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Thigh adipocyte size is inversely related to energy intake and respiratory quotient in healthy women

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Abstract

Objective—The relationship between adipocyte size and *ad libitum* energy intake has not been previously examined. We hypothesized an inverse relationship between adipocyte size and daily energy intake (DEI).

Methods—Seventy healthy adults (39M/31F; BMI 30.0±6.3) underwent dual energy X-ray absorptiometry and subcutaneous fat biopsies from the abdomen and thigh. Osmium-fixed adipocytes were sized with a Coulter counter. Volunteers self-selected food from a vending machine paradigm as the only source of energy intake over three days as inpatients. Volunteers also had 24-h respiratory quotient (RQ) measured in a whole-room indirect calorimeter.

Results—In women, the large cell peak diameter of the thigh depot was greater than that of the abdominal depot (= +15.8 μm, p<0.0001). In women, thigh peak diameter was inversely associated with DEI (β = -264.7 kcal/day per 10 μm-difference, p=0.03), adjusted for demographics and body composition. The thigh peak diameter in women was associated with 24-h RQ (r= -0.47, p=0.04), adjusted for demographics, body composition, and 24-h energy balance. These associations did not extend to men or the abdominal depot.

Conclusions—In women, thigh adipocyte size was associated with reduced DEI and 24-h RQ, indicating a special role for thigh fat in women. This depot-specific sexual dimorphism indicates common regulation of energy intake and adipocyte size in the thigh region of women.

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Keywords

human adipose tissue; energy intake; adipocytes; modeling

1. Introduction

Obesity, resulting from a prolonged imbalance between energy expenditure and energy intake, is a substantial public health problem [1, 2]. Understanding the factors regulating energy intake is therefore crucial. Despite abundant evidence from animal models that signals derived from adipose tissue convey information about energy reserves (e.g. leptin and other adipokines) to provide negative feedback on energy intake [3–5], human evidence linking fat mass (FM) and energy intake has been neither strong nor consistent, with prior studies showing no associations [6, 7] or weak negative associations [8, 9]. FM is comprised primarily of adipocytes of varying size, and adipocyte size is associated with appetite-regulating adipokines in cultured adipocytes [10], at the gene expression level in tissue [11], and in circulation [12–14], indicating a role for adipose tissue cellularity in appetite regulation. To our knowledge, whether adipocyte size is a determinant of *ad libitum* daily energy intake (DEI) has not been studied in humans.

Average adipocyte size has been shown to be directly associated with in vitro basal and isoproterenol stimulated lipolysis per cell [15]. In the same study, adipocyte size was directly associated with whole-body lipid oxidation and negatively correlated with 24-h respiratory quotient (24-h RQ) which primarily reflects the ratio of carbohydrate to fat oxidation. In previous studies, 24-h RQ was associated with greater *ad libitum* overeating (as well as future weight gain) [9, 16]. However, it is unknown if adipocyte size mediates the relationship between 24-h RQ and *ad libitum* DEI.

In the current study, the primary aim was to investigate whether adipocyte sizes from two different subcutaneous depots (i.e. abdomen and thigh) are determinants of *ad libitum* DEI using a vending machine paradigm, an objective and reproducible measure of food intake [17]. We also aimed to confirm the association between adipocyte size and 24-h RQ using whole-room indirect calorimetry and to evaluate whether adipocyte size is a determinant of DEI independent of 24-h RQ. We hypothesized that larger adipocytes are associated with lower *ad libitum* DEI, reduced 24-h RQ, and greater 24-h lipid oxidation (24-h LIPOX).

2. Methods

2.1 Participants and study design

Male and female participants were recruited from the Phoenix Metropolitan Area, Arizona, USA, between 2009 and 2016 and admitted to the clinical research unit for participation in a larger observational inpatient study (clinicaltrials.gov identifier: NCT00342732) investigating risk factors for obesity as previously described [9]. The protocol was approved by the Institutional Review Board of the National Institute of Diabetes and Digestive and Kidney Diseases (NIDDK). Written informed consent was obtained prior to participation. The present analysis was performed in 70 adults without diabetes (age < 65 years). All

participants were not currently using drugs, prescription medications or nicotine products, and healthy by screening history, physical examination, and routine laboratory testing. Women were verified to be nonpregnant with a urine pregnancy test.

