test-meal induced the secretion of FGF21 only in WT mice. Moreover, no changes in gene expression encoding neuropeptides in the hypothalamus were observed. This leads us to hypothesize that the increase in feed intake is not mediated only by the GCN2 signaling pathway in liver but involve another sensing

312 pathway and/or other organs. However, these results should be confirmed by measuring
313 protein changes of GCN2 signaling pathway.
314 We also observed that, following the meal-tolerance tests, the rates of glucose and lipid
315 oxidation were the same between wild-type and KO mice after ingestion of a NP or HP test-
316 meal but that, after ingestion of the LP test-meal, KO mice exhibited larger changes in the
317 rates of glucose and lipid oxidation. In the context of this study, one possible mechanisms is
318 that, after ingestion of the NP and HP test-meals, the flow of amino-acids to the liver was
319 sufficient to prevent the phosphorylation of GCN2. Therefore, the lack of GCN2 in KO mice
320 did not affect the metabolic fate of glucose and free-fatty acids in comparison with WT mice.
321 In contrast, in response to the LP test-meal, the reduced flow of amino acids to the liver
322 would activates the GCN2 pathway in wild-type mice. Since KO mice were not able to
323 activate this adaptive pathway, we observed in KO mice a defective post-prandial metabolic
324 fate of dietary carbohydrates and lipids characterized by an exaggerated increased in glucose
325 oxidation and an exaggerated decrease in lipid oxidation (hyperflexibility). Since WT but not
326 KO mice exhibited a huge increase in FGF21 secretion after refeeding this response was
327 probably mediated by FGF21. Accordingly, FGF21 is known to stimulate lipid oxidation (18)
328 and could therefore explain why post-meal lipid oxidation was less reduced in WT than in KO
329 mice and according to the Randle's cycle why symmetrically, glucose oxidation less
330 increased (17).
331 In addition, Xu et al. have reported that in GCN2-KO mice the expression of
332 phosphoenolpyruvate carboxykinase (PEPCK), the rate-limiting enzyme involved in
333 gluconeogenesis, is not repressed in the postprandial state compared to wild type mice both at
334 mRNA and protein level (20). Hepatic neo-synthesized glucose could therefore be oxidized in
335 peripheral tissues which could partially explain the increase in glucose oxidation. However,
336 our experiments failed to show any difference in liver PEPCK mRNA between wild-type and

GCN2-KO liver specific mice, but that remained to confirmed at the protein level. In this same study, Xu and al. reported in GCN2-KO mice that blood glucose was lower 24 and 48 h after fasting and that gluconeogenesis from exogenous pyruvate was less efficient. In line with these results we observed that, whatever the diet, fasting blood glucose was globally lower in KO mice compared to WT mice.

In conclusion, the consequences of GCN2 deletion depend on the protein content of the diet. When the dietary protein content is sufficient, The GCN2 pathway remains inactivated, and silencing of the GCN2 pathway in the liver does not affect substrates handling by the liver. In contrast, when the dietary protein content is low, GCN2 is activated in the liver of WT mice. This, in turn, induces FGF21 secretion and leads to adaptive changes in the postprandial oxidation profile of glucose and free fatty acids a response that is blunted in GNC2 KO mice.

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7. Tables

Table 1: Macronutrient composition of the HCD. Diets were prepared by the "atelier de préparation des aliments", UPAE, INRA, Jouy en Josas, France. Energy density is computed assuming a metabolizable energy of 16.7 kJ/g for carbohydrates and proteins and 37.7 kJ/g for fat.

	P20	NP	LP	HP
Weight content (g/kg)				
Milk proteins	200	140	50	530
Starch	570	622	700	287
Sucrose	93	100	113	46
Soy Oil	40	40	40	40
Minerals	35	35	35	35
Vitamins	10	10	10	10
cellulose	50	50	50	50
choline	2	2	2	2
Energy content (%)				
Protein	20.5	14.5	5.2	54.6
Carbohydrate	68.5	75.0	84.0	34.9
Fat	10.5	10.5	10.5	10.5
Energy density (kJ/g)	14.57	14.56	14.54	14.60

413 **Table 2:** Body composition, food intake and fasting glucose of WT and KO mice fed NP, LP or HP diets.

