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Altered body mass regulation in male *mPeriod* mutant mice on high fat diet

Robert Dallmann^{*} and David R. Weaver

Department of Neurobiology, University of Massachusetts Medical School, Worcester, MA, U.S.A

Abstract

The circadian clock orchestrates most physiological processes in mammals. Disruption of circadian rhythms appears to contribute to the development of obesity and metabolic syndrome. The *Period* genes *mPer1* and *mPer2* but not *mPer3* are essential for core clock function in mice. To assess the impact of *mPer* genes on body mass regulation, *mPer* mutant and control mice were fed a high-fat diet. Here we report that male *mPer1/2/3* triple deficient mice gain significantly more body mass than wild-type controls on high fat diet. Surprisingly, *mPer3* single deficient animals mimicked this phenotype suggesting a previously unrecognized role for *mPer3* in body mass regulation.

Keywords

mPeriod; diet induced obesity; mouse; circadian rhythms; metabolism

Introduction

Most organisms possess an internal biological clock that is capable of measuring time in 24-hour increments, and is reset by the daily light-dark cycle. In mammals, this circadian clock is composed of two interlocked transcriptional/(post-)translational feedback loops (Weaver and Reppert 2008). Two mammalian *Period* (*mPer*) genes, *mPer1* and *mPer2*, are essential parts of the negative arm of this core clock mechanism (Reppert and Weaver 2002; Shearman et al. 2000b). In contrast, another member of the same gene family, *mPer3*, is not necessary for the core clock mechanism; *mPer3* deficient mice show only a subtle circadian phenotype (Shearman et al. 2000a; van der Veen and Archer). Nevertheless, polymorphisms in human *hPer3* have been associated with diurnal preference, sleep structure, and altered responses to sleep deprivation (Dijk and Archer 2009).

Numerous phenotypic abnormalities have been described in mice with alterations in the core clock genes, i.e. essential parts of the molecular feedback loops. These changes include alterations in many different physiological systems as well as pathological responses (Kondratov et al. 2007), and, most importantly for the present study, alterations in body mass regulation and sleep (see below). Many of these phenotypes have been demonstrated in mice housed in light:dark cycles, where the masking influence of the lighting cycle will drive rhythmicity in several physiological functions. Thus, it is not always clear whether the phenotype is due to disruption of circadian clock function, or to another (pleiotropic) role of the clock gene being studied. These considerations led us to examine body mass regulation in mice with disruption of the *Period* genes.

^{*}Current Address for Correspondence: Dr. Robert Dallmann, Institute of Pharmacology and Toxicology, University of Zurich, Irchel Campus Y17, Winterthurerstrasse 190, 8057 Zurich, Switzerland, Robert@dallmanns.de, Phone: +41 44 635 59 59.

Various mouse lines with mutations in core clock genes have been linked to altered metabolic pathways, metabolic syndrome and obesity phenotypes (Dallmann et al. 2006; Rudic et al. 2004; Turek et al. 2005). Through which output mechanism the core circadian clockwork influences these pathways is still unclear. Recently, it has been acknowledged that metabolic syndrome and obesity are causally linked to disruption of rhythms (Bechtold 2008) and sleep (Laposky et al. 2008; Wolk and Somers 2007). Notably, high fat diet and/or diet-induced obesity have been shown to alter physiological rhythms and also to alter the amplitude and phase of clock gene and clock-controlled gene expression patterns (Barnea et al. 2009; Hsieh et al. 2010; Kohsaka et al. 2007). Even mice with targeted disruptions of *mPer1* and *mPer2* have been shown to be slightly lighter and heavier, respectively, than wild-type controls (Dallmann et al. 2006; Pilonis and Steinlechner 2008). Dependent on the genetic background of the animals used, however, results can vary between experimenters (Kennaway et al. 2007).

Here, we set out to investigate the impact of disruption of *mPer* genes on body mass development and food intake in mice under two different feeding regimes, i.e. high fat vs. control chow. *Period* triple deficient animals on high fat diet showed a late onset obesity phenotype. Most surprisingly, however, we found that even the single *PER3* deficient mice have this late onset obesity; indicating an unexpected role for *mPer3* in body mass regulation.