An overview of the inpatient study is shown in Supplementary Fig. S1. During the inpatient stay, physical activity was restricted to light activities (e.g. playing pool, television, crafts) for the entire time course of the study. A standard weight-maintaining diet (50% carbohydrate, 30% fat, and 20% protein) was provided to the volunteers from the day of the admission. Based on body weight and sex, a unit-specific equation [18] was used to calculate the weight-maintaining energy needs (WMEN) for each individual and then adjusted daily by a research dietician to maintain a body weight within 1% of the weight on the admission day. On day two, body composition was assessed using dual-energy x-ray absorptiometry (Lunar Prodigy or iDXA, GE Lunar Healthcare, Madison, WI, USA). To make absorptiometry data comparable across the two different machines, absorptiometry measures were converged using an equation previously derived from a separate study on the metabolic unit to calculate total body fat percentage, and trunk and leg fat (both expressed as a percentage of total mass in the anatomical region mass) [19]. Waist circumference was measured at the umbilicus in the supine position and thigh circumference at the gluteal fold while standing.

After at least three days on the weight-maintaining diet, glucose tolerance was assessed by a 75-g oral glucose tolerance test according to the 2003 American Diabetes Association criteria [20]. Participants with diabetes were excluded. Plasma glucose concentrations was measured by the glucose oxidase method (Glucose analyzer GM9, Analox Instruments; Lunenburg, MA, USA). Before starting the 3-day of vending machine paradigm the blood samples were drawn for the measurement of fasting adiponectin and leptin concentrations. Adiponectin and leptin were measured using the human Quantikine ELISA kits from R&D Systems (Minneapolis, MN, USA). The intra-assay and inter-assay percent coefficient variation were 3.20% and 6.03% for adiponectin and 1.34% and 1.58% for leptin, respectively.

2.2 Adipose tissue sampling and cell size analysis

Participants underwent biopsies for subcutaneous adipose tissue of the abdomen and thigh under local anesthesia with 1% lidocaine after an overnight fast and after resting for two hours. Lateral mid-thigh subcutaneous adipose tissue was sampled using a modified Bergström needle under suction. Abdominal subcutaneous adipose tissue was also sampled via needle aspiration lateral to the umbilicus. Samples of subcutaneous adipose tissue were immediately incubated in osmium tetroxide-solution (Structure Probe, West Chester, PA, USA) containing collidine (Sigma-Aldrich, St. Louis, MO, USA) in a water bath at 37°C for 48 hours as previously described [21]. The solution containing osmium-fixed cells were filtered with a 25 µm and 250 µm nylon mesh (Sefar, Buffalo, NY, USA). Following this procedure, adipocyte size was measured using the Multisizer 3 Coulter Counter (Beckman Coulter, Brea, CA, USA) with a 400-µm aperture set to count 6000 cells per run. Cell suspensions were diluted so that coincident counting was less than 10%. After collection of pulse sizes, the data was expressed on a linear scale involving 300 bins, each 0.733 µm wide,

spanning the effective cell size range of the aperture (20 to 240 μm). Each fixed cell sample was run in duplicate and the data averaged. Data from bins between 25 to 240 μm were included for subsequent analysis. Since adipocyte sizing with this method reveals a bimodal distribution, with overlapping populations of small and large adipocytes, cell size distributions were characterized using nonlinear regression implemented in the R package `nlstools` [22] to obtain peak cell diameter of the large adipocyte population, nadir diameter (defined as the mid-point between the large and small populations), and percent small adipocytes (defined as the percent of cells with diameter less than the nadir diameter), as previously described [23, 24]. A representative distribution is shown in Supplementary Fig. S2 to illustrate these adipocyte size parameters. Of the 70 participants, 52 participants had adipocyte measures from both abdominal and thigh depots, and 15 and 3 had valid measures from the abdomen only and thigh only, respectively. Missing cell size data was most often due to insufficient biopsy sample. In addition, uncommon distributions not matching the bimodal pattern, as previously reported [25], were excluded from analysis.

