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Obesity

Difference in Housing Temperature-Induced Energy Expenditure Elicits Sex-Specific Diet-Induced Metabolic Adaptations in Mice

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Objective: The aim of this study was to test whether increased energy expenditure (EE), independent of physical activity, reduces acute diet-induced weight gain through tighter coupling of energy intake to energy demand and enhanced metabolic adaptations.

Methods: Indirect calorimetry and quantitative magnetic resonance imaging were used to assess energy metabolism and body composition during 7-day high-fat/high-sucrose (HFHS) feeding in male and female mice housed at divergent temperatures (20°C vs. 30°C).

Results: As previously observed, 30°C housing resulted in lower total EE and energy intake compared with 20°C mice regardless of sex. Interestingly, housing temperature did not impact HFHS-induced weight gain in females, whereas 30°C male mice gained more weight than 20°C males. Energy intake coupling to EE during HFHS feeding was greater in 20°C versus 30°C housing, with females greater at both temperatures. Fat mass gain was greater in 30°C mice compared with 20°C mice, whereas females gained less fat mass than males. Strikingly, female 20°C mice gained considerably more fat-free mass than 30°C mice. Reduced fat mass gain was associated with greater metabolic flexibility to HFHS, whereas fat-free mass gain was associated with diet-induced adaptive thermogenesis.

Conclusions: These data reveal that EE and sex interact to impact energy homeostasis and metabolic adaptation to acute HFHS feeding, altering weight gain and body composition change.

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Study Importance

What is already known?

- Increased energy expenditure (EE) associated with physical activity hypothetically prevents or reduces short-term diet-induced weight gain by increasing coupling of energy intake to expenditure.

What does this study add?

- Our results support the energy flux hypothesis, in which increased EE results in greater energy flux, which improves energy balance via enhanced coupling of energy intake to energy demand.
- Increased EE in female mice results in predominantly fat-free mass gain during short-term high-fat/high-sucrose (HFHS) feeding.
- Increased EE is associated with greater metabolic flexibility to HFHS- and diet-induced nonshivering thermogenesis, particularly in female mice.

How might these results change the direction of research or the focus of clinical practice?

- This study highlights the importance of EE levels in the prevention of weight gain and adiposity.
- We present the first evidence that EE level may play a role in the composition of weight gained by females during acute HFHS feeding.

Introduction

Current recommendations to prevent weight gain and treat obesity include increasing physical activity or daily exercise with a goal of increasing total energy expenditure (EE) and improving energy balance (1,2). Improved energy balance at higher physical activity levels is proposed to be achieved through greater pairing of energy intake to energy demand (3,4). Hypothetically, coupling of energy intake to EE improves as energy flux, the sum of EE and energy intake, increases with increasing physical activity (4,5). This was first proposed by Jean Mayer following his studies in humans and rodents showing that food intake is more closely matched to energy demand at higher physical activity levels and assumedly greater

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EE and energy flux (6,7). However, investigating energy flux and pairing of energy intake to energy demand in human participants is complicated by the large interindividual variability of total and physical activity EE (8,9). Also, increased physical activity produces multiple systemic and tissue-specific adaptations independent of increases in EE (10,11), further complicated by sex-specific differences in physical activity level and physiological adaptation (12,13). As such, new approaches are necessary to more specifically assess the independent impact of changes in EE on energy balance and protection from weight gain.

The assessment of EE in mice using indirect calorimetry is a common experimental tool. However, indirect calorimetry experiments are impacted by several factors (14), with ambient housing temperature perhaps the most debated (15-18). This controversy centers around the well-described linear increases in cold-induced nonshivering thermogenesis as ambient temperature drops below the thermoneutral zone (19,20). This increased thermogenesis to defend body temperature is accompanied by concomitant increases in total EE and energy intake (19,21) and, as such, energy flux. Although the debate regarding rodent housing temperature is critical to the appropriate design and execution of future mouse studies, the impact of ambient temperature on EE and energy flux can be used to investigate the role of these outcomes in the adaptation to metabolic challenges.

In this study, we tested whether differences in EE, independent of physical activity level, impact energy homeostasis and metabolic adaptation of males and females during a short-term dietary challenge. We used quantitative magnetic resonance imaging (qMRI) and indirect calorimetry to assess body composition and energy metabolism outcomes in male and female mice during 1 week of high-fat/high-sucrose (HFHS) feeding while housed at either 20°C or 30°C. We observed that the composition of HFHS-induced weight gain (fat vs. fat-free mass) was different between male and female mice with different EE. Additionally, in support of the energy flux hypothesis, we observed that higher baseline EE during 20°C housing resulted in greater energy flux, coupling of energy intake to EE, and less positive energy balance. Furthermore, increased baseline EE produced greater adaptation of fat use and diet-induced nonshivering thermogenesis during short-term HFHS feeding, particularly in females.

Methods

Animals

Male and female C57Bl/6J (000664; Jackson Laboratories, Bar Harbor, Maine) mice (6 weeks old) were individually housed (with huts and cotton nestlet) at either 20°C or 30°C on a reverse light cycle (light 10 PM to 10 AM) with ad libitum access to a low-fat control diet (LFD, D12110704 [10% kilocalories from fat, 3.5% kilocalories from sucrose, 3.85 kcal/g], Research Diets, Inc., New Brunswick, New Jersey) for 3 weeks. Based on previous publications (19,21), the difference in ambient housing temperature has been proposed to produce an ~70% to 80% difference in total EE. At 9 weeks of age, animal and food weight was monitored prior to and following the 7 days of both LFD and the subsequent HFHS diet (D12451; 45% kilocalories from fat, 17% kilocalories from sucrose, 4.73 kcal/g) at the assigned ambient temperature. The animal protocols were approved by the Institutional Animal Care and Use Committee at the University of Kansas Medical Center and the Subcommittee for Animal Safety at the Kansas City Veterans' Hospital.

