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Interleukin-18 null mutation increases weight and food intake and reduces energy expenditure and lipid substrate utilization in high-fat diet fed mice

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Abstract

Objective—The proinflammatory cytokine interleukin-18 (IL-18) putatively modulates food intake and energy metabolism, but the effects of IL-18 in high-fat diet fed animals are unknown. Whether IL-18 alters basal metabolic rate or metabolic processes of living is unknown. Here, we tested the hypothesis that IL-18 modulates weight gain, energy intake, whole-body energy expenditure, and utilization of lipid as a fuel substrate in high-fat diet fed mice.

Methods—Food intake, whole-body metabolism, and motor activity of IL-18 knockout mice were compared to those of wildtype littermates; anorectic effects of intracerebroventricular IL-18 administration were compared between IL-18 receptor knockout, IL-18/IL-18R knockout and wildtype mice.

Results—Chow-reared IL-18 knockout mice were overweight at 6 months of age and then gained excess weight on both low-fat and high-fat diets, ate more high-fat diet, and showed reduced whole-body energy expenditure and increased respiratory exchange ratios. Reductions in energy expenditure of IL-18 knockout mice were seen across fasting vs. feeding conditions, low- vs. high-fat diets, high vs. low levels of physical activity and times of day, suggesting actions on basal metabolic rate. The circadian amplitude of energy expenditure, but not respiratory exchange ratio, food intake, or motor activity, also was blunted in IL-18 knockout mice. Central IL-18 administration reduced high-fat diet intake in wildtype mice, but not in mice lacking the IL-18 receptor.

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Conflict of Interest Statement The authors have no conflicts of interest with the present work.

Conclusion—The loss-of-function results support the hypothesis that endogenous IL-18 suppresses appetite and promote energy expenditure and lipid fuel substrate utilization not only during sickness, but also in healthy adults consuming high-fat diets.

Keywords

obesity or overweight; energy expenditure or metabolism; food intake or appetite; proinflammatory cytokine; body weight; high-fat diet; interleukin-18; cosinor analysis or circadian rhythm; indirect calorimetry; locomotor or physical activity

Introduction

Better understanding the molecular controls of energy metabolism may inform the treatment of obesity. Interleukin-18 (IL-18), an 18 kDa multifunctional cytokine discovered for its proinflammatory and interferon-γ-inducing properties (Okamura et al., 1995), produces diverse effects via activation of the IL-18 receptor complex (Born et al., 2000; Torigoe et al., 1997), an IL-1/Toll-like superfamily receptor. Recent findings suggest that IL-18 may be a physiologic modulator of food intake and energy metabolism. Unlike classic proinflammtory cytokines that mediate the sickness response (e.g., IL-1β, IL-6, TNF-α), IL-18 also is constitutively expressed in non-immune cells and derived partly from adipocytes. Similar to other adipocytokines, its circulating levels relate to metabolic state, including fat mass, weight loss, hyperglycemia, and dietary fat intake (Esposito et al., 2002a; Esposito et al., 2002b; Esposito et al., 2003). Intracebrebroventricular or peripheral administration of IL-18 potently suppressed chow intake, feed efficiency and weight regain in fasted mice, without promoting sickness-like behavior (Zorrilla et al., 2007). Conversely, mice partially (*Il18*$^{+/-}$) or totally deficient (*IL18*$^{-/-}$) in IL-18 overate chow and purified low-fat diet by young adulthood (Zorrilla et al., 2007). Adult *Il18*$^{-/-}$ mice showed increased feed efficiency; indirect calorimetry revealed reduced energy expenditure in low-fat diet-fed female *Il18*$^{-/-}$ mice and increased respiratory exchange ratios (RER) (VCO_2/VO_2) in mutants of both sexes (Zorrilla et al., 2007). By mid-adulthood, *Il18*$^{-/-}$ mice became obese (Netea et al., 2006; Zorrilla et al., 2007). Similar delayed-onset obesity phenotypes were observed in IL-18 receptor knockout (KO) mice and in IL-18-binding protein overexpressing mice (Netea et al., 2006).

The present studies sought to determine the effects of the IL-18 null genotype in mice fed high-fat diet. Previous calorimetry studies in IL-18 KO mice were performed using low-fat diet (Zorrilla et al., 2007). Few humans eat low-fat diets, however, and the indirect calorimetric profile of IL-18 null mice is unknown. High-fat diets can produce different rates of energy expenditure as compared with low-fat diets (Bandini et al., 1994; Ebbeling et al., 2012), in relation to the different energy and macronutrient intakes elicited by each. High-fat diets also promote greater relative utilization of lipids as a fuel substrate vs. low-fat diets (McNeill et al., 1988; Rumpler et al., 1991; Verboeket-van de Venne et al., 1994). As a result of these differences, many studies of transgenic mice have observed strikingly different metabolic phenotypes with high-fat diet exposure (Gordon et al., 2008; Klockener et al., 2011; Kusudo et al., 2012; Lee et al., 2007; Paula et al., 2010; Strader et al., 2004; Sutton et al., 2006; Wortley et al., 2004; Zigman et al., 2005). Potentially consistent with a

role for IL-18 in metabolic adaptations to high-fat diet, high-fat meals increase circulating IL-18 levels. Therefore, the present study tested the hypothesis that IL-18 null mutation also reduces whole-body energy expenditure and utilization of lipid as a fuel substrate in high-fat diet fed mice.

Energy expenditure can be subdivided into components that reflect the basal metabolic rate of minimally maintaining the organism as compared to phasic components of energy expenditure related to activities of living, including physical activity, thermic effects of food intake and adaptive thermogenesis (Even and Nadkarni, 2012). In our previous study of IL-18 KO mice, whole-body energy metabolism was studied in free-feeding mice, and the genotypes exhibited differences in food intake and motor activity (Zorrilla et al., 2007). Thus, it remains unclear whether phasic components of energy expenditure are responsible for the observed differences in total daily energy expenditure or whether IL-18 KO mice may exhibit a reduced basal metabolic rate. To differentiate between the hypotheses that basal metabolic processes vs. phasic metabolic processes (e.g., activity, absorptive phase of feeding) contribute to IL-18 genotype effects on total daily energy expenditure, the present study measured whole-body energy expenditure of IL-18 KO and wildtype mice under both fasting and feeding conditions within each of the dark cycle and light cycle. Concurrent motor activity was measured.