Materials and Methods

In this study we used mice homozygous for alleles with targeted disruptions of *mPer1*, *mPer2*, and *mPer3* (*Per1^{tm1Drw}*; *Per2^{tm1Drw}*; *Per3^{tm1Drw}*, henceforth: *Per1/2/3^{tm1Drw}*) or only *mPer3* (abbreviated *Per3^{tm1Drw}*) and congenic 129/Sv wild-type control mice. Generation and circadian phenotype of all mutant lines have been described elsewhere (Bae et al. 2001; Shearman et al. 2000a).

Unless otherwise noted, animals were single housed in individually ventilated cages (Allentown, NJ, USA, 24×16×13 cm) maintained in light tight (lights on from 06:00 h to 18:00 h each day) and climate controlled closets (21±1°C) with gamma irradiated food and tap-water available *ad libitum*. As specified below, animals were either provided with control diet (CD, Prolab 5P76 IsoPro RMH 3000, Labdiet, St. Louis, MO, USA) or high fat diet (HFD, D12451i, Research Diets, New Brunswick, NJ, USA), with 4.1 kcal/g (%kcal from carbohydrates 60, protein 26, fat 14) or 4.73 kcal/g (%kcal from carbohydrates 20, protein 35, fat 45), respectively. During the light phase, light was provided by fluorescent bulbs (ca. 100 lux) and a dim red light (>600nm) was present at all times. Animals were weighted (CS200, Ohaus, Pine Brook, NJ, USA) 2–3 hours before lights off. The content of the food hopper was determined (HH120D, Ohaus, Pine Brook, NJ, USA) at the same time and refilled. In a pilot experiment, amount of spilled food in the cage was determined for all mice of Experiment 1, but no differences for genotypes nor gender were detected (data not shown) and so spillage was not quantified in the experiments, i.e. food intake is defined as the amount of food that disappeared from the food hopper. All animal experiments were approved by the Institutional Animal Care and Use Committee at the University of Massachusetts Medical School, and were in accordance with international standards (Portugalupi et al. 2008).

Experiment 1

As baseline, *mPer1/2/3^{tm1Drw}* (n=9 males, n=10 females) and *mPer3^{tm1Drw}* (n=7 males, n=5 females) single deficient animals, with age matched (3–5 month) wild-type controls (n=10 males, n=9 females) were monitored for body mass and food intake on control diet (CD) for 8 weeks. Then, the animals were switched to high fat diet (HFD) and body mass and food

intake was recorded for 21 weeks at irregular intervals. At the end of the experiment, subcutaneous inguinal and epididymal (male) or parametrial (female) fat pads, and liver were dissected and their mass determined (HH120D, Ohaus, Pine Brook, NJ, USA). Body mass gain is defined as the increase in mass for each mouse from baseline (CD) week 8 and after 21 weeks on HFD. Experiment 1 was conducted between March and October 2006. High fat diet was introduced on 9 May 2006 and the experiment was terminated 21 weeks later.

Experiment 2

Male *mPer3^{tm1Drw}* (n=19) were compared to wild-type control (n=16) mice to confirm the unexpected finding of altered body mass regulation in males of this genotype as observed in Experiment 1. (Female mice did not show diet induced obesity in Experiment 1, and so were not included in Experiment 2). All animals were weaned and single-housed at 3 weeks of age and kept on control diet for 1 week. Then, mice in the high fat diet (HFD) group were switched to the new diet while the control mice remained on control diet (CD) until the end of the experiment.

Body mass and food intake were monitored weekly. At 8 month of age, animals were sacrificed and fat pads and liver were dissected and weighed as in Experiment 1.

Experiment 2 was carried out between December 2006 and November 2007.

Data Analysis

All data are given as mean $\pm$ standard error of the mean (SEM). Data were compared using repeated measures or one way ANOVA and Scheffé's *post-hoc* test where appropriate (StatView 5.0, SAS Institute, Cary, NC). Statistical tests were 2-tailed and the level of significance was set to $\alpha = 0.05$.