Body composition analysis

Body composition was measured by qMRI using the EchoMRI-1100 (EchoMRI, Houston, Texas). Fat-free mass was calculated as the difference between body weight and fat mass. Though not reported, lean mass was determined during the body composition analysis and it showed the same patterns as calculated fat-free mass. Body composition was determined prior to, and after, the 7-day HFHS feeding.

Indirect calorimetry and energy metabolism

Starting at 9 weeks of age ($n = 12$), energy metabolism was assessed at 20°C or 30°C ambient temperature for 7 days on LFD followed by 7 days of HFHS feeding by measuring VO_2 and VCO_2 in a Promethion continuous metabolic monitoring system (Sable Systems International, Las Vegas, Nevada). Animals were acclimated to the indirect calorimetry cages for 5 days prior to initiation of data collection. Rate of EE was calculated with a modified Weir equation (EE [kilocalories per hour] = $60 \times [0.003941 \times VO_2 + 0.001106 \times VCO_2]$) and respiratory quotient as VCO_2/VO_2. Total EE was calculated as the daily average rate of EE for each day times 24 and summed across the 7 days of each diet. Resting EE, which was determined from the average rate of EE during the 30-minute period with the lowest daily EE as kilocalories per hour, was extrapolated to 24 hours for each day and summed as with total EE. Nonresting EE was calculated as the difference between total EE and resting EE. Metabolic flexibility to HFHS was assessed as the diet-induced change in respiratory quotient as the difference in the 7-day HFHS feeding data minus the 7-day LFD data. Diet-induced adaptive nonshivering thermogenesis was calculated as the difference in resting EE on HFHS feeding and LFD feeding. Energy intake was assessed as the total food intake for each feeding period times the energy density of each diet. Energy balance was determined as the difference between the total energy intake and total EE. Energy flux represents the sum of total EE and energy intake (22). To assess the coupling of energy intake to energy demand, we calculated the percent energy coupled as follows: 1 – (energy balance/energy flux). From this calculation, the higher the percentage the greater the coupling of energy intake to EE. Food intake, energy intake, and energy balance data during HFHS feeding from two 20°C female mice were not included in data analysis because of excessive food spillage. Thermic effect of food was determined from the consensus thermic effect of food for fat (2.5%), carbohydrate (7.5%), and protein (25%), and the manufacturer provided diet information for each diet (19). As such, the thermic effect of food for HFHS feeding (D12451, Research Diets, 4.73 kcal/g, 45% kilocalories from fat, 35% kilocalories from carbohydrate, 20% kilocalories from protein) is 8.75% or 0.4139 kcal/g. This method of determining the thermic effect of food reduces the potential influence of neurobehavioral adaptations of the fed/fasted transition impacting changes in EE through calculation of thermic effect of food across the entire 7-day HFHS feeding. Activity EE was calculated as the difference between nonresting EE and the thermic effect of food. All_Meters is an assessment of cage activity, including gross and fine movements, and it is determined using the summed distances calculated from the Pythagorean theorem based on the XY second-by-second coordinates. Cost of movement was calculated as activity EE divided by total meters traveled over the 7 days of HFHS feeding. All data from one 20°C female mouse were excluded after discovering malocclusion at necropsy.

Statistical analysis

Data are presented as scatterplots with means and SE. The two-SD test was used to test for outliers in each group. SPSS Statistics version

25 (IBM Corp., Armonk, New York) was used for all statistical analysis. Two-way ANOVA analysis was performed to assess interaction and main effects of sex and temperature on outcome variables. When significant interactions or main effects were observed, post hoc analysis was performed using least significant difference to test for any specific pairwise differences. Main effects are discussed only when all pairwise treatment comparisons within that parameter were significant. Additionally, two-way ANCOVA with fat and fat-free mass as covariates was performed for total EE and energy intake to statistically adjust for body composition differences between sexes. Adjusted marginal means and partial eta-squared values as approximations of effect size were calculated. Statistical significance was set at $P < 0.05$.

Results

Housing temperature of 20°C versus 30°C produces divergent energy metabolism

In this investigation, we used the well-described capacity of different ambient housing temperatures to produce differences in mouse EE (19,20). As expected from previous publications (19-21), in using indirect calorimetry we observed male and female 30°C mice to have lower baseline total EE (~40%), energy intake (~35%), and resting EE (~55%) compared with 20°C on LFD (Table 1). Nonresting EE was not observed to be different between temperatures, whereas females were lower at both temperatures. Average daily respiratory quotient was not different across all conditions (Table 1), suggesting similar macronutrient utilization. Importantly, although female mice showed greater cage activity (All_Meters) at both temperatures (Table 1), no effect of housing temperature within sex was observed. Prior to HFHS feeding, no differences in body weight or body composition were observed between mice housed at 20°C versus 30°C, whereas females weighed less than males at both temperatures.

Increased EE produces unique short-term HFHS-induced weight gain phenotype in female mice

Interestingly, baseline EE did not impact weight gain in female mice, whereas HFHS-fed male mice housed at 30°C gained more weight compared with 20°C males and 30°C females (Figure 1A). Next, we used qMRI to assess whether baseline differences in EE influenced HFHS-induced changes in body composition. Values for fat and fat-free mass at the initiation and end of HFHS feeding are presented in Table 1. The lower EE associated with 30°C housing resulted in greater increases in fat mass during HFHS feeding compared with 20°C, whereas females gained less fat mass compared with males at either temperature (Figure 1B). Strikingly, fat-free mass increased ~2.6-fold more in 20°C female mice on HFHS feeding compared with 20°C male or 30°C females (Figure 1C). To further highlight the interaction of sex and temperature in short-term weight gain, Figure 1D displays the combined fat and fat-free mass components of the 1-week change in body weight. This representation highlights the increased proportion of HFHS-induced weight gain composed of fat-free mass in 20°C female mice (~80%) compared with all other groups (~30%). Together, these data demonstrate an interaction of sex and temperature to impact