A third goal was to determine the circadian-dependence of the IL-18 phenotype on food intake and energy expenditure. In our initial study, hyperphagia of low-fat diet was most evident during the mid-to-late dark cycle and respiratory exchange ratios were observed at some, but not other, times of day. On the other hand, genotype differences in energy expenditure of low-fat diet-fed mice and circulating IL-18 levels were consistent across a 24-hr period (Zorrilla et al., 2007). Still, circadian variations in sensitivity to IL-18 might exist, as has been seen for IL-1β and IFN-γ (Opp and Toth, 1997). We therefore performed a cosinor analysis of chronobiologic differences in the food intake, energy expenditure, respiratory exchange ratios and motor activity of high-fat diet-fed IL-18 KO vs. wildtype mice.

A final pharmacological study sought to determine whether brain IL-18 systems also modulate the control of high-fat diet intake and the mediating role of the IL-18R therein.

Methods

Ethical approval

Procedures adhered to the National Institutes of Health Guide for the Care and Use of Laboratory Animals and were approved by the Institutional Animal Care and Use Committee of The Scripps Research Institute.

Subjects

Subjects were IL-18 knockout (KO; *Il18*$^{-/-}$) mice generated on a C57BL/6J background (Takeda et al., 1998) (from Arturo Zychlinsky, New York University, NY), IL-18 receptor α-subunit KO mice (*Il18R*$^{-/-}$), double IL-18/IL-18R KO mice (*Il18*$^{-/-}$/*Il18R*$^{-/-}$), and their wildtype (WT) littermates. Mature (24-26 weeks) female mice were studied because IL-18

KO mice do not show increased feed efficiency, weight gain and adiposity until maturity and because genotype effects observed previously were slightly greater in females than in males (Netea et al., 2006; Zorrilla et al., 2007). Before studies, mice were group-housed in a 12:12 hr light cycle, humidity- (60%) and temperature-controlled (22°C) vivarium with chow and water available *ad libitum* (Harlan Teklad LM-485, 3.1 kcal/g, 17% [kcal] fat, 58% carbohydrates, 25% protein, Harlan, Indianapolis, IN).

Experimental diets

During indirect calorimetry, mice received a powdered purified low-fat diet (Research Diets D12450B; 10% [kcal] fat, 70% carbohydrate, 20% protein, 3.85 kcal/g) or its matched, high-fat/high-energy purified diet (D12492; 60% fat, 20% carbohydrate, 20% protein, 5.24 kcal/g).

Indirect calorimetry

Indirect calorimetry was performed using a computer-controlled, open-circuit system (Oxymax; Comprehensive Lab Animal Monitoring System; Columbus Instruments, Columbus, OH). Mice were tested in individual clear chambers (20 × 10 × 12.5 cm) with a stainless steel elevated wire floor. Each chamber contained a sipper tube delivering water, food tray connected to a balance, and 16 photobeams at 1.3 cm intervals situated in rows 3.3 and 7.3 cm above the floor to detect motor activity along the *x*- and *z*-axes, respectively. Room air was passed through chambers at ~0.5 L/min. The chamber exhaust was sampled for 1 min (at 16-min intervals) and passed through O_2 and CO_2 sensors for estimation of oxygen consumption (VO_2) and carbon dioxide production (VCO_2). Outdoor air reference values were sampled every 8 measurements. Gas sensors were calibrated before experiments with gas standards containing known concentrations of O_2, CO_2, and N_2 (Airgas Puritan Medical, Ontario, CA). Respiratory exchange ratio (RER) was calculated as the ratio of carbon dioxide production (VCO_2) to oxygen consumption (VO_2). Energy expenditure (heat formation [(3.815 + 1.232*RER)*VO_2 (in liters)]) was corrected for estimated metabolic mass per Kleiber's power function (Kleiber, 1975), as were differences in food intake. As an alternative correction, raw energy expenditure estimates also were subjected to analysis of covariance (ANCOVA), covarying for body weight (Arch et al., 2006).

Study design

Mice were acclimated in the calorimetry apparatus with low-fat diet and water for 5 days before the present studies. Then, mice were fasted within the calorimetry apparatus for 3 hr beginning from the onset of the dark cycle, after which low-fat diet was returned and food intake, locomotor activity and metabolism were monitored for another 3 hr. Following refeeding and normalization of intake, the fast-refeed procedure was repeated, but beginning instead from the light cycle onset. Subsequently, mice were acclimated to the high-fat diet for 4 days, after which 24-hr of uninterrupted data were collected beginning from the dark cycle onset. Thereafter, dark- and light-cycle data were collected using the fast-refeed protocol as per the low-fat diet studies. Studies were performed under ambient room temperature (~24-26°C) with water available. Mice were weighed using a scale of 0.1 g precision every 1-2 days during the study.

Intracerebroventricular IL-18 infusion and high-fat diet intake

The present study sought to determine whether IL-18 might centrally modulate the control of high-fat intake and the requirement for the IL-18R in these effects. Mice lacking the IL-18R (*Il18R*$^{-/-}$ and *Il18*$^{-/-}$/*Il18R*$^{-/-}$) and their wildtype littermates were acclimated to high-fat diet and then, under isoflurane anesthesia, stereotactically implanted with a unilateral cannula targeting the lateral ventricle as described previously (Zorrilla et al., 2007). After 1 week recovery, individually-housed mice were food-deprived for 24 hr and then received vehicle (2 μl saline) or IL-18 (2 nmol/2 μl, injected over 4 minutes) 5 min before they received renewed access to pre-weighed high-fat diet access at the dark cycle onset. Treatments were given in a counterbalanced, within-subjects design, spaced by 11 days.

Statistics

Analyses of variance (ANOVA) or Student's *t*-tests were used for comparisons involving >2 or exactly 2 levels, respectively. To analyze energy expenditure, RER and motor activity in the fast-refeed studies, a 4-way repeated-measures ANOVA was used. Within-subject factors were Feeding Condition (2: Fasting vs. Feeding), Diet (2: Low-fat vs. High-fat), and Cycle (2: Dark vs. Light). Genotype (2: IL-18 KO vs. WT) was a between-subjects factor. Energy intake during refeeding was analyzed by 3-way repeated-measures ANOVA with Diet and Cycle as within-subject factors and Genotype as a between-subjects factor. For the 23-hr *ad lib* high-fat diet study, *t*-tests were used to assess genotype differences in daily (23-hr) energy intake, energy expenditure, RER and motor activity. To compare the circadian time course of these measures, 3-way repeated measures ANOVA was used on 3-hr bin averages; Cycle (Dark vs. Light) and Time Bin (1-3, 4-6, 7-9 and 10-12 hr of each cycle) were within-subject factors, and Genotype was a between-subjects factor. Cosinor analysis was used to determine how genotype altered the daily chronobiological rhythms of energy intake and metabolism (Chen et al., 2006). The following circadian attributes were calculated: the midline estimating statistic of rhythm (MESOR; mean level around which the cosine function oscillates), amplitude [the distance from the MESOR to the extremes [peak and nadir] of the oscillation], and the acrophase (the time at which the ci peak occurs relative to a time of interest, in this case the start of the session/dark cycle). A predefined period of 24 h was used per the following equation:

$$y = MESOR + \text{amplitude} \times cos\left(\frac{2\pi\,(x - \text{acrophase})}{24}\right)$$