Diet	**NP**			**LP**			**HP**			**Genotype**	**Test diet**	**Inter**
Genotype	WT	KO		WT	KO		WT	KO				
Cumulative food intake (g)	68.26 ± 3.16	70.93 ± 2.83	A	93.64 ± 2.74	88.45 ± 3.16	B	54.26 ± 2.58	52.37 ± 3.80	C	NS	<0.001	NS
Initial Weight (g)	21.83 ± 0.28	21.76 ± 0.58		21.87 ± 0.43	21.36 ± 0.54		22.21 ± 0.48	21.44 ± 0.49		NS	NS	NS
Final Weight (g)	23.17 ± 0.48	23.54 ± 0.55		22.07 ± 0.44	21.6 ± 0.61		22.51 ± 0.89	22.56 ± 0.79		NS	NS	NS
Fat mass (g)	1.91 ± 0.11	1.98 ± 0.16	A	2.16 ± 0.16	1.97 ± 0.18	A	1.1 ± 0.13	1.2 ± 0.14	B	NS	<0.001	NS
Epididymal adipose tissue (g)	0.45 ± 0.03	0.47 ± 0.05	A	0.47 ± 0.04	0.44 ± 0.05	A	0.27 ± 0.04	0.29 ± 0.03	B	NS	<0.001	NS
Retroperitoneal adipose tissue (g)	0.14 ± 0.02	0.15 ± 0.02	A	0.2 ± 0.02	0.15 ± 0.02	A	0.07 ± 0.01	0.09 ± 0.01	B	NS	<0.001	NS
Mesenteric adipose tissue (g)	0.19 ± 0.01	0.21 ± 0.02	A	0.2 ± 0.02	0.18 ± 0.03	A	0.12 ± 0.01	0.12 ± 0.01	B	NS	<0.001	NS
Subcutaneous adipose tissue (g)	1.04 ± 0.06	1.05 ± 0.08	A	1.18 ± 0.1	1.1 ± 0.09	A	0.58 ± 0.08	0.62 ± 0.09	B	NS	<0.001	NS
Hepatic triglycerides	44.3 ± 6.94	43.42 ± 7.86	A	52.57 ± 7	56.98 ± 7.21	A	18.58 ± 2.68	26.25 ± 8.15	B	NS	<0.001	NS
Hepatic glycogen	29.44 ± 2.5	34.3 ± 2.61	A	38.96 ± 3.47	40.93 ± 3.55	B	25.12 ± 4.34	20.97 ± 2.99	C	NS	<0.001	NS
Fasting glucose	112.25 ± 14.9	91.78 ± 10.04		84 ± 13.2	62.78 ± 7.21		122.57 ± 17.86	96.83 ± 18.27		<0.05	<0.05	NS

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415 [A,B,C] Different letters within a line mean statistically different values between diets (post hoc Bonferonni tests for multiple comparisons, P<0.05).

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419 **Table 3.** mRNA abundance in liver, brown adipose tissue (BAT), white adipose tissue (WAT), muscle of WT and KO mice fed LP, NP or HP

420 diets two hours after meal onset.

Diet		**NP**			**LP**			**HP**			**Genotype**	**Test diet**	**Interaction**
Genotype		WT	KO		WT	KO		WT	KO				
Liver	GCN2	1 ± 0.23	0.16 ± 0.02		0.84 ± 0.09	0.25 ± 0.03		0.92 ± 0.14	0.20 ± 0.04		<0.0001	NS	NS
	CHOP	1 ± 0.16	0.90 ± 0.08	A	7.28 ± 2.11	1.33 ± 0.29*	B	1.27 ± 0.21	1.69 ± 0.28	A	<0.01	<0.01	<0.01
	TRB3	1 ± 0.10	0.96 ± 0.17	A	35.1 ± 12.4	2.23 ± 0.88*	B	1.20 ± 0.42	2.80 ± 1.19	A	<0.01	<0.01	<0.01
	ATF4	1 ± 0.18	0.98 ± 0.08		2.15 ± 0.43	1.09 ± 0.15*		1.25 ± 0.46	1.17 ± 0.14		0.08	0.07	NS
	FAS	1 ± 0.27	1 ± 0.27	A	0.61 ± 0.05	0.65 ± 0.14	AB	0.48 ± 0.11	0.48 ± 0.08	B	NS	<0.05	NS
	ACCa	1 ± 0.15	0.82 ± 0.13	AB	0.96 ± 0.11	0.97 ± 0.07	A	0.66 ± 0.12	0.71 ± 0.08	B	NS	<0.05	NS
	Elov16	1 ± 0.2	0.86 ± 0.12	AB	1.01 ± 0.15	1.2 ± 0.17	A	0.66 ± 0.16	0.5 ± 0.08	B	NS	<0.01	NS
	DGAT	1 ± 0.12	1.11 ± 0.18	A	1.4 ± 0.31	1.78 ± 0.19	B	1.26 ± 0.25	1.31 ± 0.16	AB	NS	<0.05	NS
	GPAT	1 ± 0.12	0.65 ± 0.04	A	1.35 ± 0.21	1.62 ± 0.21	B	0.64 ± 0.07	0.97 ± 0.17	A	NS	<0.0001	<0.05
	LPL	1 ± 0.15	2.12 ± 0.9	A	5 ± 1.87	4.8 ± 1.62	B	1.21 ± 0.22	1.52 ± 0.21	A	NS	<0.05	NS
	LDLr	1 ± 0.18	0.75 ± 0.11	A	1.31 ± 0.17	1.55 ± 0.21	B	0.69 ± 0.1	0.83 ± 0.18	A	NS	<0.001	NS
	PEPCK	1 ± 0.44	0.86 ± 0.21	A	1.07 ± 0.36	1.1 ± 0.31	AB	1.88 ± 0.54	1.77 ± 0.31	B	NS	<0.05	NS
	G6PC1	1 ± 0.18	1.05 ± 0.28	A	0.28 ± 0.06	0.62 ± 0.12	B	0.91 ± 0.21	0.92 ± 0.15	A	NS	<0.01	NS
BAT	ACCa	1 ± 0.14	0.94 ± 0.21	A	1.25 ± 0.17	1.02 ± 0.16	A	0.52 ± 0.07	0.49 ± 0.09	B	NS	<0.001	NS
	CPT1a	1 ± 0.35	0.8 ± 0.09	A	1.86 ± 0.35	1.55 ± 0.3	B	0.82 ± 0.15	1.06 ± 0.26	A	NS	<0.01	NS
	CPT1b	1 ± 0.13	1.4 ± 0.25	A	2.09 ± 0.31	1.44 ± 0.11	B	1.45 ± 0.18	1.62 ± 0.19	AB	NS	<0.05	NS
	UCP2	1 ± 0.14	0.59 ± 0.11	A	1.57 ± 0.26	1.63 ± 0.17	B	0.68 ± 0.09	0.92 ± 0.23	A	NS	<0.0001	NS
	UCP1	1 ± 1.16	1.00 ± 0.48		1.35 ± 0.75	0.79 ± 0.35		1.08 ± 0.46	1.38 ± 1.12		NS	NS	NS
WAT	ACCa	1 ± 0.12	1.13 ± 0.19	A	1.94 ± 0.21	1.67 ± 0.19	B	0.78 ± 0.15	1.49 ± 0.61	A	NS	<0.0001	NS
	FAS	1 ± 0.21	1.24 ± 0.17	A	3.05 ± 0.69	2 ± 0.41	B	0.85 ± 0.33	2.7 ± 1.87	A	NS	<0.01	NS
Muscle	GCN2	1 ± 0.26	1.11 ± 0.17		1.08 ± 0.20	0.96 ± 0.18		0.98 ± 0.12	1.21 ± 0.19		NS	NS	NS
	UCP2	1 ± 0.20	1.68 ± 0.50	A	1.86 ± 0.38	2.01 ± 0.22	B	1.14 ± 0.08	1.84 ± 0.54	AB	NS	<0.001	NS