2.3 Metabolic chamber measures

Participants spent approximately 23.5 hours inside a whole-room indirect calorimeter to measure 24-hour energy expenditure (24-h EE) and substrate oxidation in eucaloric conditions as previously described (Supplementary Fig. S1) [26]. Prescribed energy intake in the metabolic chamber was based on previously developed equations [27] and was approximately 80% of the weight maintaining diet provided outside the chamber to account for restricted physical activity inside the chamber. For an accurate calculation of intake, all unconsumed food was returned to the metabolic kitchen for weighing. Carbon dioxide production and oxygen consumption were extrapolated to 24 hours to calculate 24-h EE and 24-h RQ. The 24-hour energy balance (ENBAL) was calculated as energy intake minus 24-h EE. For better accuracy of metabolic measures, only individuals with energy balance within $\pm 20\%$ were included for analysis. From the 24-h RQ, 24-h carbohydrate oxidation (24-h CARBOX) and 24-h LIPOX rates were calculated accounting for 24-h protein oxidation (24-h PROTOX) obtained by the measurement of 24-h urinary nitrogen excretion as previously described [28]. Spontaneous physical activity (SPA) was detected by radar sensors and expressed as the percentage of time over the 24-h period in which activity was detected. Sleeping metabolic rate (SMR) was defined as the average energy expenditure between the hours of 2330 and 0500 during which SPA was $< 1.5\%$. Sleep RQ was calculated during the same time points as above.

2.4 *Ad libitum* food intake measures

The measurement of *ad libitum* food intake over three days (Supplementary Fig. S1) was assessed using a vending machine paradigm, as previously described [17]. Upon admission, a food selection questionnaire in which a 9-point Likert scale (1=dislike extremely, 5=neutral, 9=like extremely) was given to assess the food preferences of each subject to rate each food item. Based on ratings of 80 items, 40 items (scores: 4–8) were stocked in an individual-specific, computer-operated vending machines during this period. The participants had unrestricted access to food for 23.5 hours. Prior to placing the food items in the vending machines, all food was weighed by the metabolic kitchen staff. Furthermore, any uneaten food was also weighed at the end of the vending day to calculate the actual

intake. The CBORD Professional Diet Analyzer Program (CBORD, Inc., Ithaca, NY, USA) and the Food Processor database (ESHA version 10.0.0, ESHA Research, Salem, OR, USA) was used to calculate the daily total and individual macronutrient kilocalories consumed. Over three days, the average total *ad libitum* DEI was calculated and expressed as total kilocalories consumed daily. Similar calculations were performed for macronutrient intake (carbohydrate, fat, and protein). The DEI was also expressed as percentage of the WMEN determined prior to the three-day vending period.