Supplemental Figure 1 illustrates the cosinor analysis measures. Cosinor functions were fit individually to obtain the MESOR, amplitude, acrophase, and goodness of fit (*r*) for each mouse. Peaks were calculated as the MESOR + amplitude; nadirs were calculated as the MESOR − amplitude. Genotype differences in these parameters were evaluated by *t*-test. Parameters were cumulated into 3-h bins to facilitate modeling of the hypothesized underlying circadian rhythm. Mixed design, two-way ANOVA was used to determine the effects of IL-18 treatment (within-subjects factor), in interaction with Genotype (between-

subjects factor), on 6-hr high-fat diet intake. The software used was Systat 12.0 (SPSS, Chicago, IL) and DataFit 9.0 (Oakdale Engineering, Oakdale, PA).

Results

Body weight

As Table 1 shows, age-matched, chow-reared female *Il18*$^{-/-}$ mice weighed 32% (7.5 g) more than WT mice at the study onset. By completion of the high-fat diet studies, IL-18 KO weighed 50% (11.4 g) more than WT controls. A Genotype main effect indicated that IL-18 KO mice gained more weight than WT mice ($F_{1,11}$=10.04, p< 0.01), irrespective of the diet available (Genotype × Diet: $F_{1,11}$=0.76, p>0.40). A Diet main effect reflected that mice of both genotypes gained weight faster during the high-fat diet, than low-fat diet, phase of the study ($F_{1,11}$=33.38, p<0.0001) (see Table 1).

Fast-refeed protocol

Food intake—Energy intake during the 3-hour refeeding periods did not differ per main effects or interactions that involved Genotype or Diet (all *p*s>0.1; Genotype vs. WT [*M* ±SEM kcal] for low-fat dark cycle, 2.61±0.11 vs. 3.06±0.33, light cycle, 2.84±0.26 vs. 2.89±0.28; for high-fat dark cycle, 2.79±0.56 vs. 3.05±0.45, light cycle, 2.71±0.34 vs. 3.44±0.50).

Energy expenditure—Figure 1 shows that, across times of day, diets and feeding state, female *Il18*$^{-/-}$ mice expended less energy than WT littermates (Genotype: $F_{1,11}$=6.01, p<0.05). A trend for a Genotype × Diet × Fasted interaction ($F_{1,11}$=4.51, p=0.057) indicated that the magnitude of IL-18 genotype differences in heat tended to vary in relation to the diet and feeding state of the mice. Genotype differences in energy expenditure tended to be even greater in mice fasted from low-fat diet, than in those fasted from high-fat diet (Genotype × Diet in fasted mice, p=0.07). Specifically, WT mice showed greater energy expenditure when fasted from low-fat diet, than from high-fat diet (p=0.05), a difference not evident in IL-18 KO mice (p=0.97). In contrast, genotype differences were of similar magnitude in the fed state between diet conditions. No other interactions involving Genotype approached significance (all *p*s>0.11). Main effects indicated that fasting ($F_{1,11}$=19.62, p<0.001) and high-fat diet ($F_{1,11}$=4.75, p=0.05) independently reduced whole-body heat expenditure vs. their respective fed and low-fat diet conditions (see Figure 1).

Respiratory exchange ratio—Figure 1 shows the large main effects of Diet ($F_{1,11}$=339.57, p<0.001), Light Cycle ($F_{1,11}$=64.46, p<0.001) and Fasting ($F_{1,11}$=334.00, p<0.001) on respiratory exchange ratios (RER). Consistent with greater relative utilization of fat as a fuel substrate, lower RERs were seen during fasting, the high-fat diet condition, and the early dark cycle (i.e., after the light cycle, during which comparatively little is eaten). Diet × Cycle ($F_{1,11}$=36.16, p<0.001), Diet × Fasting ($F_{1,11}$=165.94, p<0.001), Fasting × Cycle ($F_{1,11}$=4.65, p=0.05) and Diet × Fasting × Cycle interactions ($F_{1,11}$=19.15, p<0.001), reflected that: 1) refeeding on low-fat diet increased RER more than did refeeding on high-fat diet, 2) RERs of mice fasting from low-fat diet were not as low as those of mice fasting from high-fat diet, and 3) the latter difference was greater at the beginning of the

light cycle, when mice had just completed their active (feeding) cycle. Genotype-related differences in RER were not detected in the context of these large effects (all *p*s>0.15) (see Figure 1).

Motor activity—Table 2 shows horizontal and vertical motor activity of mice during calorimetry testing. Motor activity in both dimensions was greater during the dark cycle, than light cycle, and especially during the fasting (vs. feeding) phases of testing (Fasting × Cycle: Fs$_{1,11}$>8.40, *p*s<0.015; Cycle: Fs$_{1,11}$>20.37, *p*s<0.001). However, a substantial Diet × Fasting interaction reflected that motor activity was greater during the feeding than fasting period in high fat diet-fed mice, whereas the reverse was true in low fat diet-fed mice (Fs$_{1,11}$>20.94, *p*s<0.001); this pattern was significantly more prominent in WT mice than in IL-18 KO mice (Genotype × Diet × Fasting: Fs$_{1,11}$>5.08, *p*s<0.05). Genotype effects on motor activity could not (fully) account for differences in energy expenditure, because the latter were still present even during time bins that IL-18 KO mice moved as much as or more than WT mice (see Table 2).

23-hr *ad libitum* high-fat diet protocol

Table 3 shows that during the 23-hr *ad libitum* high-fat diet study, female *Il18*$^{-/-}$ mice ate 44% more high-fat diet (t_{11}=4.18, *p*<0.005), expended 18% less energy (t_{11}=−2.47, *p*<0.05), and showed a significantly increased mean RER (t_{11}=5.17, *p*<0.0005) vs. WT mice. *Il18*$^{-/-}$ mice still ate more even after controlling for differences in non-fat body weight (1.47+0.08 vs. 1.23+0.06 kcal/g$^{0.75}$, *p*<0.05). There were no genotype differences in total horizontal or vertical motor activity (t_{11}=−0.88 and 0.29, respectively; *p*s>0.4) (see Table 3). Figure 2 shows the observed (panels A, C, E) and cosinor-fitted (B, D, F) time course of food intake and energy metabolism measures during the study. Table 3 shows the parameters of the cosinor functions, which fit the observed data well, especially beginning ~2 hr into the session, by when effects of initiating the session had dissipated.