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422 [A,B,C] Different letters within a line mean statistically different values between diets (post hoc Bonferonni tests for multiple comparisons, P<0.05).

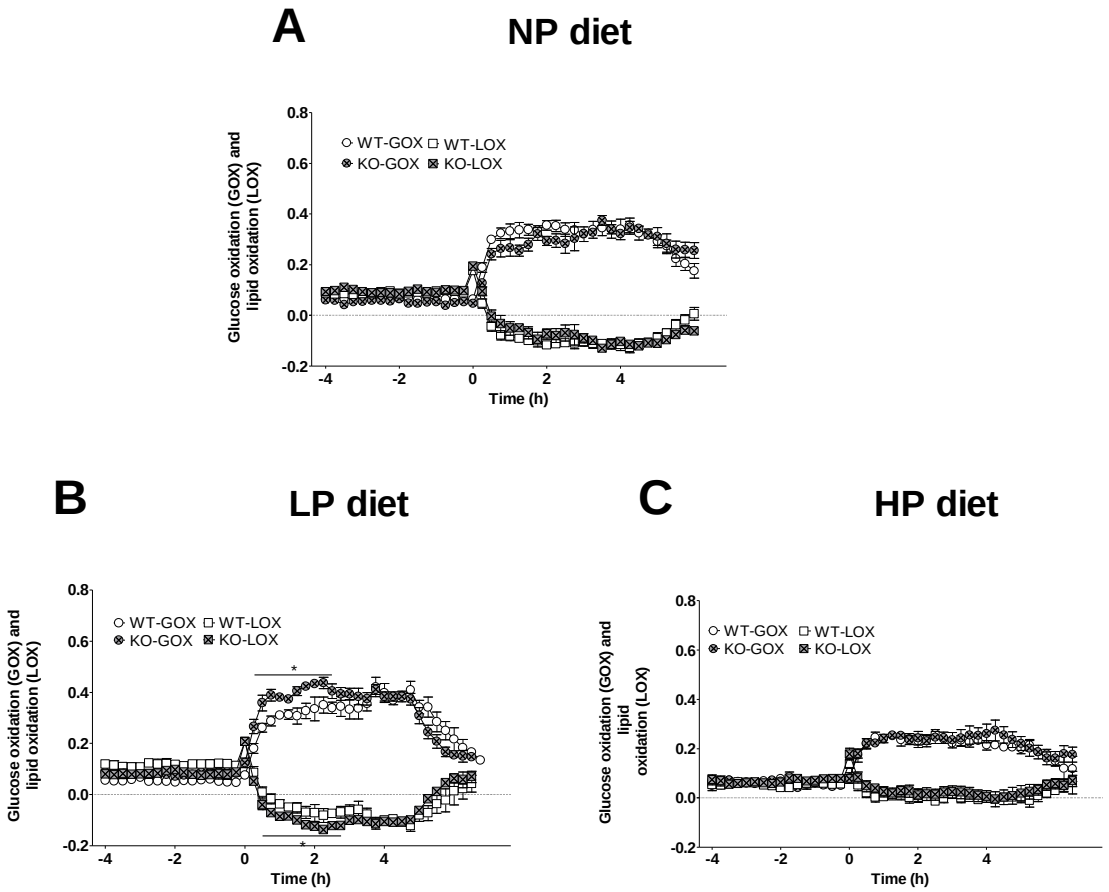

Fig. 1: Meal-induced changes in glucose and lipid oxidation following the ingestion of a test-meal. (A) Glucose and lipid oxidation in WT and KO mice fed the NP diet. (B) Glucose and lipid oxidation in WT and KO mice fed the LP diet. (C) Glucose and lipid oxidation in WT and KO mice fed the HP diet. Fasted mice were fed at 9:00 (T=0) with 1 g of their respective maintenance diet. Data are mean ± SEM (n =6-8). *, $P < 0.05$ between WT and KO mice