Results

Experiment 1

The age of the mice at the beginning of the experiment was counter-balanced between genotypes, but was quite variable, spanning 2 months. Body mass did not differ between males of the three genotypes at the beginning of the experiment. After 21 weeks on HFD, body mass of *mPer1/2/3^{tm1Drw}* and *mPer3^{tm1Drw}* male mice was significantly higher than wild-type controls (Figure 1). The females did not show body mass differences between genotypes although there was a trend toward elevated body mass in the two mutant lines, relative to wild-type females. In fact, females did not show a significant diet induced body mass gain in any genotype even after 21 weeks on HFD (Figure 1, Table 1).

Food intake did not differ between *mPer3^{tm1Drw}* animals and wild-type controls, within either sex (Table 1). In *mPer1/2/3^{tm1Drw}* mice, however, a slightly higher intake of CD was observed in both males and females, relative to the wild-type controls. This difference in food intake disappeared during HFD. All groups reduced their food intake after the transition from CD to HFD diet (Table 1).

Body composition, judged by the mass of subcutaneous and perigonadal fat pads, was quite different between genotypes; male *mPer1/2/3^{tm1Drw}* and *mPer3^{tm1Drw}* mice had more fat than controls. Differences in female mice were more subtle. Nevertheless, perigonadal fat pads of *mPer3^{tm1Drw}* females were heavier than those of the two other lines. In addition, relative liver mass of the mutant lines was decreased compare to wild-type mice (Table 1).

Experiment 2

Due to the lack of major differences in body mass between the genotypes in female mice, we conducted Experiment 2 with male mice only. Experiment 2 was also modified to make the initial age at transfer to HFD more consistent. The results of Experiment 2 confirmed that male *mPer3^{tm1Drw}* mice had a delayed onset obesity phenotype when fed a high-fat diet, relative to wild-type controls (Table 2).

Body mass of *mPer3^{tm1Drw}* mice was indistinguishable from controls until 20 weeks of age, although they were already on HFD for 16 weeks at that time. Only subsequently, the mutant mice in the HFD group became heavier, and at 35 weeks of age the body mass difference between *mPer3^{tm1Drw}* and control mice was 13.1 ± 1.7 g ($p < 0.001$). In the CD group, the difference between the genotypes was much smaller (3.5 ± 1.0 g, $p = 0.08$, n.s.). Interestingly, only the mutant animals showed a difference between the two feeding conditions (7.7 g, $p < 0.01$); wild-type HFD mice did not differ significantly in body mass from those fed CD (2.1 g, $p = 0.23$).

Food intake did not differ between the genotypes until 20 weeks of age. Counter-intuitively, however, the *mPer3^{tm1Drw}* mutants had lower food intake, both in the HFD and CD groups. Also, both genotypes had lower food intake on the HFD compared to CD (Figure 3). Interestingly, body mass differences became apparent shortly after the animals reached 20 weeks of age.

At the end of the experiment (35 weeks of age), fat pads of *mPer3^{tm1Drw}* mice in the HFD group weighed more than double that of wild-type controls. Even in the CD group, mutant mice had significantly heavier fat pads than controls. This finding was present both when data were compared as absolute amount (per mouse) or relative (per 30 g mouse). Liver mass, however, did not differ between groups or genotypes (Table 2).

Discussion

Our data indicate that *mPer* genes are involved in body mass control in male mice. More specifically, we found that disrupting the circadian clock in *mPer1/2/3^{tm1Drw}* male mice led to higher body mass on a high fat diet. Surprisingly, however, *mPer3^{tm1Drw}* mice had a similar diet-induced obesity phenotype as the triple mutant mice. This was unexpected because *mPer3^{tm1Drw}* mice do not have any overt disruption of circadian rhythms (Shearman et al. 2000a). It has been proposed that *mPer3* plays a role in the output pathway of the core oscillator (Bae et al. 2001; Shearman et al. 2000a). Downstream targets of this output have not yet been identified, however. Nevertheless, subtle effects on the core circadian clock could translate to sizeable downstream effects on whole body physiology.