Food intake—Time course analysis showed that IL-18 KO mice ate more high-fat diet than WT mice (Genotype: $F_{1,11}$=16.52, *p*<0.002), especially during the light cycle (Genotype × Cycle: $F_{1,11}$=7.74, *p*<0.02) and at particular times within a cycle (Genotype × Bin: $F_{3,33}$=5.42, *p*<0.005). IL-18 KO mice had a greater MESOR, peak and nadir of their circadian rhythm for high-fat diet intake vs WT mice (ts$_{11}$=4.08, 3.86 and 3.47, respectively, *p*s<0.005). No genotype difference was seen in the amplitude of the food intake rhythm (t_{11}=−0.25, *p*>0.80). As expected, mice ate less during the light cycle than dark cycle ($F_{1,11}$=7.44, *p*=0.02), and intake declined across the dark cycle and increased across the light cycle (linear contrast Cycle × Bin: $F_{1,11}$=81.28, *p*<0.001). The feeding rhythm of both genotypes was adequately fit by the cosinor function and had a circadian peak (acrophase) ~1-2 hr into the dark cycle (see Figure 2A-B and Table 3).

Energy expenditure—IL-18 KO mice expended less energy than WT mice (Genotype: $F_{1,11}$=5.97, *p*<0.05), irrespective of the cycle (Genotype × Cycle: $F_{1,11}$=1.90, *p*>0.19), the time bin within the cycle (Genotype × Bin: $F_{3,33}$=2.30, *p*>0.09), and their 3-way interaction (Genotype × Cycle × Bin: $F_{3,33}$=1.33, *p*>0.28). IL-18 KO mice not only had a reduced MESOR (t_{11}=−2.45, *p*<0.05), peak (t_{11}=−2.32, *p*<0.05), and nadir (t_{11}=−2.16, *p*=0.05) of

their circadian rhythm for energy expenditure, but also a reduced amplitude (t_{11}=−2.16, p=0.05). Genotype differences were not seen in the timing of the circadian peak for heat, which occurred ~2-3 hr into the dark cycle for both genotypes (acrophase: t_{11}=−1.00, p>0.3), or in the goodness-of-fit of the cosinor function (t_{11}=−0.99, p>0.3) (see Figure 2C-D and Table 3). As expected, energy expenditure was lower during the light cycle than dark cycle ($F_{1,11}$=48.89, p<0.001) and decreased across the dark cycle and increased across the light cycle (linear contrast Cycle × Bin: $F_{1,11}$=110.83, p<0.001).

IL-18 KO mice also expended less energy if ANCOVA, covarying for body weight, was used as the method of analysis. Differences manifested in Genotype × Cycle × Bin ($F_{3,30}$=5.27, p<0.005) and Genotype × Cycle interactions ($F_{1,10}$=4.54, p=0.05). The interactions again reflected the blunted circadian amplitude of energy expenditure of IL-18 KO mice, with reduced energy expenditure rate evident throughout the dark cycle (weight-adjusted lsmeans + standard error for KO vs.WT, Hr 1-3: 0.61+0.06 vs. 0.47+0.06, Hr 4-6: 0.54+0.05 vs. 0.40+0.05, Hr 7-9: 0.50+0.05 vs. 0.39+0.05, Hr 10-12: 0.52+0.05 vs. 0.36+0.05 kcal hr^{-1}).

Respiratory exchange ratio—IL-18 KO mice also consistently had higher RERs than WT mice (Genotype: $F_{1,11}$=24.88, p<0.001), irrespective of the cycle (Genotype × Cycle: $F_{1,11}$=1.89, p>0.19), the time bin within the cycle (Genotype × Bin: $F_{3,33}$=0.28, p>0.83), or their 3-way interaction (Genotype × Cycle × Bin: $F_{3,33}$=0.85, p>0.47). IL-18 KO mice had a greater MESOR, peak and nadir of their circadian rhythm for RERs as compared with WT mice (ts$_{11}$=4.68, 4.32 and 3.51, respectively, ps<0.005). However, unlike the rhythm for heat, no genotype difference was seen in the amplitude of RER rhythm (t_{11}=1.53, p>0.15). The RER rhythm of both genotypes was adequately fit by the cosinor function and had an acrophase just before the onset of the dark cycle (see Figure 2E-F and Table 3). As expected, RERs declined across the dark cycle and increased across the light cycle (linear contrast Cycle × Bin: $F_{1,11}$=86.48, p<0.001).

Motor activity—Figure 3 shows that IL-18 KO mice did not differ reliably from WT mice in horizontal or vertical motor activity, as evidenced by nonsignificant main and interaction effects that involved genotype (all ps>0.07). As expected, motor activity was greater during the dark (vs. light) cycle (Cycle: $F_{1,11}$=97.76, p<0.001), and varied within cycles as a function of time (Bin: $F_{1,11}$=97.76, p<0.001). Because genotype effects were not seen, cosinor analyses were not performed.

Central IL-18 infusion reduces high-fat diet intake via an IL-18R dependent mechanism—Finally, as shown in Figure 4, acute intracerebroventricular infusion of IL-18 reduced 6-hr high-fat diet intake in fasted wildtype mice, but not in *Il18R*$^{-/-}$ mice or *Il18*$^{-/-}$/*Il18R*$^{-/-}$ mice, as reflected in significant Genotype × Dose ($F_{2,12}$=4.03, p<0.05) and Genotype ($F_{2,12}$=8.83, p<0.005) effects.

Discussion

The present study confirms that IL-18 deficiency promotes positive energy balance in healthy female mice across times of day and dietary feeding conditions. Chow-reared female

IL-18 KO mice were overweight at ~6 months of age, gained excess weight on both low-fat and high-fat purified diets, ate more high-fat diet, and showed reduced whole-body energy expenditure and decreased relative utilization of lipid as a fuel substrate vs. WT mice. The results further implicate endogenous IL-18 as a catabolic regulator of food intake and energy metabolism during adulthood.