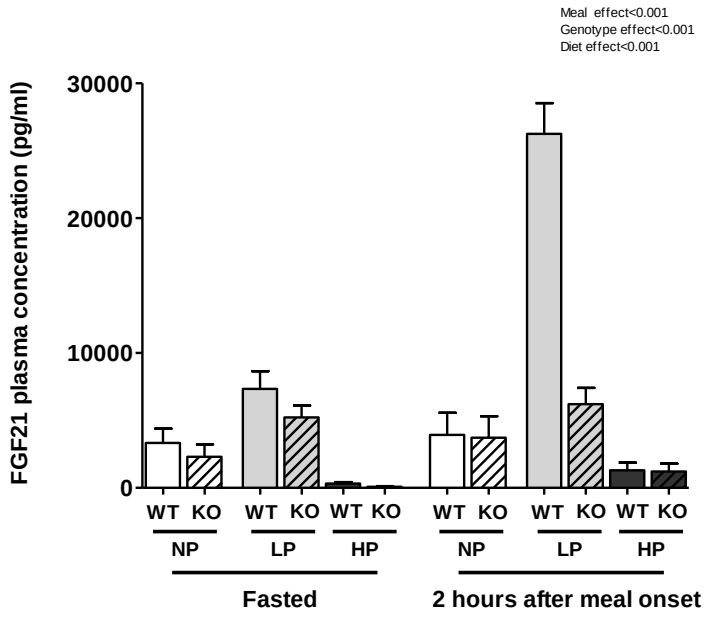

Fig. 2: FGF21 concentration. Modulation of FGF21 protein abundance in the plasma in the fasting state and two hours after meal onset. Data are mean ± SEM (n =7-9). *, $P < 0.05$ between WT and KO mice.

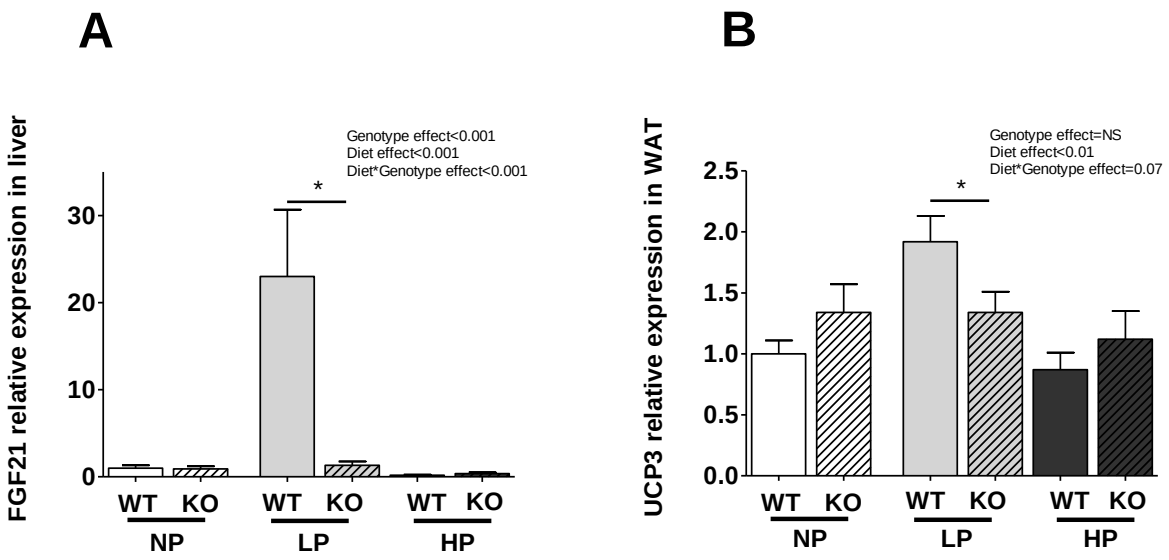

Fig. 3: FGF21 and UCP3 gene expressions. (A) Modulation of FGF21 mRNA abundance in the liver of mice two hours after meal onset. (B) Modulation of UCP3 mRNA abundance in the white adipose tissue (WAT) of mice two hours after meal onset. Data are mean ± SEM (n =7-9). *, $P < 0.05$ between WT and KO mice.