2.5 Statistical Analysis

Statistical analyses were performed using SAS 9.4 (SAS Institute, Cary, NC) and R 3.5.2 (The R Foundation for Statistical Computing, Vienna, Austria). Data are expressed as mean \pm standard deviation. Categorical variables were compared with the χ^2 test. As no differences in adipocyte parameters were observed between races using ANOVA, non-Native American individuals (n=38) were combined into one group (other races) and compared to Native Americans (n=32). Unpaired *t*-test were used to assess difference by sex and race, and the paired *t*-test was used to assess differences in adipocyte size between the abdominal and thigh depots. Correlations between normally distributed data were assessed by the Pearson's correlation coefficient. Similar analyses were performed to assess the association between adipocyte size and *ad libitum* energy intake measures (i.e. DEI and macronutrient intake). Analyses were conducted by sex and depot to address the known influence of these variables on adipocyte morphology and function [29, 30]. To correct for multiple comparisons, a Bonferroni-corrected threshold for significance equal to 0.0125 was obtained by dividing the nominal threshold for significance (0.05) by the number of comparison groups (4 = 2 sexes + 2 depots). Linear regression analysis was used to calculate residuals of total *ad libitum* daily energy intake and residuals of macronutrient intake, adjusting for age, race, FM, and fat-free mass (FFM). Additionally, in a sensitivity analysis, height-normalized indices of both fat mass and fat-free mass (kg/m^2) were used instead in place of the original covariates. Linear regression analysis was also performed to calculate residuals of 24-h RQ and substrate oxidation (adjusting for age, race, body fat percentage, and ENBAL) and to calculate residual 24-h EE and its components after adjustment for age, race, FM, and FFM. To increase the precision of regression estimates, residuals were calculated using the entire cohort of volunteers who completed the study which included participants who did not have adipose tissue biopsies (n=135).

3. Results

Demographic and anthropometric characteristics are reported in Table 1. Participants ranged in age from 19–65 years (mean 39.0 ± 11.9) and Native American race was the most common racial group (46%). BMI ranged from 20.2–47.2 kg/m^2 (mean 30.0 ± 6.3). As expected, women had greater body fat percentage ($40.2 \pm 5.3\%$), and FM (34.2 ± 11.7 kg), and BMI (32.8 ± 7.4) compared to men ($26.8 \pm 5.1\%$; 23.5 ± 8.4 kg; 27.8 ± 4.1 , respectively).

3.1 Determinants of adipocyte measurements

Adipocyte sizes are reported in Table 2. Compared with women, men had lower peak and nadir diameters in the abdominal depot (= $-9.2 \mu\text{m}$, $p < 0.011$ and = $-6.5 \mu\text{m}$ $p = 0.015$,

respectively). Similarly, in the thigh depot, the peak and nadir diameters were lower in men than in women ($\Delta = -20.5 \mu\text{m}$, $p < 0.0001$ and $\Delta = -12.4 \mu\text{m}$, $p < 0.0001$, respectively). After adjustment for BMI, the significant difference in peak and nadir diameters in the thigh depot between men and women remained unchanged ($\Delta = -15.5 \mu\text{m}$, $p < 0.0001$ and $\Delta = -9.8 \mu\text{m}$, $p < 0.0001$, respectively), whereas there were no differences in either size parameter in the abdomen (all $p > 0.48$). No differences in percent small cell between men and women in either depot was observed ($p > 0.4$). No differences in the adipocyte size (peak diameter, nadir point, and percent small cells) in abdomen and thigh locations were observed between different races (Native American vs. other races, all $p > 0.1$). Adipocyte size was not associated with age in either anatomical region (all $p > 0.3$).

In participants with adipocyte size measurements in both the abdomen and thigh, intrasubject correlation between thigh and abdominal regions for peak diameter, nadir diameter, and percent small cells was assessed. In men, peak diameter of the abdomen was correlated with peak diameter of the thigh ($r = 0.45$, $p = 0.02$, Supplemental Fig. S3A), whereas there was no correlation between nadir diameter and percent small cells between the two regions (all $p > 0.1$, Supplemental Fig. S3B and Supplemental Fig. S3C). In women, peak and nadir diameters in abdomen were moderately correlated with the same adipocyte size measure in the thigh (peak cell diameter, $r = 0.57$, $p = 0.002$; nadir diameter $r = 0.52$, $p = 0.006$, Supplemental Fig. S3D–E), whereas no correlation was observed between abdomen and thigh percent small cell ($p = 0.8$, Supplemental Fig. S3C).

In women ($n = 27$), the thigh peak and nadir diameters were greater compared to the abdominal region ($\Delta = +15.8 \mu\text{m}$, 95% CI: 10.0 to 17.5, $p = 0.0001$, Fig. 1B; $\Delta = +9.6 \mu\text{m}$, 95% CI: 8.7 to 15.1, $p < 0.0001$, Fig. 1D) whereas no differences were observed in men (all $p > 0.4$, $n = 25$, Fig. 1A–C–E). No difference was observed in percent small cell between the two fat depots in either sex ($p > 0.2$, Fig. 1E–F).

