Metabolic Responses to 24-Hour Fasting and Mild Cold Exposure in Overweight Individuals Are Correlated and Accompanied by Changes in FGF21 Concentration

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METABOLISM

A greater decrease in 24-h energy expenditure (24 EE) during 24-h fasting defines a "thriftier" metabolic phenotype prone to weight gain during overfeeding and resistant to weight loss during caloric restriction. As the thermogenic response to mild cold exposure (COLD) may similarly characterize this human phenotype identified by acute fasting conditions, we analyzed changes in 24 EE and sleeping metabolic rate (SLEEP) in a whole-room indirect calorimeter during 24-h fasting at thermoneutrality (24°C) and during energy balance both at thermoneutrality (24°C) and mild cold (19°C) in 20 healthy volunteers (80% male; aged 36.6 ± 11.4 years; percentage body fat 34.8 ± 10.5%). Greater decrease in 24 EE during fasting (thriftier phenotype) was associated with less increase in 24 EE during COLD (i.e., less cold-induced thermogenesis). Greater decreases in plasma fibroblast growth factor 21 (FGF21) after 24-h fasting and after COLD were highly correlated and associated with greater decreases in SLEEP in both conditions. We conclude that the metabolic responses to short-term fasting and COLD are associated with and mediated by the liver-derived hormone FGF21. Thus, the 24 EE response to COLD further identifies the "thrifty" versus "spendthrift" phenotype, providing an additional setting to investigate the physiological mechanisms underlying the human metabolic phenotype and characterizing the individual susceptibility to weight change.

The measurement of the energy expenditure (EE) response to short-term fasting identifies a metabolic phenotype associated with the degree of susceptibility to weight change (1). That is, a greater decrease in 24-h EE (24 EE) during acute fasting (indicative of a "thrifty" phenotype), likely mediated by less epinephrine secretion (2), predicts greater weight gain in free-living conditions and during controlled overfeeding as well as less weight loss during caloric restriction (1).

Similar to short-term fasting, mild cold exposure (COLD) also leads to an adaptive EE response, termed cold-induced thermogenesis (CIT) (3), mediated by sympathetic nervous system activity, nonshivering muscle activity, and/or brown adipose tissue (BAT) activity (4,5). In predominantly lean individuals, COLD leads to increased CIT and fibroblast growth factor 21 (FGF21) secretion (6).

Rodent studies show an association between CIT and diet-induced thermogenesis (7), indicating common, biological mechanisms underlying the metabolic changes during feeding and COLD. It is unclear if CIT is different between "thrifty" versus "spendthrift" phenotypes. Therefore, we assessed the relationship between CIT and the EE response to fasting over 24 h (as a reliable measure of the metabolic phenotype) by measuring 24 EE and previously established hormonal markers such as FGF21 and urinary 24-h catecholamine concentrations in carefully controlled dietary conditions of energy balance (ENBAL) during thermoneutrality and COLD at 19°C and during fasting at thermoneutrality. We hypothesized that a greater decrease in 24 EE during acute fasting is associated with limited capacity to increase 24 EE during COLD.

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RESEARCH DESIGN AND METHODS

This study examines data from an ongoing clinical trial (ClinicalTrials.gov identifier NCT02939404) (Supplementary Fig. 1). On admission to the clinical research unit, volunteers were placed on a weight-maintaining diet (50% carbohydrate, 30% fat, and 20% protein) with total daily intake calculated using unit-specific equations based on weight and sex (8). Daily fasting body weight measured by calibrated scale was maintained within 1% of admission weight by daily adjusting intake (9). All participants were without diabetes based on oral glucose tolerance test performed 3 days after admission (10). All participants provided written informed consent prior to beginning the study. The Institutional Review Board of the National Institute of Diabetes and Digestive and Kidney Diseases approved the study.

Measurements of Energy Metabolism

The 24 EE and respiratory quotient were assessed in a large, open-circuit, indirect whole-room calorimeter (11,12). Participants' spontaneous physical activity (SPA) was measured by radar sensors and expressed as percentage of time when activity was detected (13). Sleeping metabolic rate (SLEEP) was calculated as average EE between 11:30 P.M. and 5:00 A.M. overnight when participant movement was <1.5% (<0.9 s/min) and extrapolated to 24 h (11).