Supplemental Table S1: Primer sequences used for liver, muscle, adipose tissues, hypothalamus, nucleus accumbens, epithelial intestinal mRNA analysis.

PROTEINE	GENES	FULL-LENGTH NAME	FORWARD PRIMER (5' TO 3')	REVERSE PRIMER (3' TO 5')
-	*18S*	ribosomal RNA	ACGGAAGGGCACCACCAGGAG	GCACCACCACCCACGGAAAC
ACCa	*Acaca*	acetyl-CoA carboxylase a	TGGTGCAGAGGTACCGAAGTG	CGTAGTGGCCGTTCTGAAACT
ACCb	*Acacb*	acetyl-CoA carboxylase b	GGGCTCCCTGGATGACAAC	GCTCTTCCGGGAGGAGTTCT
AGRP	*Agrp*	agouti related neuropeptide	GTTCCCAGAGTTCCCAGGTCTAA	GTGTCTCAGGTCTGCAGTTA
ATF4	*Atf4*	Activating transcription factor 4	TCGATGCTCTGTTTCGAATG	AGAATGTAAAGG
CART	*Cartp*	cocaine- and amphetamine-regulated transcript	CCGAGCCCTGGACATCTACTC	AAATACTGACCAGCTCCTTCT
CB1	*Cb1*	cannabinoid receptor 1	GCTGGTATCAAATGCCAAGGAA	GTTCTCATCTGGTAGTTGGGC
CD36	*Cd36*	cluster of differenciation 36	CATGATTAATGGCACAGATGCA	GCAAATGTCAGAGGAAAAGA
CRH	*Crh*	corticotropin releasing hormone	CAACCTCAGCCGGTTCTGA	CCCCAGGCGGAGGAAGTA
CHOP	*Ddit3*	DNA damage inducible transcript 3	CTTGACCCTGCATCCCTAGCT	AGGGCTTTGGGA
CPT1a	*Cpt1a*	carnitine palmitoyl transferase 1a	TCTCTGGATGCGGTAGAAAAGG	CTCTATATCCCTGTTCCGATT
CPT1b	*Cpt1b*	carnitine palmitoyl transferase 1b	CAGCCATGCCACCAAGATC	CTTGGGCAGTGATGTTTGGA
DGAT	*Dgat1*	diacylglycerol O-acyltransferase 1	ACTCCAGTGGGTTCCGTGTTT	GCGGCACCACAGGTTGAC
DOR	*Oprd1*	opioid receptor, delta 1	CGTGCTCGTCATGTTTGGAA	AAGGCCAGATTGAAGATGTA
DR2	*Drd2*	opioid receptor, delta 2	CCATCAGCATTGACAGGTACACA	CAGTAACTCGGCGCTTGGA
DR3	*Drd3*	opioid receptor, delta 3	GCTTCCCTCAGCAGTCTTCTT'	CCCTTATTGAAAACTGCCGAA
ELOVL6	*Elovl6*	family member 6, elongation of long chain fatty	CGTAGCGACTCCGAAGATCAG	ACAGCGGAGAA
FAS	*Fasn*	fatty acid synthase	TGCTCCCAGCTGCAGGC	GCCCGGTAGCTCTGGGTGTA
FGF21	*Fgf21*	fibroblast growth factor 21	CAGGGAGGATGGAACAGTGGTA	GCTGTTGGCAAAGAAACCTA
G6PC1	*G6pc*	glucose-6-Phosphatase, Catalytic	GTGCAGCTGAACGTCTGTCTGTG	TCCGGAGGCTGGC
GCN2	*Eif2ak4*	Eukaryotic initiation factor 2 alpha kinase 4	AAAAAGCTACTGCTGTGCTGGTAA	TAGTGCAGTGTTTGTTCCCCA
GLUT1	*Slc2a1*	solute carrier family 2 member 1	GCCCCCAGAAGGTTATTGA	CGTGGTGAGTGTGGTGGA
GLP1	*Gcg*	glucagon-	GCCGAGGAAGGCGAGACT	GGCCGAGTTCCTCAGCAAT
GPAT	*GPAT*	glycerol-3-phosphate acyltransferase	CAACACCATCCCCGACATC	GTGACCTTCGATTATGCGAT
HK2	*Hk2*	hexokinase 2	AACCGAACAAGCTGGTGTAC	TGCACACATCTATAGGTGGC
HSL	*Lipe*	hormone-sensitive lipase	CCTACATGGCTCAACTCC	CGTTCTTGACTATGGGTGA
HMG lyase	*Hmgcl*	3-hydroxy-3-methylglutaryl-Coenzyme A lyase	CTGCTCTATAGAGGAGAGTTTC	TGGCAGTGGACAGCCAATGC
ACAA	*Acaa*	Acetyl-Coenzyme A acyltransferase	TCACGGCAGAAGCAGGATGC	TGCTCCATCACTCACCTGACT