TABLE 1 Low-fat diet energy metabolism and high-fat/high-sucrose diet anthropometrics

	20°C		30°C	
	Male	Female	Male	Female
Baseline energy metabolism (1-week low-fat diet)				
Total EE (kcal)	81.77 ± 1.34	74.51 ± 2.20††	48.79 ± 0.97*	44.80 ± 0.78*
Energy intake (kcal)	92.21 ± 2.91	78.77 ± 3.15††	58.33 ± 1.22*	52.98 ± 1.41*
Resting EE (kcal)	56.13 ± 1.77	53.33 ± 1.69	25.58 ± 0.59*	23.80 ± 0.51*
Nonresting EE (kcal)	24.24 ± 0.83	21.53 ± 0.72†	22.92 ± 0.48	21.00 ± 0.55†
Respiratory quotient	0.860 ± 0.010	0.850 ± 0.008	0.861 ± 0.007	0.841 ± 0.008
All_Meters (m)	1,839.8 ± 117.2	2,149.4 ± 185.5†	2,007.3 ± 97.8	2,308.6 ± 108.9†
Body weight (1-week HFHS)				
Day 0	24.21 ± 0.34	18.67 ± 0.31†	25.26 ± 0.35	19.46 ± 0.24†
Day 7	26.91 ± 0.52	21.13 ± 0.48†	28.98 ± 0.51**	21.76 ± 0.40†
Body composition (1-week HFHS)				
Fat mass (g)				
Day 0	1.74 ± 0.17	1.53 ± 0.08	2.47 ± 0.14*	1.84 ± 0.09†
Day 7	3.37 ± 0.32	2.13 ± 0.18†	5.18 ± 0.30*	3.28 ± 0.28*,†
Fat-free mass (g)				
Day 0	22.72 ± 0.43	17.21 ± 0.33†	22.79 ± 0.26	17.63 ± 0.24†
Day 7	23.54 ± 0.34	19.01 ± 0.38†	23.80 ± 0.27	18.48 ± 0.20†

*$P < 0.05$, main effect of 20°C vs. 30°C.
** $P < 0.05$, 20°C vs. 30°C within sex.
†$P < 0.05$, main effect of male vs. female.
††$P < 0.05$, male vs. female within temperature.
All values expressed as means ± SEM ($n = 11$-16).
EE, energy expenditure; HFHS, high fat/high sucrose.