Interventions

Participants completed three 24-h sessions inside the calorimeter at least 4 days after admission in the following order: during thermoneutral ENBAL (ambient temperature: 23.6 ± 0.3°C [mean ± SD]), during COLD in isocaloric conditions (19.0 ± 0.3°C), and during thermoneutral fasting (23.6 ± 0.3°C). Energy intake during the eucaloric assessment was calculated using unit-specific formulas as previously described (11) and was 20% less compared with the weight-maintaining diet outside the calorimeter to account for limited physical activity. During COLD, energy intake was the same compared with the eucaloric intervention, and standardized clothing (hospital gown, pants, and ankle-length socks) was provided. For sleep (11:00 P.M. to 6:00 A.M.), three blankets were provided so that participants could create their own microenvironment to achieve a level of comfort. Participants were asked to report shivering, in which case an extra blanket was provided. During fasting, volunteers were instructed to keep themselves hydrated by drinking water.

Analytical Measurements

Blood plasma samples were collected in the morning after an overnight fast prior to and upon exiting the calorimeter, while 24-h urine was collected continuously during each session. Samples were stored in a freezer at −70°C for later measurements by the National Institute of Diabetes and Digestive and Kidney Diseases Core laboratory. Plasma insulin concentrations were measured with an automated immunoenzymometric assay (Tosoh Bioscience Inc., Tessenderlo, Belgium). Plasma FGF21 and leptin were measured by ELISA (R&D Systems), and the intra- and interassay coefficients of variation were 2.5% and 5.2% and 1.3% and 1.6%, respectively. Urinary catecholamines were measured via high-performance liquid chromatography at Mayo Collaborative Services, LLC (Rochester, MN).

Statistical Analysis

Statistical data analysis was performed using the SAS Statistical Software package (SAS Enterprise Guide version 7.15; SAS Institute, Cary, NC). Data were expressed as mean ± SD or mean with 95% CI. Skewed variables were $\log_{10}$-transformed before analyses to meet the assumptions of parametric tests.

Data and Resource Availability

The data sets analyzed during the current study are available from the corresponding author upon reasonable request.

RESULTS

Baseline characteristics of the study cohort are presented in Table 1. Study participants were mainly overweight or with obesity (85% had BMI >25 kg/m^2; range 21.9–46.5) (Supplementary Fig. 3).

Changes in Energy Metabolism During 24-h Fasting and Cold Exposure

The relationships between 24 EE during ENBAL and COLD/fasting are shown in Supplementary Fig. 4. Compared with ENBAL, 24 EE during COLD did not change overall ($P = 0.26$) but showed a high interindividual variability (SD 96 kcal/day), whereas 24 EE during fasting decreased in all participants by an average of 162 kcal/day ($P < 0.0001$) (Table 2).

The individual change in 24 EE during COLD correlated with the decrease in 24 EE during fasting ($r = 0.84$; $P < 0.0001$) (Fig. 1*A*), such that lesser decrease in 24 EE during fasting by 100 kcal/day was associated with greater increase in 24 EE during COLD by an average of 64 kcal/day. Similar results were obtained in sensitivity analyses: *1*) considering individual changes in adjusted 24 EE ($r = 0.84$; $P < 0.0001$) (Supplementary Fig. 5*A*), and *2*) controlling for 24 EE during ENBAL as partial variable (partial $r = 0.83$; $P < 0.0001$) (Supplementary Fig. 5*B*). Individual changes in 24 EE during fasting or COLD were not associated with BMI or percentage body fat (PFAT) (all $P > 0.16$).

For illustrative purposes and to confirm our results obtained using continuous 24 EE data, we also performed groupwise comparisons of thrifty versus spendthrift individuals (Fig. 1*B*) as arbitrarily defined by the median