KOR	*Oprk1*	opioid receptor, kappa 1	TGTGGTATTTGTGGTGGGCTTA	TCTTCGTGTATCGGATGATGA
LDLr	*Ldlr*	low density lipoproteine receptor	GCTCCATAGGCTATCTGCTCTTCA	GCGGTCCAGGGTCATCTTC
LPL	*Lpl*	lipoprotein lipase	TGAAAGTGGGTTTTCCTGAGTAT	GGTTAGCCACCGTTTAATATT
MC4R	*Mc4r*	melanocortin 4 receptor	CCGAACCCAGAAGAGACCAA	CTAGGAGCAGGGTCAGAAGC
NPY	*Npy*	neuropeptide Y	GGGAGCCTGAGAAACGGC	CCTGGTGGTGGCATGCAT
Y2R	*Npy2r*	neuropeptide Y receptor Y2	CCGCTCCTGCTTCTGACTC	ACCCAAAGCAGGTCCGATT
Y5R	*Npy5r*	neuropeptide Y receptor Y5	AACCTTTGGCTCAGCATTGC	CAGAGGGCCATGACTCAACA
PEPCK	*Pck1*	phosphoenolpyruvate carboxykinase	GGAAAGTTGAATGTGTGGGTGAT	TTCTGGGTTGATGGCCCTTA
LPK	*Pklr*	l-Pyruvate kinase	AACCATGAAGGCGTGAAGAAGT	TGGGATCTCAATGCCAAGGT
MOR	*Oprm1*	opioid receptor, mu 1	CACGGCTAATACAGTGGATCGA	GGGCAATGGAGCAGTTTCTG
POMC	*Pomc*	proopiomelanocortin	AGGCCTTTCCCCTAGAGTTCAA	GTCGGCCTTCTCGGTATCC
PYY	*Pyy*	peptide YY	CGGCAGCGGTATGGAAAA	TGTGAAGAGCAGTTTGGAGA
SIRT1	*Sirt1*	sirtuin 1	TCACACGCCAGCTCTAGTGACT	CCAATCATGAGATGTTGCTGA
TRB3	*Trib3*	Tribbles homolog 3	TACCTCCCGCCTCAGACTTG	TTGCCTTGCTCTCGTTCCA
UCP1	*Ucp1*	mitochondrial uncoupling protein 1	CGTACCAAGCTGTGCGATGT	GACCCGAGTCGCAGAAAAGA
UCP2	*Ucp2*	mitochondrial uncoupling protein 2	TGAAAGCCAACCTCATGACAGA	CAATGACGGTGGTGCAGAAG
UCP3	*Ucp3*	mitochondrial uncoupling protein 3	CTGGGAGCTTGGAACGTGAT	AAACGGAGATTCCCGCAGTA

Supplemental Table S2: Plasma Metabolite profile of WT and KO mice fed LP, NP or HP diets before or two hours after meal onset.

Diet		**NP**		**LP**		**HP**		**Genotype**	**Test diet**	**Interaction**
Genotype		WT	KO	WT	KO	WT	KO			
Plasma Urea (mmol/l)	Fasted	17 ± 2.21	15.21 ± 2.6	[A] 19.78 ± 2.62	13.89 ± 1.74	[A] 30.06 ± 4.62	27.71 ± 4.18	[B] NS	<0.0001	NS
	2h	30 ± 3.76	34.18 ± 3.88	[A] 31.31 ± 3.52	22.08 ± 3.53	[A] 54.91 ± 6.13	58.79 ± 9.53	[B] NS	<0.0001	NS
Plasma NEFA (mmol/l)	Fasted	1.77 ± 0.27	1.92 ± 0.54	2.31 ± 0.44	3.29 ± 0.59	1.73 ± 0.23	1.95 ± 0.32	NS	NS	NS
	2h	0.77 ± 0.18	0.75 ± 0.29	0.6 ± 0.12	0.6 ± 0.19	0.48 ± 0.1	0.44 ± 0.16	NS	NS	NS
Plasma Chol (mmol/l)	Fasted	5.49 ± 0.84	4.84 ± 1.06	5.46 ± 1.13	4.5 ± 0.72	6.16 ± 0.75	5.47 ± 1.21	NS	NS	NS
	2h	4.98 ± 1.02	5.28 ± 0.87	4.38 ± 0.63	4.18 ± 0.83	4.06 ± 0.58	3.88 ± 0.77	NS	NS	NS
Plasma Trig (mmol/l)	Fasted	2.43 ± 0.37	1.91 ± 0.48	2.6 ± 0.42	2.52 ± 0.35	1.9 ± 0.2	2 ± 0.43	NS	NS	NS
	2h	1.74 ± 0.29	2.02 ± 0.32	2.11 ± 0.2	2.12 ± 0.33	1.63 ± 0.23	1.89 ± 0.38	NS	NS	NS
Plasma HDL (mmol/l)	Fasted	4.13 ± 0.6	3.54 ± 0.76	3.82 ± 0.65	3.17 ± 0.53	4.6 ± 0.55	4.08 ± 0.87	NS	NS	NS
	2h	3.76 ± 0.77	4.3 ± 0.69	3.28 ± 0.46	3.1 ± 0.58	3.19 ± 0.41	3.04 ± 0.66	NS	NS	NS
Plasma β-hydroxybutrate (mmol/l)	Fasted	1.1 ± 0.14	1.07 ± 0.3	[AB] 1.94 ± 0.54	1.89 ± 0.46	[A] 0.89 ± 0.16	0.83 ± 0.17	[B] NS	<0.01	NS
	2h	0.22 ± 0.05	0.33 ± 0.05	[A] 0.33 ± 0.05	0.30 ± 0.08	[A] 0.51 ± 0.07	0.52 ± 0.09	[B] NS	<0.01	NS
Plasma insulin	Fasted	0.19 ± 0.05	0.22 ± 0.07	0.39 ± 0.23	0.33 ± 0.05	0.19 ± 0.04	0.23 ± 0.07	NS	NS	NS
	2h	1.51 ± 0.36	1.9 ± 0.52	0.94 ± 0.23	1.44 ± 0.45	2.58 ± 0.88	2.64 ± 0.8	NS	NS	NS