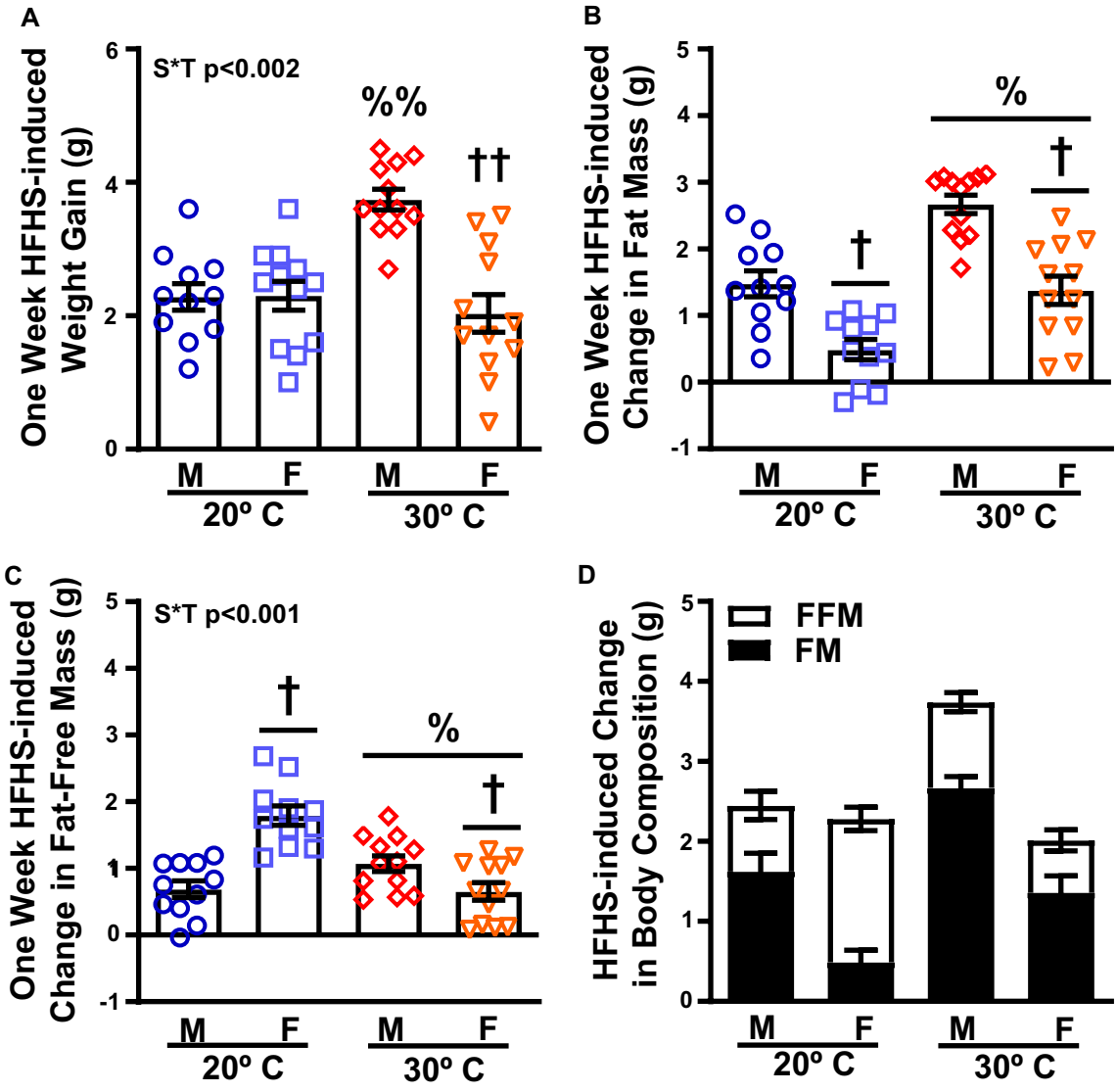

Figure 1 Higher energy metabolism during 20°C housing changes composition of weight gained in female mice during 1 week of high-fat/high-sucrose (HFHS) feeding. Body weight and body composition analysis using quantitative MRI was performed before and after 7 days of HFHS feeding. (**A**) One-week change in body weight ($n = 10$-16). Differences in the initial and final values during 1 week of HFHS feeding are displayed as (**B**) change in fat mass (FM) and (**C**) fat-free mass (FFM). (**D**) One-week change in body weight presented as change in FM and FFM. Values are means ± SEM. $n = 10$-16. $^{\%}P < 0.05$, main effect of 20°C vs. 30°C; $^{\%\%}P < 0.05$, 20ºC vs. 30ºC within sex; $^{\dagger}P < 0.05$, main effect of sex.

diet-induced weight gain and changes in body composition, particularly in females.

Short-term HFHS-induced weight gain is not associated with energy balance or energy intake coupling in female mice

From the indirect calorimetry data, we assessed whether the observed sex-specific HFHS-induced weight gain phenotypes were associated with energy balance and coupling of energy intake to EE. Interestingly, energy balance during short-term HFHS feeding did not mirror the observed weight gain but it was associated with change in fat mass (Figure 2A). Furthermore, energy balance during HFHS feeding was more positive in 30°C male and female mice, whereas female mice had less positive energy balance compared with males regardless of temperature. The primary components of energy balance, total EE and energy intake, are presented in Figure 2B-2C, respectively. As with the LFD baseline, total EE was lower during HFHS feeding in 30°C mice compared with 20°C for both sexes, and 30°C females were lower than males. Notably, differences in total EE because of temperature were not associated with any differences in activity level or activity EE during HFHS feeding (Supporting Information Figure S1). Energy intake was lower in 30°C mice regardless of sex, and females at both temperatures had lower intake during 1 week of HFHS feeding.

Increased EE has been proposed as a mechanism to increase energy flux, the sum of total EE and energy intake, which improves energy balance through greater pairing of energy intake to EE (3-5). Energy