Table 1—Baseline characteristics of the study group

	Total (n = 20)	Thrifty (n = 10)	Spendthrift (n = 10)	P value
Demographic characteristics				
Men, n (%)	16 (80)	8 (80)	8 (80)	1.0
Race/ethnicity, n	3 BLK, 4 CAU, 3 HIS, 10 NA	1 CAU, 3 HIS, 6 NA	3 BLK, 3 CAU, 4 NA	
Age (years)	36.6 ± 11.4 (19.3, 55.4)	37.4 ± 10.7 (19.4, 52.5)	35.9 ± 12.6 (19.3, 55.4)	0.8
Body composition measurements				
Height (cm)	170.5 ± 8.1 (153.5, 186.0)	168.9 ± 8.3 (153.5, 177.6)	172.1 ± 8.0 (163.0, 186.0)	0.4
Body weight (kg)	90.1 ± 22.9 (62.5, 139.2)	89.2 ± 23.3 (63.6, 139.2)	91.1 ± 23.6 (62.5, 124.0)	0.9
BMI (kg/m^2)	31.0 ± 7.6 (21.9, 46.5)	31.1 ± 6.9 (23.5, 46.0)	30.9 ± 8.6 (21.9, 46.5)	1.0
Body fat (%)	34.8 ± 10.5 (18.6, 52.4)	36.0 ± 8.4 (25.1, 52.4)	33.5 ± 12.6 (18.6, 52.1)	0.6
FM (kg)	32.8 ± 16.3 (11.6, 64.2)	33.2 ± 15 (17.4, 64.2)	32.4 ± 18.4 (11.6, 62.5)	0.9
FFM (kg)	57.3 ± 10.6 (40.0, 75.0)	56.0 ± 11.5 (40.0, 75.0)	58.6 ± 10.0 (40.0, 70.9)	0.6
EE during ENBAL				
24 EE during ENBAL (kcal/day)	2,227 ± 372 (1,770, 3,188)	2,289 ± 409 (1,849, 3,188)	2,164 ± 341 (1,770, 2,719)	0.5
Food intake during ENBAL (kcal/day)	2,334 ± 278 (1,906, 2,956)	2,351 ± 272 (2,030, 2,956)	2,317 ± 298 (1,906, 2,777)	0.8
ENBAL (%)	5.9 ± 10.0 (−7.3, 29.9)	4.0 ± 11.2 (−7.3, 29.9)	7.8 ± 8.7 (−7.1, 23.7)	0.4
Measurements of glucose metabolism				
Fasting glucose (mg/dL)[2]	91.2 ± 8.3 (77.5, 113.0)	90.1 ± 8.8 (77.5, 105.0)	92.4 ± 8.1 (83.5, 113.0)	0.6
2-h glucose during OGTT (mg/dL)[2]	122.6 ± 35.5 (50.0, 181.0)	114.6 ± 42.3 (50.0, 181.0)	130.5 ± 27.1 (85.0, 174.0)	0.3
Fasting insulin (μIU/mL)	13.8[1] [3.6, 32.4]	11[1] [3.6, 20.4]	15.8[1] [4.5, 32.4]	0.4
2-h insulin during OGTT (μIU/mL)	63.3[1] [3.0, 509.8]	44.8[1] [3.0, 273.5]	63.3[1] [34.0, 509.8]	0.6

Data are mean ± SD for continuous variables with minimum and maximum in parentheses or as otherwise specified for categorical variables. Body composition was assessed by DXA (Lunar iDXA; GE Healthcare, Madison, WI) on the second day after admission. Body fat mass (FM) and fat-free mass (FFM) were calculated from total body weight and the PFAT obtained from DXA scan. Columns show baseline data from all participants (Total column), from thrifty individuals, and from spendthrift individuals and P values for statistical differences between both groups. Characterization of thrifty/spendthrift individuals is based on the median decrease in 24 EE from ENBAL to fasting (−147 kcal/day). BLK, black; CAU, Caucasian; HIS, Hispanic; NA, Native American; OGTT, oral glucose tolerance test. [1]Skewed values are expressed as medians with interquartile ranges in brackets. [2]Plasma glucose concentrations were measured using the Analox GM9 glucose oxidase method (Analox Instruments USA Inc., Lunenburg, MA).

decrease (−147 kcal/day) in 24 EE from ENBAL during 24-h fasting, as done previously (14,15) (Table 1). Thrifty individuals decreased, on average, 24 EE by 82 kcal/day (P = 0.02), while spendthrift individuals did not change 24 EE during COLD (P = 0.12), with an average between-group difference of 115 kcal/day (P = 0.004). Similar results were observed when individuals were classified as thrifty/spendthrift based on the two lower/upper quintiles of the decrease in 24 EE during fasting (Supplementary Fig. 6*A*).