Reductions in energy expenditure of IL-18 KO mice were seen across feeding conditions (fasting, refeeding, *ad libitum*) and diets (low-fat, high-fat), newly demonstrating that at least some component of the reductions are independent of thermic effects of feeding. Differences in energy expenditure were evident using Kleiber power-function (Kleiber, 1975) or ANCOVA-based analysis to correct for differences in metabolic mass (Arch et al., 2006). IL-18 KO mice expended less energy during physically active (dark cycle) and less active (light cycle) phases of the day and also showed reduced energy output even when the genotypes exhibited similar levels of physical activity. Genotype differences were seen even while mice were fasting during the light cycle, a period with similarly low physical activity. Collectively, the findings extend our previous calorimetry studies in chow-fed mice by providing strong evidence for a reduction in basal metabolic rate. Accordingly, IL-18 deficiency led to a reduced MESOR of the circadian function for energy expenditure and an increased MESOR of the circadian function for respiratory exchange ratio, consistent with positive energy balance and decreased lipid substrate utilization, respectively. IFN-γ, which is induced by IL-18, pharmacologically increases resting metabolic rate in humans (de Metz et al., 1999) and mediates not only anorectic, but also hypermetabolic responses to inflammatory stimuli (Arsenijevic et al., 2000), making IFN-γ a potential mechanism by which IL-18 alters metabolism.

Additionally, several genotype differences in non-basal metabolic processes were newly revealed and merit mention. First, IL-18 KO mice failed to show the heightened level of energy expenditure when fasting from low-fat diet vs. high-fat diet that was seen in wildtype mice. This increase in wildtype mice was accompanied by a greater increase in motor activity as compared with IL-18 null mutants. Second, IL-18 KO mice only showed increased RERs when provided high-fat diet *ad libitum*, during which they overate relative to wildtype controls. No genotype differences in RER were seen when mice were studied during fasting or refeeding conditions, during which intake levels were equivalent across genotypes. Third, the amplitude of the circadian rhythm of energy expenditure was blunted in IL-18 KO mice, and the amplitude of this rhythm reflects non-maintenance metabolic processes (Berger and Phillips, 1988). An increased frequency or quantity of energy intake, as was observed here, has been associated with a blunted amplitude of energy expenditure rhythms (Rashotte et al., 1995). Thus, the present study revealed that IL-18 KO mice differ not only in basal metabolic rate, but also in phasic aspects of energy metabolism.

While peripheral sites of IL-18 action also are possible, (Conti et al., 1997; Penttila et al., 2003; Sugama et al., 2000; Wang et al., 2006; Zorrilla et al., 2007), several previous and current findings implicate central actions of IL-18. Previously we showed that central IL-18 administration suppressed low-fat chow intake, feed efficiency and weight regain in fasted mice with an anorectic potency (≤1 nmol; (Zorrilla et al., 2007)) on the same order as many anorexigenic molecules with central modes of action (Barrachina et al., 1997; Mounien et

al., 2009; Semjonous et al., 2009). IL-18 itself is constitutively expressed in third ventricle ependymal cells, which may function as bidirectional intermediaries between the CSF and periventricular energy balance-regulating hypothalamic neurocircuitry, as occurs for leptin (Amat et al., 1999; Cheunsuang et al., 2006). IL-18 modulates neuronal activity (Cumiskey et al., 2007; Curran and O'Connor, 2001; Kanno et al., 2004) and is also centrally produced in medial habenula, cortex, striatum, and glia (Alboni et al., 2010; Conti et al., 1999; Culhane et al., 1998; Sugama et al., 2002). Peripheral immune challenge elicits the hypothalamic synthesis of IL-18 and its receptor, suggesting that central IL-18 activation might contribute to anorectic and metabolic components of the "sickness syndrome." (Alboni et al., 2010; Alboni et al., 2011).

Also consistent with a central mode of action, IL-18 receptor complex subunits are constitutively expressed in mouse hypothalamus (Alboni et al., 2009; Alboni et al., 2010; Alboni et al., 2011; Wheeler et al., 2000), notably in the paraventricular nucleus and ventromedial hypothalamic nucleus. Both the IL-18Rα and Rβ subunits also are present in the hindbrain, including the nucleus tractus solitarius. The reviewed appetite-regulatory brain regions express not only the canonical IL-18Rs but also three additional isoforms predicted to be either decoy receptors or negative regulators of IL-18 action (Alboni et al., 2010; Alboni et al., 2011). Consistent with this distribution, here we found that central IL-18 administration also suppressed high-fat diet intake and did so via an IL-18R-dependent mechanism. Additional transgenic models, including conditional and region-specific IL-18Rs KO under development in the laboratory, will hopefully prove helpful in determining the exact sites and mechanisms of action.

Mice deficient in proinflammatory cytokines (or their receptors) other than IL-18 also display an obese phenotype, including those null for IL-1R1 (Garcia et al., 2006), IL-6 (Wallenius et al., 2002) and leptin (Zhang et al., 1994). With the exception of leptin, however, the mechanisms by which IL-6, IL-18 and IL-1β systems modulate energy homeostasis remain poorly understood from both physiological and molecular perspectives. The present manuscript demonstrates a key metabolic role for IL-18 that is still present during high-fat diet exposure. When considering the specificity vs. generality of IL-18 actions with respect to other proinflammatory cytokines, several differences and similarities bear discussion. First, through distinct receptors, both IL-18 and IL-1 activate MyD88-IRAK-NF-kB signaling, whereas IL-6 and leptin both activate JAKSTAT signaling, indicating the convergence of different cytokines to two pathways. Second, despite their apparent similarities, IL-1 and IL-18 are molecularly dissociable and present differences that may be exploited pharmacologically. From a translational perspective, two key differences are that unlike IL-1β, IL-18 does not potently produce fever (Gatti et al., 2002) or malaise (Zorrilla et al., 2007), raising the hypothesis that the IL-18/IL-18R system might be targeted to modulate energy homeostasis without inducing as many "sickness syndrome" side effects that limited targeting of the IL-1 system.