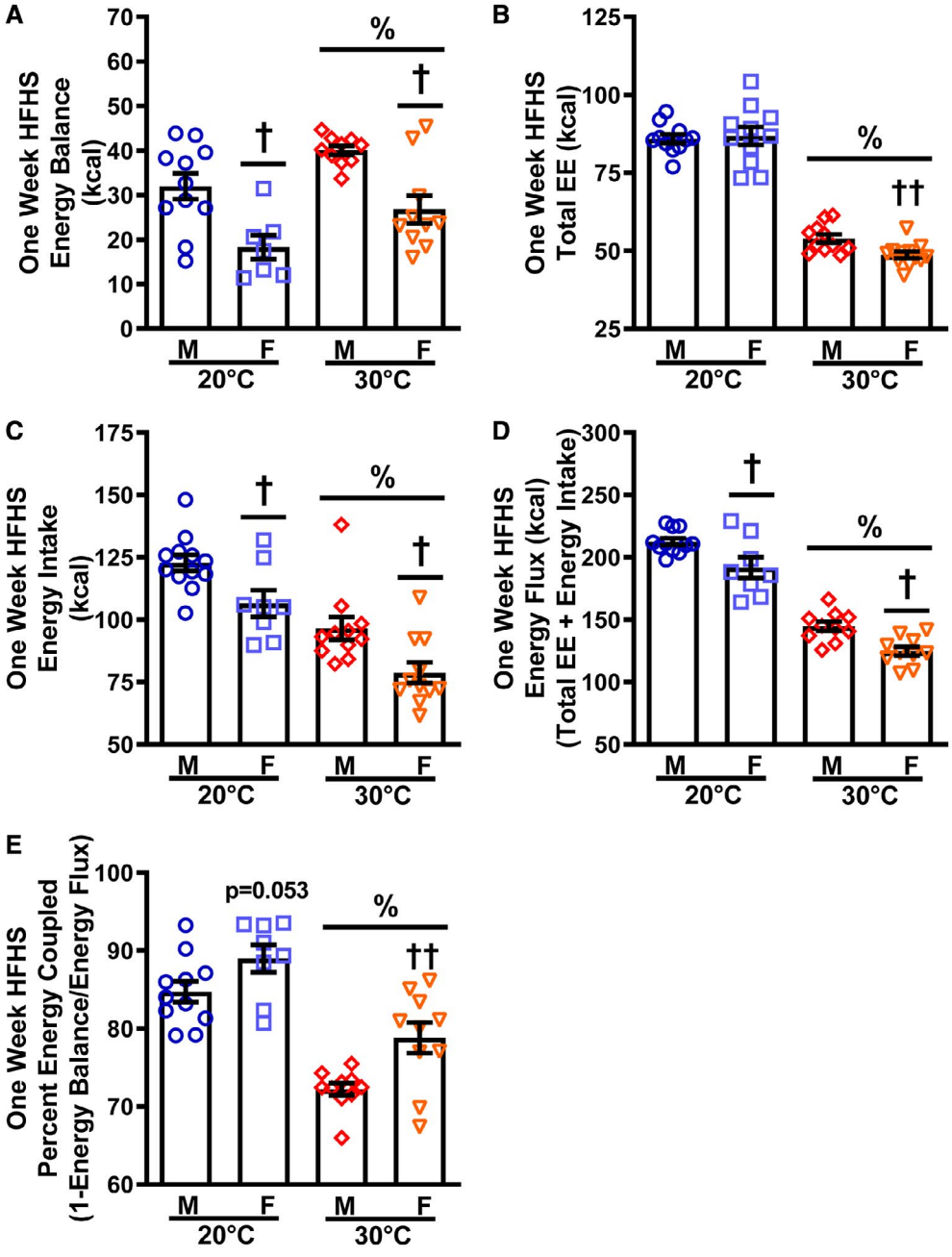

Figure 2 Female mice have reduced energy balance and improved coupling of energy intake to energy expenditure (EE) regardless of housing temperature. Indirect calorimetry was used to assess energy metabolism in male and female C57Bl/6J mice during 7 days of high-fat/high-sucrose (HFHS) feeding ($n = 8$-12). (**A**) Energy balance was calculated as the difference in energy intake and total EE for each mouse during each diet exposure. (**B**) Total EE was determined as the sum of the 24-hour EE during 7 days of HFHS feeding. (**C**) Energy intake during each dietary exposure was determined as the sum of food intake (grams) times the energy density (kilocalories per gram) for each diet. (**D**) Energy flux was calculated as the sum of 1-week total EE and energy intake. (**E**) Percentage of energy coupled represents coupling of energy intake to EE and was calculated as 1 minus energy balance divided by energy flux. Values are means ± SEM. $^{\%}P < 0.05$, main effect of 20°C vs. 30°C; $^{\dagger}P < 0.05$, main effect of sex; $^{\dagger\dagger}P < 0.05$, male vs. female within temperature.

flux was lower in HFHS-fed 30°C mice compared with 20°C for both sexes (Figure 2D). Largely because of the observed lower energy intake in female mice, energy flux was lower at both temperatures in females compared with males. In Figure 2E, the efficiency of coupling energy intake to EE was calculated as 1 minus the energy balance divided by energy flux (percent energy coupled). Male and female mice housed at 30°C had reduced energy coupling during 1 week of HFHS feeding compared with 20°C. Furthermore, although 20°C female mice tended

to have greater energy coupling compared with males, only the 30°C females were observed to have significantly greater energy coupling compared with 30°C males. Together, these data support the hypothesis that increased EE can improve energy balance through increased energy flux and coupling of energy intake to EE. Furthermore, these data highlight that energy balance is a powerful predictor of acute diet-induced fat mass gain in both sexes but that it does not predict weight gain in female mice.

Female mice have greater total EE following ANCOVA

The observed sex-specific differences in HFHS-induced weight gain and changes in body composition suggest that the differences in body size may impact the energy metabolism outcomes. Covariate analysis was performed to assess the effect of differences in the components of body weight (fat and fat-free mass) on the interpretation of total EE and energy intake. Adjusted estimated marginal means and estimate of covariate effect size, partial eta-squared values, are shown in Table 2. Following ANCOVA adjustment for differences in fat and fat-free mass, females had higher total EE at both temperatures compared with males, whereas total EE in 20°C mice remained higher than 30°C as observed in absolute total EE data (Figure 2B). Importantly, fat mass was not a significant covariate of total EE, even though fat-free mass was significant, with a moderate effect size (partial eta-squared, 0.38) on total EE. During ANCOVA of energy intake, neither fat or fat-free mass was found to be a significant covariate; therefore, no data are presented. These findings suggest that the smaller female mice have greater inherent EE per unit of fat-free mass, which is associated with reduced energy balance and improved energy coupling.

Reduced EE of 30°C housing is associated with impaired metabolic flexibility

Human patients with obesity do not increase fat use to the same extent as lean patients when following a high-fat diet (23), and this lack of metabolic flexibility to acute high-fat diet is predictive of future weight gain (24). To assess whether differences in baseline EE impacted substrate use and the capacity to adapt substrate use during short-term HFHS feeding, we determined daily respiratory quotient. As described earlier, respiratory quotient was not different on LFD (Table 1). During HFHS feeding, daily average respiratory quotient was higher in 30°C males compared with both 20°C males and 30°C females (Figure 3A), suggesting lower fat use. No difference was observed between females. We calculated metabolic flexibility, the capacity to adapt substrate use based on changes in substrate availability, as the difference in average daily respiratory quotient during HFHS feeding and LFD feeding (Figure 3B) (25,26). Mice kept in 30°C housing were less metabolically flexible during short-term HFHS feeding as revealed by the smaller HFHS diet–induced reduction in daily average respiratory quotient compared with 20°C mice. However, 30°C female mice had greater HFHS diet–induced changes in respiratory quotient compared with males. Figure 3C shows the average change in daily respiratory quotient during the transition from the LFD to HFHS feeding. The figure highlights the rapid respiratory quotient decrease in all groups and the slower more transient response of the 30°C mice. These data show greater baseline EE results in increased metabolic flexibility during short-term HFHS feeding, which is associated with improved energy balance, coupling of energy intake to EE, and reduced fat mass gain.