On average, SLEEP was unchanged both during COLD and fasting (all $P > 0.3$) (Table 2) but showed a high interindividual variability (−25 ± 108 and 20 ± 142 kcal/day, respectively). The individual change in SLEEP during COLD was related to

Table 2—Changes in EE measures and hormones during/after 24-h cold exposure in isocaloric conditions and fasting at thermoneutrality

	Cold exposure	P value	Fasting	P value
Changes in EE measures				
24 EE (kcal/day)	−25.1 ± 96.3 (−70.2, 20.0)	0.26	**−162.1 ± 126.8 (−221.4, −102.8)**	**<0.0001**
SLEEP (kcal/day)	−25.2 ± 107.7 (−75.6, 25.2)	0.31	19.6 ± 142 (−46.8, 86.0)	0.54
RQ (ratio)	−0.005 ± 0.017 (−0.013, 0.003)	0.22	**−0.08 ± 0.03 (−0.09, −0.07)**	**<0.0001**
SPA (%)	**−1.2 ± 1.3 (−1.8, −0.6)**	**0.0006**	−0.5 ± 1.2 (−1.1, 0.1)	0.09
Hormonal changes				
Insulin (μU/mL)[1]	5.4 ± 52.0 (−13.4, 28.2)	0.58	**−40.4 ± 63.0 (−52.9, −24.5)**	**0.0002**
FGF21 (pg/mL)	**−93.3 ± 115.6 (−147.4, −39.2)**	**0.002**	**−57.9 ± 96.6 (−103.1, −12.7)**	**0.01**
Leptin (ng/mL)	**−7.3 ± 8.0 (−11, −3.5)**	**0.0006**	**−25.9 ± 28.2 (−39.1, −12.6)**	**0.0006**
Urinary epinephrine (μg/24 h)[2]	**2.3 ± 2.8 (1.0, 3.6)**	**0.0017**	0.9 ± 3.0 (−0.5, 2.3)	0.18
Urinary norepinephrine (μg/24 h)[2]	−4.3 ± 15.1 (−11.3, 2.8)	0.22	−2.1 ± 25.5 (−14.1, 9.8)	0.71

Absolute values are presented as mean ± SD with 95% CI in parentheses and P values. Statistical significance determined by Student paired t test. Statistically significant results are highlighted in boldface. RQ, respiratory quotient. Changes in EE measures and 24-h urinary epinephrine and norepinephrine were calculated as difference from isocaloric conditions. [1]Changes in insulin shown as percentage changes due to skewed distribution. [2]Changes in urinary epinephrine and norepinephrine concentrations during 24-h cold exposure and fasting conditions were calculated as 24-h concentration during fasting minus 24-h concentration during ENBAL.