[A,B,C] Different letters within a line mean statistically different values between diets (post hoc Bonferonni tests for multiple comparisons, P<0.05).

Supplemental Table S3: mRNA abundance in liver, muscle, white adipose tissue (WAT), brown adipose tissue (BAT), hypothalamus, nucleus accumbens (NACC), ileum, duodenum, jejunum of WT and KO mice fed LP, NP or HP diets two hours after meal onset

Diet		**NP**		**LP**		**HP**		**Genotype**	**Test diet**	**Interaction**
Genotype		WT	KO	WT	KO	WT	KO			
Liver	Cpt1a	1 ± 0.28	2.42 ± 1.55	1.06 ± 0.24	1.06 ± 0.21	1.25 ± 0.34	0.69 ± 0.22	NS	NS	NS
	ACAA	1 ± 0.18	0.93 ± 0.16	0.95 ± 0.19	1.1 ± 0.11	0.64 ± 0.16	0.9 ± 0.09	NS	NS	NS
	HMG lyase	1 ± 0.2	0.97 ± 0.13	1.34 ± 0.13	1.34 ± 0.22	0.7 ± 0.13	1.13 ± 0.2	NS	NS	NS
	LPK	1 ± 0.17	0.95 ± 0.2	0.6 ± 0.08	0.63 ± 0.12	0.74 ± 0.27	0.47 ± 0.05	NS	<0.05	NS
Muscle	ACCb	1 ± 0.2	1.17 ± 0.26	0.95 ± 0.17	1.11 ± 0.11	1.04 ± 0.13	1.28 ± 0.3	NS	NS	NS
	CD36	1 ± 0.39	1.19 ± 0.24	1.06 ± 0.23	1.06 ± 0.09	1.07 ± 0.14	1.24 ± 0.27	NS	NS	NS
	CPT1b	1 ± 0.16	1.57 ± 0.34	1.12 ± 0.28	1.04 ± 0.1	1.28 ± 0.17	1.32 ± 0.36	NS	NS	NS
	FAS	1 ± 0.31	0.72 ± 0.17	0.81 ± 0.23	0.95 ± 0.2	0.61 ± 0.07	0.94 ± 0.18	NS	NS	NS
	HK2	1 ± 0.22	1.22 ± 0.27	0.92 ± 0.19	0.91 ± 0.1	0.99 ± 0.14	0.94 ± 0.14	NS	NS	NS
	LDLr	1 ± 0.28	1.2 ± 0.28	1.11 ± 0.32	0.93 ± 0.15	1.1 ± 0.23	1.02 ± 0.18	NS	NS	NS
	UCP3	1 ± 0.21	1.61 ± 0.27	1.34 ± 0.33	1.64 ± 0.13	1.2 ± 0.17	1.5 ± 0.54	NS	NS	NS
WAT	GPAT	1 ± 0.23	0.88 ± 0.19	1.14 ± 0.21	1.22 ± 0.29	1.21 ± 0.24	1.27 ± 0.27	NS	NS	NS
	CD36	1 ± 0.27	0.83 ± 0.1	1.09 ± 0.09	0.9 ± 0.13	0.71 ± 0.12	0.7 ± 0.12	NS	NS	NS
	HSL	1 ± 0.24	1.02 ± 0.26	1.88 ± 0.43	1.31 ± 0.44	0.98 ± 0.32	0.99 ± 0.4	NS	NS	NS
	Sirt1	1 ± 0.18	0.97 ± 0.08	1.24 ± 0.17	0.89 ± 0.1	1.11 ± 0.17	0.92 ± 0.2	NS	NS	NS
	UCP2	1 ± 0.23	1.11 ± 0.23	1.36 ± 0.23	1.26 ± 0.29	0.96 ± 0.18	1 ± 0.29	NS	NS	NS
	GPAT	1 ± 0.23	0.88 ± 0.19	1.14 ± 0.21	1.22 ± 0.29	1.21 ± 0.24	1.27 ± 0.27	NS	NS	NS
BAT	ACCb	1 ± 0.15	1 ± 0.14	1.29 ± 0.2	1.1 ± 0.23	0.81 ± 0.19	0.89 ± 0.15	NS	NS	NS
	GLUT1	1 ± 0.09	1.64 ± 0.28	1.8 ± 0.27	1.45 ± 0.19	1.64 ± 0.2	1.64 ± 0.2	NS	NS	NS
	HSL	1 ± 0.16	1.01 ± 0.19	1.25 ± 0.24	1.35 ± 0.14	1.35 ± 0.15	0.83 ± 0.12	NS	NS	NS
	UCP1	1 ± 0.44	1 ± 0.19	1.35 ± 0.27	0.79 ± 0.13	1.08 ± 0.21	1.38 ± 0.46	NS	NS	NS
	UCP3	1 ± 0.38	1.13 ± 0.16	1.77 ± 0.35	1.63 ± 0.32	1.46 ± 0.19	1.26 ± 0.19	NS	NS	NS
	ACCb	1 ± 0.15	1 ± 0.14	1.29 ± 0.2	1.1 ± 0.23	0.81 ± 0.19	0.89 ± 0.15	NS	NS	NS