Certain allelic variations of the *hPer3* gene are associated with delayed sleep phase and sleep architecture (Archer et al. 2003; Ebisawa et al. 2001; Pereira et al. 2005; Viola et al. 2007). Moreover, significant differences in *hPer3* allelic frequencies between human populations have been reported (Ciarleglio et al. 2008; Nadkarni et al. 2005). In the case of the *bona fide* clock gene, *Clock*, an association between polymorphisms in *Clock* and obesity has been reported (Scott et al. 2008; Sookoian et al. 2008). In light of our data, it would be interesting to assess the association of polymorphisms in *hPer3* with obesity and other metabolic endpoints.

A link between sleep and metabolic syndrome has been established (Laposky et al. 2008; Wolk and Somers 2007). Notably, however, *Per3^{tm1Drw}* mice do not have an overt sleep phenotype (Shiromani et al. 2004). It thus seems that the propensity to obesity in this strain of mice is not secondary to a gross alteration in sleep.

In a recent publication, disruption of *mPer3* was shown to influence the period and phase of rhythms recorded *in vitro* from lung and pituitary explants, but SCN rhythms were unaffected (Pendergast et al. 2009). This finding raises the possibility that altered body mass regulation in mPER3-deficient mice is the result of “internal misalignment” in which the relative phasing of peripheral organs differs from wild-type (Scheer et al. 2009). Indeed, it has been shown that phase of food intake matters; day-fed mice gain more weight than night-fed mice (Arble et al. 2009). The results of Pendergast et al. (2009) were obtained with mPER3 deficient animals backcrossed extensively to C57BL/6 mice. Here, we used mice on a 129 genetic background. This particular strain, however, is not widely used in obesity research and is rather resistant to diet induced obesity (Fink et al. 2007; Kokkotou et al. 2005; Su et al. 2008; present results).

In Experiment 1, there was a trend toward higher body mass in the female *mPer* mutant mice, but this was not significant (Table 1). The *mPer3* mutant females did have heavier perigonadal fat pads and reduced relative liver mass, compared to wild-type controls, but even the wild-type females did not develop obesity on HFD. In this regard, the female mice appear more resistant to HFD-induced obesity than male mice on this genetic background. Some studies find that females tend to be more resistant to the effects of high fat diet (e.g., Shockley et al. 2009), but others do not find such sex dimorphism (e.g. Su et al. 2008). It may simply be that the increase in body mass with HFD is generally higher in males, and that in some studies, this increase is below the threshold of significance. While this sexual dimorphism is interesting, we focused our efforts on male mice in Experiment 2, providing an independent confirmation of an effect of *mPer3* disruption on diet-induced obesity.

Also, the differential effect on body mass became apparent only late in the experiments. Future studies should use mPER3 deficient mice backcrossed to C57BL/6J mice, in which diet-induced obesity is of larger magnitude, and address important questions beyond the scope of this report, and on which we do not have any data at this time, such as the time-course of alterations in body composition, metabolic rate, and energy balance, as well as biochemical parameters such as glucose and fatty acid metabolism. Metabolic rate measurements should be especially interesting in view of the paradoxical reduction in food intake but a higher body mass gain in *Per3^{tm1Drw}* mutant mice, compared to wild-type animals.

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References

- Arble DM, Bass J, Laposky AD, Vitaterna MH, Turek FW. Circadian timing of food intake contributes to weight gain. *Obesity* 2009;17:2100–2102. [PubMed: 19730426]
- Archer SN, Robilliard DL, Skene DJ, Smits M, Williams A, Arendt J, von Schantz M. A length polymorphism in the circadian clock gene *Per3* is linked to delayed sleep phase syndrome and extreme diurnal preference. *Sleep* 2003;26:413–415. [PubMed: 12841365]
- Bae K, Jin X, Maywood ES, Hastings MH, Reppert SM, Weaver DR. Differential functions of *mPer1*, *mPer2*, and *mPer3* in the SCN circadian clock. *Neuron* 2001;30:525–536. [PubMed: 11395012]
- Barnea M, Madar Z, Froy O. High-fat diet delays and fasting advances the circadian expression of adiponectin signaling components in mouse liver. *Endocrinology* 2009;150:161–168. [PubMed: 18801899]
- Bechtold DA. Energy-responsive timekeeping. *J Genet* 2008;87:447–458. [PubMed: 19147933]