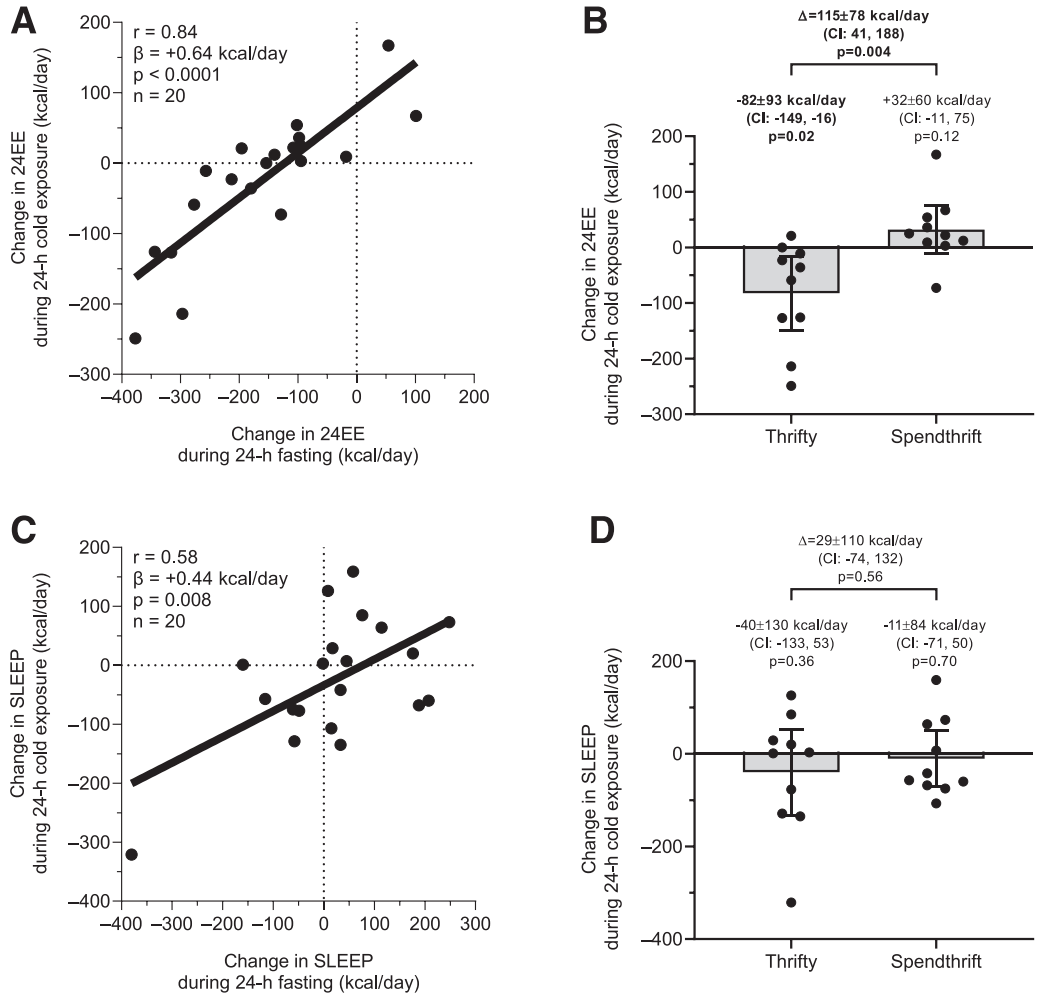

Figure 1— Thriftier individuals have less capacity to increase CIT during 24-h COLD. Associations between changes in 24 EE (*A*) and SLEEP (*C*) during fasting at thermoneutrality and 24-h cold exposure in isocaloric conditions in the whole cohort and between metabolically thrifty and spendthrift individuals (*B* and *D*). Individual changes (Δ) in 24 EE and SLEEP were calculated as the difference between cold exposure and fasting condition minus ENBAL condition. Individuals were categorized as thrifty or spendthrift based on the median value of the difference in 24 EE between ENBAL and fasting (−147 kcal/day). Paired *t* test was used to evaluate the within-group changes in EE measures between fasting/cold exposure and eucaloric conditions, while unpaired *t* test was used to compare between-group differences in EE measures. Spearman nonparametric correlation analyses were also performed as sensitivity analyses to account for influential cases that may have inflated Pearson correlation values, and similar results were obtained (*A*: Spearman $\rho = 0.84$, $P < 0.0001$; *C*: Spearman $\rho = 0.46$, $P = 0.04$). Sensitivity analyses of EE measures were performed considering adjusted values obtained via multivariate regression analysis, including fat-free mass and fat mass as covariates (Supplementary Fig. 5). Supplementary Figure 2 shows the association between fat-free mass and 24 EE during ENBAL, cold exposure, and fasting.

the individual change in SLEEP during 24-h fasting ($r = 0.58$; $P = 0.008$) (Fig. 1*C*), such that lesser decrease in SLEEP during 24-h fasting by 100 kcal/day was associated with greater increase in SLEEP during COLD by an average of 44 kcal/day. In groupwise comparisons, thrifty and spendthrift individuals did not change SLEEP during COLD (all $P > 0.35$), with no difference between groups ($P = 0.56$) (Fig. 1*D*) and also when classifying individuals as thrifty/spendthrift based on the two lower/upper quintiles of the decrease in 24 EE during fasting (Supplementary Fig. 6*B*).

On average, SPA decreased by 1.2% during COLD ($P = 0.0006$) (Table 2) but remained unchanged during 24-h fasting ($P = 0.09$). The decrease in SPA during COLD was not related to the concomitant individual change in 24 EE and SLEEP (both $P > 0.73$) and thus was similar between thrifty and spendthrift individuals ($P = 1.0$).

Hormonal Changes During 24-h Fasting and Cold Exposure

Hormonal changes of FGF21, leptin, and urinary catecholamines are presented in Table 2 and Supplementary Fig. 7.