In humans, circulating IL-18 levels positively correlate with body mass index, adiposity, and metabolic syndrome disorders (Bruun et al., 2007; Esposito et al., 2002b; Hung et al., 2005; Leick et al., 2007; Sun et al., 2011). Such a finding is consistent with the increased adipocytokine release from adipose tissue in obesity (Fain, 2006). Accordingly, fat-resident

monocyte/macrophage lineage cells are major sources of circulating IL-18 (Fain, 2006), adipocytes from obese humans secrete 3-fold more IL-18 than those from lean donors (Skurk et al., 2005), and subcutaneous adipose tissue expression of IL-18 is elevated in obesity and metabolic syndrome disorders (Bruun et al., 2007; Leick et al., 2007; Membrez et al., 2009; Weiss et al., 2011). Consistent with a signal of metabolic state, circulating IL-18 levels are increased by hyperglycemia or a high-fat meal (Esposito et al., 2002a; Esposito et al., 2003), and intermittent glucose exposure increases the secretion of IL-18 by adipocytes (Sun et al., 2009). Conversely, weight loss and exercise decrease IL-18 levels (Bruun et al., 2007; Esposito et al., 2003; Leick et al., 2007; Madsen et al., 2009; Vilarrasa et al., 2007). Some adipocytokines whose levels increase with obesity (e.g, leptin) oppose positive energy balance in negative feedback fashion (Plata-Salaman, 2001; Wong and Pinkney, 2004). Perhaps reminiscent of obesity-related leptin resistance, leukocytes from patients with obesity or type 2 diabetes are resistant to IL-18 (Zilverschoon et al., 2008). Polymorphisms of the IL-18 gene or its receptor have been associated with obesity (Melen et al., 2010) and metabolic syndrome disorders (Evans et al., 2007; Koch et al., 2011; Presta et al., 2009) in humans. The present and previous (Netea et al., 2006; Zorrilla et al., 2007) loss-of-function and pharmacological results support the hypothesis that endogenous IL-18 suppresses appetite and promotes energy expenditure and lipid fuel substrate utilization and may do so not only during sickness, but also in healthy adults.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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Highlight

Results from interleukin-18 knockout mice suggest that it may curb appetite and promote lipid utilization in healthy adults consuming high-fat diets.

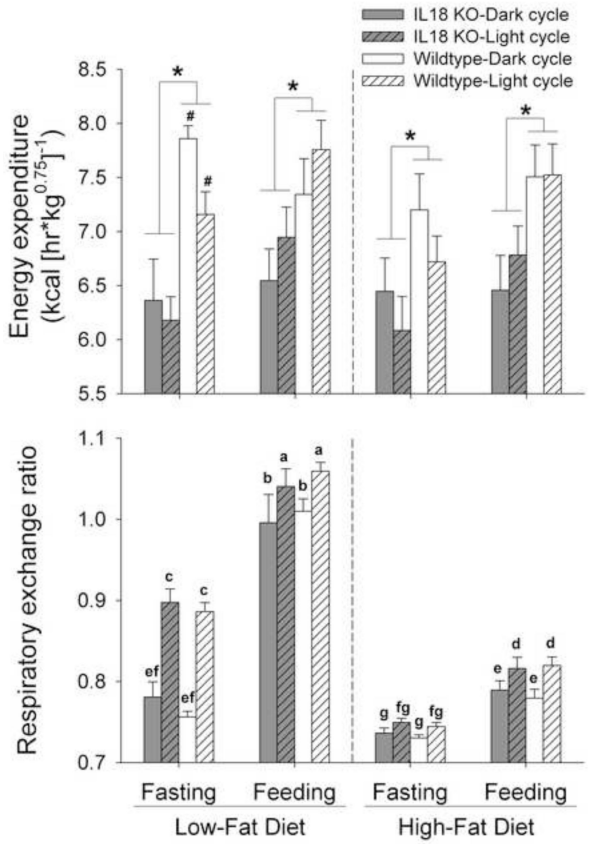

Figure 1.

Energy expenditure and respiratory exchange ratios in interleukin-18 knockout (IL-18 KO) and wildtype (WT) mice during brief fast-refeed conditions. Data express *M* (+SEM) weight-normalized energy expenditure (Panel *A*) and respiratory exchange ratios *(B)* of mature female mice measured across a 3-hr fasting period and a subsequent 3-hr refeeding period. In each subject, the fast-refeed protocol was performed during both the dark and light cycle and while receiving low-fat or high-fat diet. Panel *A* shows that IL-18 KO mice ($n = 7$) expended less energy than WT mice ($n = 6$) across all conditions, indicated by a main effect of Genotype; a Genotype × Diet × Fasted interaction reflected that the difference was most pronounced when mice were fasting from low-fat diet. Not denoted with symbols, main effects indicated that mice expended less energy when fasting (than feeding) and when maintained on high-fat (than low-fat) diet. Panel *B* shows that Genotype differences in respiratory exchange ratios were not evident in the context of large group differences that resulted from main and interaction effects of Fasting, Cycle, and Diet (see Results). *$p <$ 0.05, main effect of Genotype, #$p < 0.05$ vs. respective high-fat condition. In panel *B*, groups with different letter symbols differ from another, as determined via post hoc Fisher's protected least significant difference (LSD) tests.

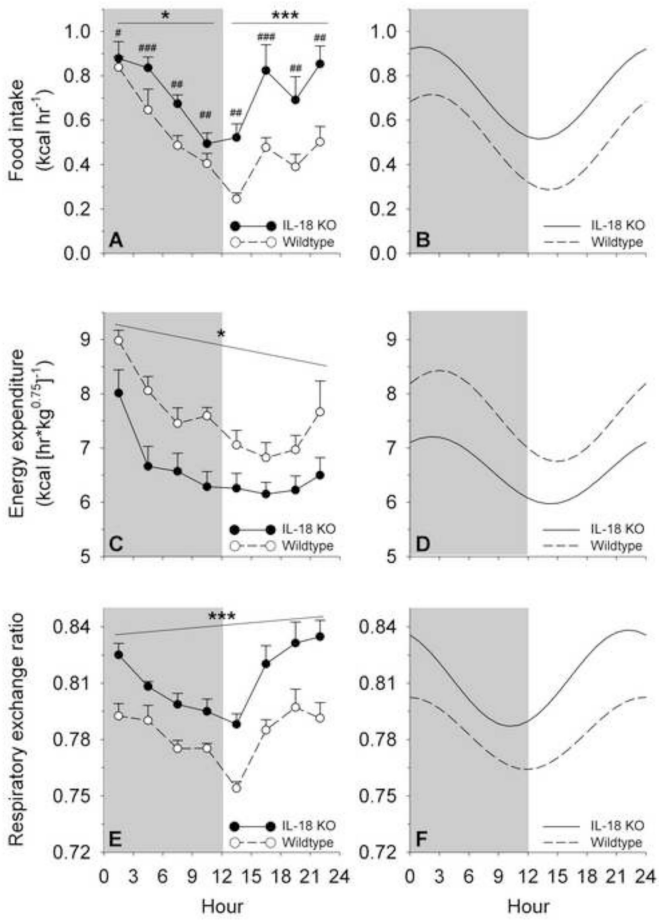

Figure 2.