Hypothalamus	CRH	1 ± 0.26	0.84 ± 0.07	1.24 ± 0.4	1.31 ± 0.17	1.14 ± 0.23	1.55 ± 0.15	NS	NS	NS
	MC4R	1 ± 0.21	0.87 ± 0.1	1.36 ± 0.33	1.11 ± 0.17	1.11 ± 0.1	1.06 ± 0.2	NS	NS	NS
	NPY	1 ± 0.16	1.01 ± 0.13	0.97 ± 0.11	0.92 ± 0.11	1.14 ± 0.16	1.07 ± 0.26	NS	NS	NS
	POMC	1 ± 0.23	1.07 ± 0.17	0.88 ± 0.11	1.09 ± 0.15	1.16 ± 0.31	1.38 ± 0.21	NS	NS	NS
	AGRP	1 ± 0.15	1.31 ± 0.3	1.06 ± 0.14	1.1 ± 0.36	1.3 ± 0.15	1.4 ± 0.3	NS	NS	NS
	CART	1 ± 0.43	0.77 ± 0.1	1.51 ± 0.53	0.85 ± 0.14	0.96 ± 0.15	0.96 ± 0.12	NS	NS	NS
	Y5R	1 ± 0.06	0.9 ± 0.11	0.94 ± 0.15	0.77 ± 0.09	0.86 ± 0.11	0.79 ± 0.14	NS	NS	NS
	MOR	1 ± 0.12	0.98 ± 0.11	1.04 ± 0.27	0.88 ± 0.29	1.70 ± 0.16	1.14 ± 0.23	NS	NS	NS
	Y2R	1 ± 0.13	0.66 ± 0.15	0.87 ± 0.14	0.87 ± 0.16	0.71 ± 0.12	1.03 ± 0.21	NS	NS	NS
NACC	DR2	1 ± 0.17	0.96 ± 0.15	1.21 ± 0.18	0.83 ± 0.18	1.02 ± 0.08	0.88 ± 0.21	NS	NS	NS
	DR3	1 ± 0.18	0.97 ± 0.17	1.15 ± 0.18	0.76 ± 0.17	1.13 ± 0.13	0.83 ± 0.18	NS	NS	NS
	CB1	1 ± 0.09	1.05 ± 0.14	1.08 ± 0.14	0.82 ± 0.09	1.07 ± 0.12	0.87 ± 0.13	NS	NS	NS
	DOR	1 ± 0.12	1.25 ± 0.26	1.2 ± 0.19	0.84 ± 0.1	0.92 ± 0.08	0.85 ± 0.14	NS	NS	NS
	KOR	1 ± 0.18	1.49 ± 0.33	1.16 ± 0.17	0.75 ± 0.12	1.1 ± 0.13	1.01 ± 0.15	NS	NS	NS
	MOR	1 ± 0.19	1.29 ± 0.26	1.33 ± 0.25	0.98 ± 0.19	1.19 ± 0.15	1.22 ± 0.1	NS	NS	NS
Ileum	PYY	1 ± 0.13	0.71 ± 0.05	0.93 ± 0.14	0.75 ± 0.07	1.09 ± 0.19	1.43 ± 0.34	NS	NS	NS
	GLP1	1 ± 0.13	0.93 ± 0.06	1.14 ± 0.29	0.81 ± 0.13	0.95 ± 0.14	1.16 ± 0.15	NS	NS	NS
Jejunum	PYY	1 ± 0.15	1.44 ± 0.19	1.38 ± 0.3	1.36 ± 0.22	1.3 ± 0.18	1.42 ± 0.3	NS	NS	NS
	GLP1	1 ± 0.14	0.78 ± 0.11	0.95 ± 0.15	0.89 ± 0.11	0.81 ± 0.1	0.74 ± 0.13	NS	NS	NS