Sex and baseline EE interact in the nonshivering thermogenic response to short-term HFHS feeding

Diet-induced nonshivering thermogenesis is the centrally mediated adaptive capacity to increase EE in response to increases in energy intake and it is proposed as a compensatory mechanism for limiting increased energy balance during transitions to energy-dense diets (27,28). Diet-induced nonshivering thermogenesis is calculated as the difference in resting EE during HFHS feeding and LFD feeding (diet-induced resting EE). Interestingly, the lower baseline EE of 30°C mice was associated with lower diet-induced nonshivering thermogenesis in both male and female mice compared with 20°C mice Figure 4A). Additionally, 20°C females had greater diet-induced nonshivering thermogenesis compared with males. Figure 4B depicts the daily increase in resting EE because of HFHS feeding and demonstrates the rapid and sustained responses observed across the 7-day intervention. In Figure 4C, the diet-induced nonshivering thermogenesis is presented as the percentage of total EE to highlight the relative contribution of this adaptive response on overall EE during

TABLE 2 High-fat/high-sucrose energy expenditure component analysis and ANCOVA

	20°C		30°C	
	Male	Female	Male	Female
1-week HFHS energy expenditure component analysis				
Resting EE (kcal)	67.41 ± 1.71	66.44 ± 2.08	31.69 ± 0.83*	27.97 ± 0.86*
Nonresting EE (kcal)	19.49 ± 0.72	18.69 ± 0.67	22.00 ± 0.67*	20.74 ± 0.50*
Weight-adjusted energy expenditure				
Total EE (kcal/d, covariate(s) fat and fat-free mass)	76.66 ± 2.12	93.83 ± 2.21†	45.21 ± 2.54*	58.86 ± 2.45*,†
Covariate effect size	Covariate(s)	P value	Partial Eta^2	
	Fat-free mass	$P < 0.001$	0.388	
	Fat mass	$P = 0.261$	0.032	

All resting and nonresting EE values expressed as means ± SEM (n = 10-12). All ANCOVA values expressed as estimated marginal means ± SEM (n = 10-12). Estimated effect size of covariates for ANCOVA analysis presented as partial eta-squared.
*$P < 0.05$, main effect of 20°C vs. 30°C.
†$P < 0.05$, main effect of male vs. female.
EE, energy expenditure; HFHS, high fat/high sucrose.

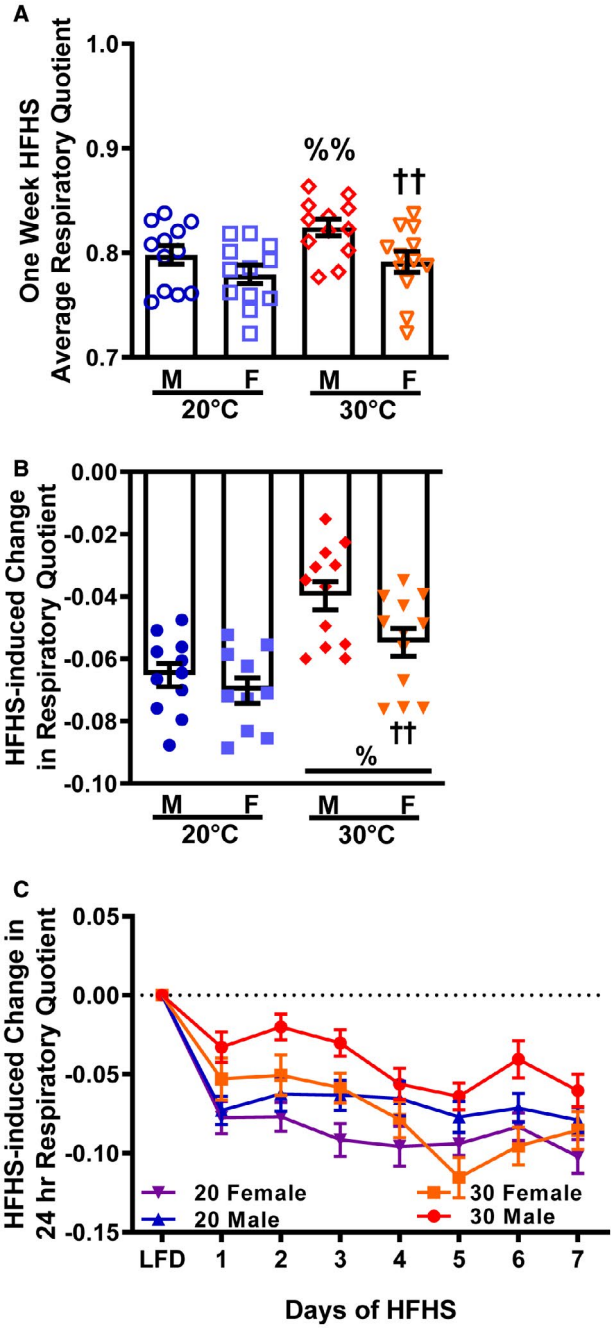

Figure 3 Decreased energy metabolism in mice housed at 30°C produces reduced metabolic flexibility to short-term high-fat/high-sucrose (HFHS) diets. (**A**) Indirect calorimetry was used to determine average daily respiratory quotient (RQ). (**B**) Metabolic flexibility to HFHS feeding was assessed as the difference in average daily RQ during HFHS feeding and average daily RQ during low-fat control diet (LFD) feeding. (**C**) RQ change from LFD RQ during each day of the HFHS exposure. Values are means ± SEM. $n = 10$-16. $^{\%}P < 0.05$, main effect of 20°C vs. 30°C; $^{\%\%}P < 0.05$, 20°C vs. 30°C within sex; $^{\dagger\dagger}P < 0.05$, male vs. female within temperature by diet group.

HFHS feeding. Although no difference was observed between male mice, female mice showed a divergent response, with 30°C mice being higher and 20°C being lower than males. Importantly, these nonshivering thermogenesis findings were not associated with the thermic effect of food (Supporting Information Figure S1), which necessarily closely matched food intake (Supporting Information Figure S2) based on the calculation method. Diet-induced changes in resting EE could result in alterations in the other major component of total EE, nonresting EE. Diet-induced changes in nonresting EE were lower in 20°C mice compared with 30°C mice, with males having the greatest decrease. Interestingly, very little change in nonresting EE was observed in 30°C mice (Figure 4D). These data demonstrate that differences in baseline EE produce divergent adaptive thermogenic responses to HFHS feeding, particularly in females, which are associated with the observed HFHS-induced gains in fat-free mass.