- Ciarleglio CM, Ryckman KK, Servick SV, Hida A, Robbins S, Wells N, Hicks J, Larson SA, Wiedermann JP, Carver K, Hamilton N, Kidd KK, Kidd JR, Smith JR, Friedlaender J, McMahon DG, Williams SM, Summar ML, Johnson CH. Genetic differences in human circadian clock genes among worldwide populations. *J Biol Rhythm* 2008;23:330–340.
- Dallmann R, Touma C, Palme R, Albrecht U, Steinlechner S. Impaired daily glucocorticoid rhythm in *Per1^{Brd}* mice. *J Comp Physiol A* 2006;192:769–775.
- Dijk DJ, Archer SN. PERIOD3, circadian phenotypes, and sleep homeostasis. *Sleep Med Rev*. 2009;10.1016/j.smrv.2009.07.002
- Ebisawa T, Uchiyama M, Kajimura N, Mishima K, Kamei Y, Katoh M, Watanabe T, Sekimoto M, Shibui K, Kim K, Kudo Y, Ozeki Y, Sugishita M, Toyoshima R, Inoue Y, Yamada N, Nagase T, Ozaki N, Ohara O, Ishida N, Okawa M, Takahashi K, Yamauchi T. Association of structural polymorphisms in the human *period3* gene with delayed sleep phase syndrome. *EMBO Rep* 2001;2:342–346. [PubMed: 11306557]
- Fink BD, Herlein JA, Almind K, Cinti S, Kahn CR, Sivitz WI. Mitochondrial proton leak in obesity-resistant and obesity-prone mice. *Am J Physiol Reg-I* 2007;293:R1773–1780.
- Hsieh MC, Yang SC, Tseng HL, Hwang LL, Chen CT, Shieh KR. Abnormal expressions of circadian-clock and circadian clock-controlled genes in the livers and kidneys of long-term, high-fat-diet-treated mice. *Int J Obes* 2010;34:227–239.
- Kennaway DJ, Owens JA, Voultsios A, Boden MJ, Varcoe TJ. Metabolic homeostasis in mice with disrupted Clock gene expression in peripheral tissues. *Am J Physiol Reg-I* 2007;293:R1528–1537.
- Kohsaka A, Laposky AD, Ramsey KM, Estrada C, Joshu C, Kobayashi Y, Turek FW, Bass J. High-fat diet disrupts behavioral and molecular circadian rhythms in mice. *Cell Metab* 2007;6:414–421. [PubMed: 17983587]
- Kokkotou E, Jeon JY, Wang X, Marino FE, Carlson M, Trombly DJ, Maratos-Flier E. Mice with MCH ablation resist diet-induced obesity through strain-specific mechanisms. *Am J Physiol Reg-I* 2005;289:R117–124.
- Kondratov RV, Gorbacheva VY, Antoch MP. The role of mammalian circadian proteins in normal physiology and genotoxic stress responses. *Cur Top Dev Bio* 2007;78:173–216.
- Laposky AD, Bass J, Kohsaka A, Turek FW. Sleep and circadian rhythms: key components in the regulation of energy metabolism. *FEBS Lett* 2008;582:142–151. [PubMed: 17707819]
- Nadkarni NA, Weale ME, von Schantz M, Thomas MG. Evolution of a length polymorphism in the human PER3 gene, a component of the circadian system. *J Biol Rhythm* 2005;20:490–499.
- Pendergast JS, Nakamura W, Friday RC, Hatanaka F, Takumi T, Yamazaki S. Robust food anticipatory activity in BMAL1-deficient mice. *PloS One* 2009;4:e4860. [PubMed: 19300505]
- Pereira DS, Tufik S, Louzada FM, Benedito-Silva AA, Lopez AR, Lemos NA, Korczak AL, D'Almeida V, Pedrazzoli M. Association of the length polymorphism in the human Per3 gene with the delayed sleep-phase syndrome: does latitude have an influence upon it? *Sleep* 2005;28:29–32. [PubMed: 15700718]
- Pilorz V, Steinlechner S. Low reproductive success in Per1 and Per2 mutant mouse females due to accelerated ageing? *Reproduction* 2008;135:559–568. [PubMed: 18367514]
- Portaluppi F, Touitou Y, Smolensky MH. Ethical and methodological standards for laboratory and medical biological rhythm research. *Chronobiol Int* 2008;25:999–1016. [PubMed: 19005901]
- Reppert SM, Weaver DR. Coordination of circadian timing in mammals. *Nature* 2002;418:935–941. [PubMed: 12198538]
- Rudic RD, McNamara P, Curtis AM, Boston RC, Panda S, Hogenesch JB, Fitzgerald GA. BMAL1 and CLOCK, two essential components of the circadian clock, are involved in glucose homeostasis. *PLoS Biol* 2004;2:e377. [PubMed: 15523558]
- Scheer FA, Hilton MF, Mantzoros CS, Shea SA. Adverse metabolic and cardiovascular consequences of circadian misalignment. *PNAS* 2009;106:4453–4458. [PubMed: 19255424]
- Scott EM, Carter AM, Grant PJ. Association between polymorphisms in the Clock gene, obesity and the metabolic syndrome in man. *Int J Obesity* 2008;32:658–662.
- Shearman LP, Jin X, Lee C, Reppert SM, Weaver DR. Targeted disruption of the *mPer3* gene: subtle effects on circadian clock function. *Mol Cell Biol* 2000a;20:6269–6275. [PubMed: 10938103]