Circadian time course of food intake, energy expenditure and respiratory exchange ratio in high-fat diet-fed interleukin-18 knockout (IL-18 KO) and wildtype (WT) mice. Data express *M* (+SEM) rates of food intake (Panel *A*), weight-normalized energy expenditure (*C*) and average respiratory exchange ratios *(E)* of mature female mice averaged across 3-hr bins during a daily observation period in indirect calorimetry chambers. Panels *B, D* and *F* show the respective cosinor functions, interpolated from the observed data; cosinor parameters are presented in Table 3. The grey shading indicates the dark cycle. Panels *A* and *B* illustrate that IL-18 KO mice ate significantly more high-fat diet than WT mice; this difference was even greater during the light cycle than dark cycle and was reflected in an increased circadian MESOR, peak and nadir. Genotype differences pooled across the cycle $*p = 0.06$, $***p < 0.0001$ (Genotype × Cycle interaction followed by Fisher's protected least significance difference tests); pooled across corresponding bins $^{\#}p < 0.05$, $^{\#\#}p < 0.005$, $^{\#\#\#}p < 0.001$ (Genotype × Bin interaction followed by Fisher's protected least significance difference tests). Panels *C* and *D* show that IL-18 KO mice expended significantly less energy than WT mice across the entire day and also had a blunted circadian rhythm; these differences were reflected in a reduced circadian amplitude, MESOR, peak and nadir. $*p < 0.05$, Genotype main effect. Panels *E* and *F* show that IL-18 KO mice had greater mean respiratory exchange ratios than WT mice across the entire light/dark cycle, differences that

were reflected in an increased circadian MESOR, peak and nadir. ***$p < 0.001$, Genotype main effect. Genotypes did not differ in the acrophase of rhythms.

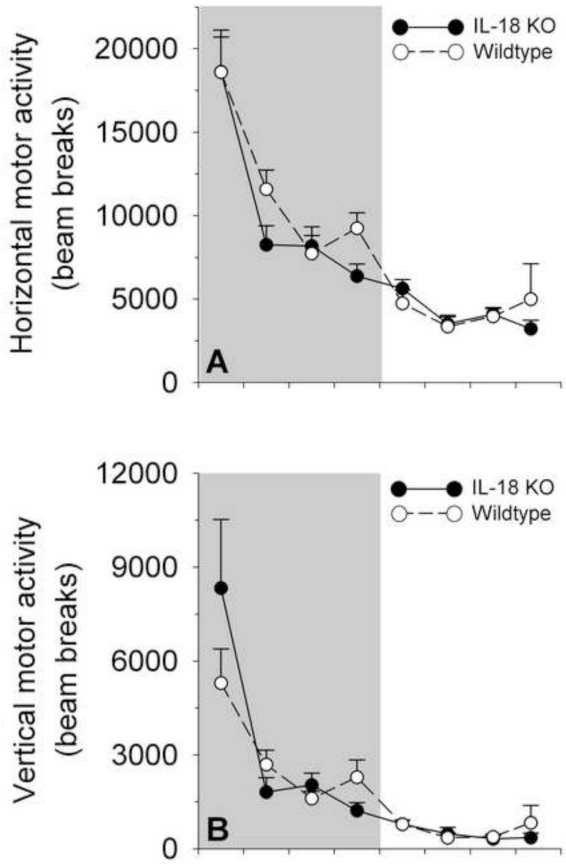

Figure 3.

Circadian time course of motor activity in high-fat diet-fed interleukin-18 knockout (IL-18 KO) and wildtype (WT) mice. Data express *M* (+SEM) photocell interruptions in the *x*-dimension (horizontal activity, Panel *A*) or *z*-dimension (vertical activity, Panel *B*) by mature female mice averaged across 3-hr bins during a daily observation period in indirect calorimetry chambers. The grey shading indicates the dark cycle. Panels *A* and *B* illustrate that no significant main or interaction effects that involved Genotype were observed on motor activity measures. As expected, irrespective of genotype, motor activity was greater during the dark (vs. light) cycle and decreased across the dark cycle, but not light cycle (see Results for statistics).

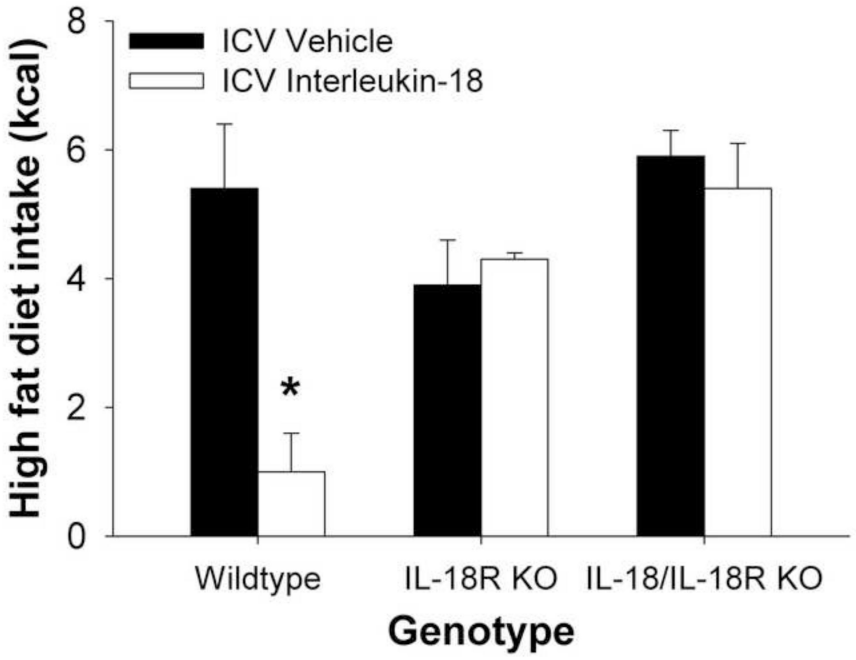

Figure 4.

Acute intracerebroventricular administration of interleukin-18 (2 nmol/2 μl) reduced 6-hr, nocturnal high-fat diet intake in 24-hr fasted wildtype mice, but not in transgenic knockout mice that lacked the interleukin-18 receptor alpha subunit (*Il18R*$^{-/--/-}$ or *Il18*$^{-/-}$/*Il18R*$^{-/-}$ mice), yielding a significant Genotype × Dose effect. Data express *M* (+SEM) high-fat diet energy intake. *$p < 0.05$, differs from respective vehicle-treated condition.