Discussion

Optimal maintenance of energy balance and body weight is putatively achieved at higher levels of EE via greater energy flux and improved coupling of energy intake to energy demand (3-5). However, the direct impact of EE on energy balance and weight gain is complicated by potentially confounding factors produced by the common experimental tools (e.g., physical activity, chemical uncouplers). Also, there have been limited studies examining the interaction of EE and metabolic adaptations on weight gain regulation between sexes. Herein, we used differing ambient housing temperatures (20°C vs. 30°C) to modulate EE in male and female mice as a novel experimental tool to assess the independent role of EE on diet-induced weight gain and adaptation of energy metabolism.

The early work of Mayer et al. (6,7) and Edholm et al. (29,30) independently demonstrated in rodents and humans that energy intake is highly regulated at higher levels of EE and measured as oxygen consumption or increased physical activity. This work has been extended to describe two zones. The regulated zone occurs when energy intake and greater EE are highly coupled, and the unregulated zone occurs at lower EE in which the relationship between EE and energy intake becomes uncoupled (unregulated zone) (31). Recently, increased coupling of energy intake to EE and associated improved energy balance were observed during stepwise increases in physical activity and EE (32). Furthermore, higher EE was associated with better pairing of energy intake to energy demand and increased fat oxidation during either energy balance feeding or overfeeding (33). Here, we show that using different housing temperatures produces divergent EE in mice independent of physical activity. Leveraging this effect shows that greater EE results in increased energy flux, less positive energy balance, and greater coupling of energy intake to EE during HFHS feeding in both males and females. However, female mice had less positive energy balance and greater energy intake coupling compared with males at either EE level. The previously cited studies did not investigate whether the increases in energy intake coupling and energy balance associated with higher EE were different in males versus females (32,33). Future work is necessary to specifically investigate the observed sex differences in coupling of energy intake to EE and the responses in energy metabolism to increased EE. Together, these findings confirm and support that higher EE aids in improving energy balance regulation and body weight homeostasis while also revealing critical differences between sexes.

Previous studies have consistently revealed sexual dimorphism for weight gain and anatomical location of fat mass gains during hypercaloric conditions. In this study, female mice at higher EE gained less fat mass despite having the same change in body weight as lower EE

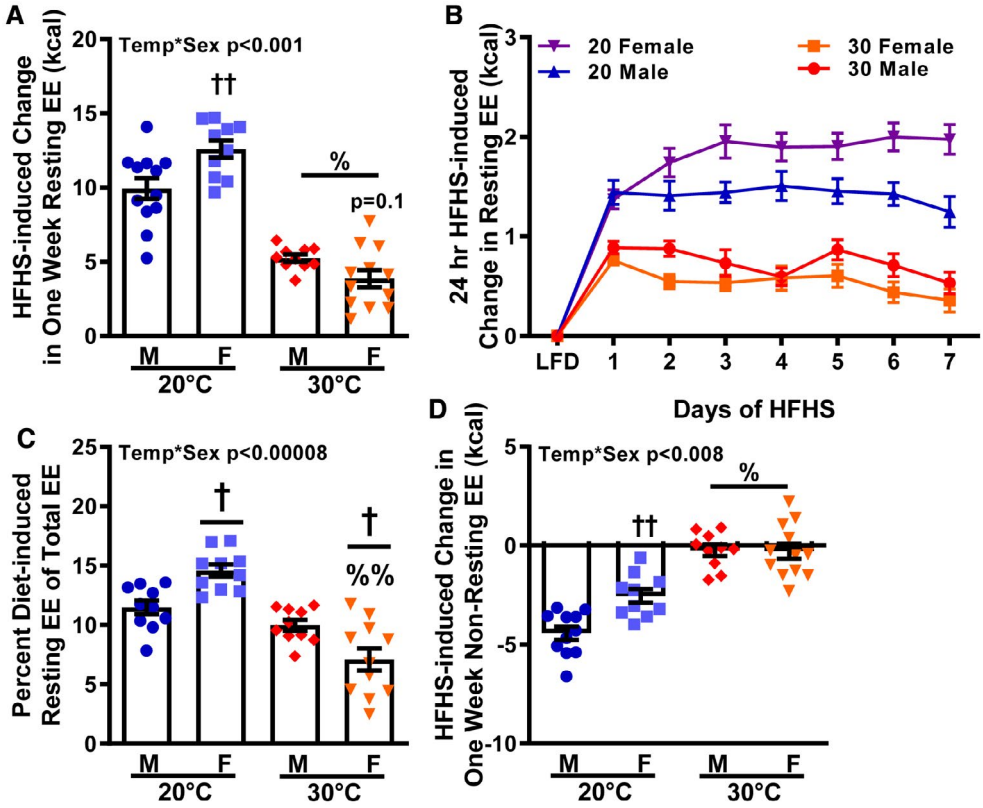

Figure 4 Baseline energy expenditure (EE) and sex interact, impacting high-fat/high-sucrose (HFHS)-induced nonshivering adaptive thermogenesis. (**A**) HFHS-induced nonshivering thermogenesis was calculated as the diet-induced change in resting EE as the difference of 1-week HFHS values and the 1-week low-fat control diet (LFD) values. (**B**) Daily HFHS-induced change in resting EE above the 1-week average LFD resting EE. (**C**) Percentage of total EE composed of diet-induced resting EE during HFHS feeding. (**D**) HFHS-induced change in nonresting EE. Values are means ± SEM. $n = 10$-12. $^{\%}P < 0.05$, main effect of 20°C vs. 30°C; $^{\dagger}P < 0.05$, main effect of sex; $^{\dagger\dagger}P < 0.05$, male vs. female within temperature; $^{\%\%}P < 0.05$, 20°C vs. 30°C within sex.

females. Interestingly, the higher EE female mice (20°C) were the only group to gain more fat-free mass than fat mass during HFHS feeding. Thus, the fat mass gain in the 20°C females represented only 20% of the body weight gain, compared with ~70% of the weight gain for the other three groups. Typically, between 60% and 80% of weight gained during most periods of positive energy balance is fat mass (34). Human data showed that individuals with reduced EE and reduced coupling of energy intake to EE had increased fat mass gain over time (35). However, other research observed an increase in fat-free mass with a decrease in fat mass in women with overweight following 3 months of physical training, which was not observed in male participants (36). These data highlight both a unique metabolic response to short-term HFHS feeding in females with higher EE and the need for more studies on how differences in EE produce sex-specific responses in body composition and weight gain during increased energy intake.