During COLD, greater decrease in FGF21 was related to greater concomitant decrease in SLEEP ($r = 0.59$; $P = 0.007$) (Fig. 2*A*). The same relationship was observed during fasting ($r = 0.83$; $P < 0.0001$) (Fig. 2*B*). The changes in FGF21 during 24-h fasting and COLD correlated with each other ($r = 0.74$; $P = 0.0002$) (Fig. 2*C*). There were no such

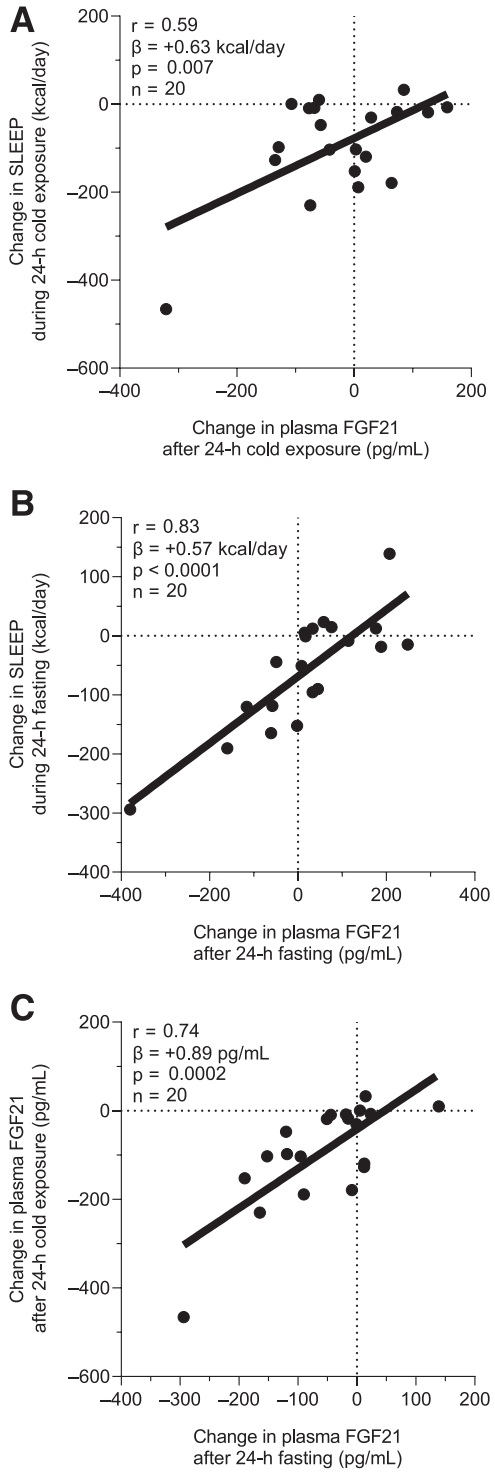

Figure 2—Changes in plasma FGF21 concentration after both 24-h cold exposure and fasting conditions are associated with concomitant changes in SLEEP. Association between changes in plasma FGF21 and SLEEP during cold exposure (*A*) during isocaloric conditions and fasting conditions (*B*) at thermoneutrality. *C*: Association between changes in FGF21 during fasting and cold exposure conditions. Individual changes (Δ) in SLEEP and FGF21 were calculated as the difference between cold exposure and fasting condition minus ENBAL condition. As the pre-chamber values of FGF21 were significantly different in all three EE measurements (Supplementary Fig. 7), we performed a sensitivity analysis by recalculating the changes in FGF21 during cold exposure and fasting using the averaged pre-FGF21 of all three EE assessments and found results similar to the ones presented in this study. There were no significant associations between changes in FGF21 during cold exposure and fasting and concomitant changes in other EE measures (all $P > 0.08$). Spearman nonparametric correlation analyses were also performed as sensitivity analyses to account for influential cases that may have inflated Pearson correlation values, and similar results were obtained for correlations shown in *B* (Spearman $\rho = 0.77$; $P < 0.0001$) and *C* (Spearman $\rho = 0.63$; $P = 0.003$), while the correlation was attenuated for *A* (Spearman $\rho = 0.25$; $P = 0.28$).

relationships with norepinephrine, epinephrine, leptin, and insulin (data not shown).