Table 1

Interleukin-18 genotype differences in body weight and weight change across the study period

Body weight measurement	IL-18 knockout (*n* = 7)	Wildtype control (*n* = 6)
Low-fat diet phase		
Onset (g)	30.6 ± 2.2 *	23.1 ± 1.1
Completion (g)	30.3 ± 2.2 **	21.4 ± 0.6
Net daily change (dg/day)	−0.6 ± 0.8 ##	−3.5 ± 1.2
High-fat diet phase		
Onset (g)	32.0 ± 2.0 **	23.1 ± 0.8
Completion (g)	34.1 ± 2.3 ***	22.7 ± 0.7
Net daily change (dg/day)	3.5 ± 0.7 ##, 1	−0.5 ± 0.8 1

Body weight progression of adult female *Il18*$^{-/-}$ and wildtype littermate mice across the 5-day low-fat diet phase and 6-day high-fat diet phase of the study. Data are expressed as M ± SEM. Superscripts denote significant differences from wildtype controls

* $p < 0.05$,

** $p < 0.005$,

*** p = 0.001 (Student's *t*-test),

$p < 0.01$ (main effect of Genotype) and from respective low-fat diet measure

1 $p < 0.001$ (main effect of Diet).

Table 2

Motor activity of interleukin-18 knockout (IL-18 KO) and wildtype mice during the fast-refeed protocol

	Horizontal Activity				Vertical Activity			
	Fasting		Feeding		Fasting		Feeding	
	Low-Fat	High-Fat	Low-Fat	High-Fat	Low-Fat	High-Fat	Low-Fat	High-Fat
Dark cycle								
IL-18 KO	25.3 ± 2.9	20.0 ± 2.5	17.5 ± 2.5	18.1 ± 1.9	5.2 ± 1.3	4.5 ± 1.7	3.3 ± 1.0	4.0 ± 1.5
Wildtype	31.8 ± 5.8***	19.4 ± 2.7	13.2 ± 2.3	23.9 ± 2.2##	6.5 ± 1.5**	3.6 ± 0.7	2.0 ± 0.6	4.5 ± 0.5#
Light cycle								
IL-18 KO	6.0 ± 1.2	9.7 ± 0.9	6.0 ± 0.9	15.3 ± 1.7	1.4 ± 0.3	1.4 ± 0.4	1.6 ± 0.3	1.8 ± 0.4
Wildtype	6.1 ± 1.7***	10.6 ± 1.2	6.0 ± 1.0	16.7 ± 2.4##	2.0 ± 0.7**	0.9 ± 0.2	2.2 ± 0.6	2.9 ± 0.8

Data express *M* (±SEM) photocell interruptions (x100) in the *x*-dimension (horizontal activity) or *z*-dimension (vertical activity) in mature female IL-18 knockout (KO) mice ($n = 7$) and their wildtype controls ($n = 6$) during a 3-hr fasting period and a subsequent 3-hr refeeding period. In each subject, the fast-refeed protocol was performed during both the dark and light cycle and while receiving low-fat or high-fat diet. Potential genotype effects on motor activity could not account for differences in energy expenditure, because mice expended less energy even during periods when IL-18 KO mice moved as much as or more than WT mice. The only significant effect involving Genotype was a Genotype × Diet × Fasting interaction. Post hoc deconvolution of this interaction, shown as symbols in the Table, indicated that wildtype mice moved significantly less during the feeding vs. fasting period in low-fat diet testing, but more in the feeding vs. fasting period during high-fat diet testing, and the corresponding differences were smaller or absent in IL-18 KO mice. Irrespective of genotype, mice moved significantly more during the dark cycle (than light cycle), especially during the fasting (vs. feeding) phase of testing (see Results for statistics). Symbols represent

** $p < 0.01$,

*** $p < 0.001$ greater than wildtype mice fasting from high-fat diet or feeding on low-fat diet;

# $p < 0.05$,

$p < 0.005$ greater than wildtype mice feeding on low-fat diet or fasting from high-fat diet.

Table 3

Interleukin-18 (IL-18) knockout and high-fat diet intake, metabolism and motor activity

Measure	IL-18 knockout (*n* = 7)	Wildtype control (*n* = 6)
High-fat diet intake, kcal	16.5 ± 1.0 **	11.5 ± 0.6
Cosinor parameters		
MESOR, kcal hr^{-1}	0.72 ± 0.04 **	0.50 ± 0.03
Amplitude, kcal hr^{-1}	0.21 ± 0.01	0.21 ± 0.02
Peak, kcal hr^{-1}	0.93 ± 0.05 **	0.72 ± 0.03
Nadir, kcal hr^{-1}	0.51 ± 0.05 **	0.29 ± 0.03
Acrophase, hr	1.2 ± 1.2	2.1 ± 0.5
Goodness-of-fit, *r*	0.70 ± 0.07	0.76 ± 0.03
Total energy expenditure, $kcal/kg^{0.75}$	151.5 ± 6.9 *	174.1 ± 5.7
Cosinor parameters		
MESOR, kcal $(hr^{*}kg^{0.75})^{-1}$	6.59 ± 0.30 *	7.59 ± 0.26
Amplitude, kcal $(hr^{*}kg^{0.75})^{-1}$	0.62 ± 0.08 *	0.84 ± 0.05
Peak, kcal $(hr^{*}kg^{0.75})^{-1}$	7.22 ± 0.38 *	8.42 ± 0.28
Nadir, kcal $(hr^{*}kg^{0.75})^{-1}$	5.97 ± 0.23 *	6.75 ± 0.25
Acrophase, hr	2.3 ± 0.5	3.0 ± 0.5
Goodness-of-fit, *r*	0.72 ± 0.04	0.78 ± 0.02
Respiratory exchange ratio	0.812 ± 0.005 ***	0.783 ± 0.003
Cosinor parameters		
MESOR	0.813 ± 0.005 ***	0.783 ± 0.004
0.783 ± 0.004	0.026 ± 0.003	0.019 ± 0.002
Peak	0.838 ± 0.006 ***	0.803 ± 0.006
Nadir	0.787 ± 0.006 ***	0.764 ± 0.002
Acrophase, hr	−1.8 ± 0.8	−0.1 ± 0.6
Goodness-of-fit, *r*	0.86 ± 0.04 *	0.73 ± 0.03
Horizontal motor activity, beam breaks	57,879 ± 5189	64,189 ± 4879
Vertical motor activity, beam breaks	15,337 ± 3340	14,215 ± 1566

Daily and cosinor parameters of food intake, energy expenditure, respiratory exchange ratio and motor activity of female $Il18^{-/-}$ mice and wildtype littermate tested across 23 hr in a CLAMS indirect calorimetry system. Please see Methods for definition of cosinor parameters. Data express *M* ± SEM. Symbols denote differences from wildtype controls

* $p < 0.05$,

** $p < 0.005$,

*** $p < 0.0005$ (Student's *t*-test).