The capacity to adapt energy metabolism through changes in substrate use and nonshivering thermogenesis likely drives susceptibility for obesity and metabolic disease and it is thus a focus of treatment (25,27,28,37,38). The capacity to adapt substrate use from fat (low respiratory quotient) to carbohydrate (high respiratory quotient) during the fed-to-fasted transition was coined as metabolic flexibility (39) and was demonstrated to be reduced in patients with obesity compared with lean patients. The definition of metabolic flexibility has expanded to encompass adaptation of substrate use in response to high-fat diet feeding (26), and human data demonstrate that a lower ratio of fat to carbohydrate oxidation or the inability to increase fat use during high-fat overfeeding predicts future weight gain (24,40). Previously, we observed increased weight gain in a rodent model with reduced metabolic flexibility and reduced fat oxidation during short-term HFHS feeding (41). In this study, although 7 days of HFHS feeding resulted in reduced average daily respiratory quotient in all mice, increased baseline EE was associated with more extensive lowering of average daily respiratory quotient following the HFHS transition. Additionally, females demonstrated greater metabolic flexibility to short-term HFHS feeding. The causative role of metabolic flexibility in the development and progression of obesity and metabolic disease is still unclear; however, these data support an association of increased EE with improved metabolic flexibility and reduced gains in adiposity during HFHS feeding.

Nonshivering adaptive thermogenesis is the centrally mediated increase in heat production in response to either cold or diet stimuli (28). With recent discoveries of thermogenic adaptations in both adipose depots and skeletal muscle, many laboratories have

explored nonshivering thermogenesis as a target for obesity treatment (27,28,37). Rodent work has highlighted the potential importance of diet-induced nonshivering thermogenesis through findings of increased susceptibility or protection for diet-induced weight gain following knockout (42-45) or overexpression (46) of genes involved in various thermogenic pathways. In this study, we observed that HFHS feeding produced rapid and sustained increases in nonshivering thermogenesis (diet-induced changes in resting EE). Similar findings in human participants have been observed as change in sleeping EE from 1 day (47,48), up to 56 days (48), of overfeeding. Importantly, we observed that mice with higher baseline EE had a greater diet-induced nonshivering thermogenic response, which was further increased in 20°C female mice, in which diet-induced nonshivering thermogenesis represented approximately twice the proportion of total EE (~14%) as 30°C females (~7%). Interestingly, diet-induced nonshivering thermogenesis was closely associated with the observed changes in fat-free mass in female mice during short-term HFHS feeding. This suggests that the increased nonshivering thermogenesis of the 20°C female mice is due to the greater gain in fat-free mass that was found only in these mice. However, because of the experimental design, it is not possible to determine the daily change in fat-free mass across the HFHS feeding. Together, these findings are the first to show that EE and sex interact to alter short-term thermogenic response to an energy-dense diet.

Limitations: Despite the wide breadth of energy metabolism data collected during these experiments, several potentially confounding factors and limitations should be considered. First, although the C57Bl/6J mouse strain is extensively used in obesity studies, the use of other inbred and outbred mouse strains for future studies, particularly related to assessment of sex differences, is necessary. Second, the increased EE of mice at subthermoneutral ambient temperatures is primarily mediated by centrally regulated nonshivering thermogenic pathways in adipose and skeletal muscle. These pathways differ from those potentially activated through increased physical activity or exercise, and thus, findings may not be directly comparable. Furthermore, from the data herein, we cannot determine the magnitude of activation of the different nonshivering thermogenic tissues, which could potentially differ by baseline EE or sex. Also, it is not clear how the use of different ambient housing temperatures impacts other metabolic homeostatic systems (e.g., glucose homeostasis), which could influence the outcomes of the current study. Third, the assessment of diet-induced weight gain in 9- to 11-week-old mice could be confounded by the previously observed dependence of weight gain on age of diet initiation and sex (49). Fourth, previous mouse work has demonstrated that male mice defend different body core temperatures at different ambient temperatures (19,50). The lack of these thermal biology data prevents a comprehensive dissection of sexual differences in energy metabolism and the impact on metabolic responses to short-term HFHS feeding. Also, no determination of protein use for energy is possible under the experimental conditions. Finally, the lack of fecal energy excretion data prevents the calculation of net energy intake during both the LFD and HFHS feeding and potentially confounds the calculation of energy balance.

Conclusion

Because the prevention of weight gain is putatively easier than weight loss (3), it is critical that mechanisms underlying the protection or susceptibility to weight gain during short-term hypercaloric conditions be elucidated. This study used ambient temperature to determine whether higher or lower EE in male and female mice would change metabolic adaptations and weight gain during a transition to acute HFHS feeding. Herein, these data support that higher levels of EE enhance coupling of energy intake to energy demand, producing lower positive energy balance with reduced weight and adipositygain during short-term HFHS feeding. Additionally, these data demonstrate that EE level plays an important role in determining weight gain and body composition changes in female mice exposed to acute HFHS feeding. Furthermore, this study demonstrates the utility of different mouse housing temperature as a tool to study the independent role of EE in metabolic phenotypes. Finally, these findings have further significance when one considers that the vast majority of obesity research conducted in mice occurs at subthermoneutral housing, near the 20°C temperature.**O**

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Supporting information: Additional Supporting Information may be found in the online version of this article.

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