In this study, we also assessed changes in core and skin body temperatures (Supplementary Table 1). Their associations with concomitant changes in 24 EE during COLD are shown in Supplementary Fig. 8. Their associations with PFAT are shown in Supplementary Fig. 9.

DISCUSSION

The human body possesses thermoregulatory mechanisms to protect itself against cold. CIT is one of these mechanisms and comprises nonshivering and shivering muscle activity, as well as BAT activation, all of which can increase EE by up to 36% above that measured during thermoneutrality depending on experimental settings (3). The CIT capacity varies among individuals due to individual differences in shivering threshold, BAT activity, age, and adiposity (3).

In this present study, we found that a greater decrease in 24 EE during acute fasting (indicative of a "thriftier" phenotype [1]) was associated with less CIT. This result was validated in confirmatory analyses by arbitrarily defining thrifty/spendthrift individuals based on a greater/smaller-than-median decrease in 24 EE during 24-h fasting, respectively. Our results are independent of the degree of adiposity, but as these subjects are more overweight, they may be considered overall thriftier. Our findings are supported by animal (7) and human (4) studies showing a relationship between CIT and diet-induced thermogenesis, although one study did not find this association (16). Our present data provide evidence that a reduced CIT capacity is a novel feature of the thrifty phenotype and, as such, may be informative for the individual susceptibility to weight gain.

We observed an overall decrease in plasma FGF21 after both short-term COLD and fasting, and, interestingly, these changes were correlated within individuals. While in humans the decrease in FGF21 during short-term fasting has been described previously (17), changes in FGF21 during COLD are inconclusive. One study reported an increased (6) while others reported a decreased FGF21 secretion (18–20), most likely due to different measurement time points, as FGF21 undergoes a circadian rhythm, with nightly increase and daily decrease (21).

We found that changes in FGF21 after both COLD and fasting were associated with concomitant changes in SLEEP,

a surrogate for resting metabolic rate. Our findings confirm data obtained in a larger cohort (2). Our results suggest that FGF21 may represent a mutual hormonal mediator of the change in SLEEP during these interventions. They are supported by mice studies showing that FGF21 regulates EE (22,23), but so far, no human study has found a relationship between changes in FGF21 and concomitant changes in EE. The effect of FGF21 on SLEEP observed during 24-h fasting and COLD did not translate to increased 24 EE, which could be due to the timing of FGF21 measurements that were obtained in the morning after an overnight fast, and thus shortly after FGF21 peaked at night. Accordingly, the fasting FGF21 measurement may not reflect the diurnal changes in FGF21 concentration in response to feeding and SPA, which might influence daily EE. In general, due to the diurnal changes of FGF21 and timing of blood collection, our findings should be interpreted with caution and merit further investigation including FGF21 kinetics.

Limitations

Our study has several limitations. Because this was a secondary analysis, fasting and COLD interventions were not randomized. Only half of our participants increased 24 EE during COLD, which may be due to the anthropometrics of our study group, as we mainly recruited individuals with overweight or obesity who are likely to be better insulated from cold due to *1*) higher basal heat generation, *2*) larger surface-to-volume ratio, and *3*) more subcutaneous fat providing additional thermal insulation and thus demonstrating a lower or even absent CIT (5,24,25). However, our results indicate that the individual thermogenic mechanisms elicited by these interventions can be observed even in a situation in which the overall increase in 24 EE during COLD is not achieved in all individuals. Furthermore, we mainly recruited men ($n = 16$) and only four premenopausal women. In sensitivity analyses including only male participants, main results were unchanged. Further, shivering was self-reported by participants instead of being assessed via electromyography. Lastly, our participants live in a warm climate area, and 50% were Native Americans, which limits generalizability.

Conclusion

Greater decrease in 24 EE during 24-h fasting at thermoneutrality was associated with less CIT and thus further characterizes the thrifty versus spendthrift phenotype, providing additional metabolic information to investigate the physiologic mechanisms by which dynamic changes in 24 EE (during overfeeding, fasting, and now COLD) quantify the individual susceptibility to future weight gain. Although we could not determine a mutual hormonal mediator underlying the overall 24 EE responses to COLD and fasting, we found that changes in FGF21 paralleled changes in SLEEP during both conditions. Our results may open up new pathways to identify and potentially ameliorate thriftiness (i.e., possibly by administering FGF21 exogenously to thrifty individuals to increase BAT activity).

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