- Shearman LP, Sriram S, Weaver DR, Maywood ES, Chaves I, Zheng B, Kume K, Lee CC, van der Horst GT, Hastings MH, Reppert SM. Interacting molecular loops in the mammalian circadian clock. *Science* 2000b;288:1013–1019. [PubMed: 10807566]
- Shiromani PJ, Xu M, Winston EM, Shiromani SN, Gerashchenko D, Weaver DR. Sleep rhythmicity and homeostasis in mice with targeted disruption of *mPeriod* genes. *Am J Physiol Reg-I* 2004;287:R47–57.
- Shockley KR, Witmer D, Burgess-Herbert SL, Paigen B, Churchill GA. Effects of atherogenic diet on hepatic gene expression across mouse strains. *Physiol Genomics* 2009;39:172–182. [PubMed: 19671657]
- Sookoian S, Gemma C, Gianotti TF, Burgueno A, Castano G, Pirola CJ. Genetic variants of Clock transcription factor are associated with individual susceptibility to obesity. *Am J Clin Nutr* 2008;87:1606–1615. [PubMed: 18541547]
- Su Z, Korstanje R, Tsaih SW, Paigen B. Candidate genes for obesity revealed from a C57BL/6J × 129S1/SvImJ intercross. *Int J Obes* 2008;32:1180–1189.
- Turek FW, Joshu C, Kohsaka A, Lin E, Ivanova G, McDearmon E, Laposky A, Losee-Olson S, Easton A, Jensen DR, Eckel RH, Takahashi JS, Bass J. Obesity and metabolic syndrome in circadian *Clock* mutant mice. *Science* 2005;308:1043–1045. [PubMed: 15845877]
- van der Veen DR, Archer SN. Light-dependent behavioral phenotypes in PER3-deficient mice. *J Biol Rhythm* 25:3–8.
- Viola AU, Archer SN, James LM, Groeger JA, Lo JC, Skene DJ, von Schantz M, Dijk DJ. PER3 polymorphism predicts sleep structure and waking performance. *Curr Biol* 2007;17:613–618. [PubMed: 17346965]
- Weaver, DR.; Reppert, SM. Circadian Timekeeping. In: Squire, LR., et al., editors. *Fundamental Neuroscience*. 3. Elsevier/Academic Press; Amsterdam; Boston: 2008. p. 931-958.
- Wolk R, Somers VK. Sleep and the metabolic syndrome. *Exp Physiol* 2007;92:67–78. [PubMed: 17085678]

Figure 1. Body mass development in male (closed symbols) and female (open symbols) *Per1/2/3^{tm1Drw}*, *Per3^{tm1Drw}* and wild-type (WT) mice in Experiment 1. See also Table 1.

Figure 2.
Body mass development of male *Per3^{tm1Drw}* and wild-type (WT) mice in Experiment 2.

Figure 3. Monthly mean values ($\pm$ SEM) for daily food intake of male *Per3^{tm1Drw}* and wild-type mice on different diets in Experiment 2 (HFD = High fat diet, CD = Control diet). Where SEM bars are not visible, they fall within the symbol.

Table 1

Body mass gain on HFD, mean daily food intake on control (CD) and high fat diet (HFD), subcutaneous and perigonadal fat and liver mass of mice used in Experiment 1 (mean±SEM, in g or g/30g mouse).

Genotype	N	Body mass gain	CD food intake		HFD food intake		Subcutaneous fat		Perigonadal fat		Liver	
			Abs.	Rel.	Abs.	Rel.	Abs.	Rel.	Abs.	Rel.	Abs.	Rel.
Males												
<i>Per1/2/3^{mt}Drw</i>	9	9.4±1.9***	4.8±0.1	6.0±0.1***	2.3±0.1*	2.4±0.1	1.1±0.2***	0.9±0.1***	2.1±0.3***	1.8±0.2***	1.6±0.2	1.3±0.1
<i>Per3^{mt}Drw</i>	7	13.1±1.7***	4.9±0.2	5.6±0.1	2.3±0.1	2.4±0.1	1.5±0.2***	1.1±0.1***	2.6±0.3***	1.8±0.1***	1.9±0.2	1.4±0.1
wild-type	10	1.4±1.5	5.2±0.3	5.4±0.1	2.7±0.1	2.5±0.1	0.6±0.1	0.5±0.1	1.0±0.2	1.0±0.1	1.7±0.1	1.7±0.1
Females												
<i>Per1/2/3^{mt}Drw</i>	10	2.5±0.7	5.0±0.1	6.8±0.1***	2.1±0.1	2.7±0.1	0.5±0.1	0.6±0.1	1.0±0.2	1.3±0.2	1.1±0.1	1.4±0.1*
<i>Per3^{mt}Drw</i>	5	2.3±1.1	4.8±0.3	5.9±0.1	2.2±0.1	2.4±0.1	0.5±0.1	0.6±0.1	1.2±0.1*	1.4±0.1*	1.1±0.1	1.3±0.1*
wild-type	9	0.0±0.7	5.2±0.3	6.1±0.1	2.4±0.1	2.6±0.1	0.3±0.1	0.4±0.1	0.7±0.1	0.8±0.1	1.5±0.1	1.8±0.1

Abbreviations: M = male, F = female, Abs. = absolute per mouse, Rel. = relative per 30 g mouse.

* p<0.05 and

*** p<0.001 vs. wild-type of same sex.

Body mass gain on HFD, subcutaneous and epididymal fat and liver mass of male mice used in Experiment 2 (mean±SEM, in g or g/30g mouse).

Table 2

Genotype	Diet	N	Body mass gain	Subcutaneous fat		Epididymal fat		Liver	
				Abs.	Rel.	Abs.	Rel.	Abs.	Rel.
<i>Per3^{mlDrw}</i>	HFD	10	22.6±1.3 ^{****}	1.6±0.2 ^{***}	1.2±0.1 ^{***}	2.8±0.2 ^{***}	2.1±0.1 ^{***}	1.6±0.1 [*]	1.1±0.1
	CD	9	14.8±1.2	0.7±0.1 ^{***}	0.5±0.1 ^{***}	1.2±0.1 ^{***}	0.8±0.2 ^{***}	1.3±0.1	1.5±0.1
wild-type	HFD	7	9.6±1.8	0.5±0.2	0.6±0.1	0.8±0.3	1.1±0.1	1.3±0.1	1.3±0.1
	CD	9	10.3±0.8	0.3±0.1	0.3±0.1	0.5±0.1	0.5±0.1	1.4±0.1	1.5±0.1

Abbreviations: HFD = high fat diet, CD = control, Abs. = absolute per mouse, Rel. = relative per 30 g mouse,

* p<0.05 and

*** p<0.01 *Per3^{mlDrw}* vs. wild-type on same diet.