In both abdominal and thigh regions of men, peak diameter was positively correlated with FM ($r = 0.53$, $p = 0.0007$, Fig. 2A; $r = 0.64$, $p = 0.0003$, Fig. 3A), body fat percentage ($r = 0.56$, $p = 0.0002$, Fig. 2C; $r = 0.57$, $p = 0.002$, Fig. 3C), and BMI ($r = 0.47$, $p = 0.0035$, Fig. 2E; $r = 0.64$, $p = 0.0003$, Fig. 3E) respectively. In men, the peak diameter of the thigh region was directly associated with FFM ($r = 0.58$, $p = 0.0013$) whereas no association was observed in the abdominal region ($p = 0.1$). In women, the peak diameter of the abdominal region was directly associated with FM ($r = 0.50$, $p = 0.005$, Fig. 2B), FFM ($r = 0.36$, $p = 0.04$), % fat ($r = 0.46$, $p = 0.009$, Fig. 2D), and BMI ($r = 0.55$, $p = 0.001$, Fig. 2F). Furthermore, the peak diameter of the thigh was positively associated BMI ($r = 0.40$, $p = 0.03$, Fig. 3F) and showed a trend with FM ($r = 0.35$, $p = 0.06$, Fig. 3B), but no association was observed with body fat percentage ($p = 0.21$, Fig. 3D).

In women, waist circumference and trunk fat percentage were associated with peak diameter of the abdomen ($r = 0.54$, $p = 0.002$ and $r = 0.37$, $p = 0.04$, respectively). Thigh circumference was associated with peak diameter of the thigh ($r = 0.40$, $p = 0.03$). No association was observed between leg fat percentage and peak diameter of the thigh ($p = 0.3$). In men, peak diameter of the abdomen was associated with waist circumference ($r = 0.42$, $p = 0.009$) but not trunk fat percentage ($p = 0.09$). Both thigh circumference and leg fat percentage were

associated with peak diameter of the thigh ($r=0.67$, $p=0.0001$ and $r=0.51$, $p=0.008$, respectively). Adipocyte size was not significantly associated with circulating leptin or adiponectin levels independent of age, race, and body composition in either sex or depot (all $p>0.35$).

Adipocyte size (peak diameter and nadir) was also analyzed in relation to fat distribution expressed as the ratio of trunk or leg fat mass to the total body fat mass or the waist-thigh-ratio. In unadjusted and adjusted analysis (for BMI), the adipocyte size in the abdomen of men was associated with the ratio of trunk fat mass to total body fat mass (both $p=0.02$). There was no similar association observed in women ($p>0.20$). Furthermore, in either sex, there was no association between adipocyte size in the thigh and the ratio of leg fat mass to total body fat mass (both $p>0.4$). Furthermore, there were no observed associations between thigh or abdomen adipocyte size with the waist-to-thigh ratio in either sex (all $p>0.10$).

3.2 Relationship between adipocyte size and *ad libitum* food intake

Ad libitum food intake measurements are described in Table 3. For the entire cohort, the average DEI was 3229.4 ± 1103.8 kcal/day (or $125.4 \pm 38.7\%$ when expressed as percentage of WMEN).

3.2.1 Thigh Depot—The peak diameter of thigh adipocytes in women was negatively associated with residuals of DEI ($\beta = -264.7$ kcal/day per 10 μm -difference in peak diameter, CI: -508.2 to -21.2 , $p=0.03$, Fig. 4B, Supplementary Table 1), daily protein intake ($\beta = -31.3$ kcal/day per 10 μm -difference in peak diameter, CI: -60.5 to -2.1 , $p=0.04$, Fig. 4H) and showed a trend with carbohydrate intake ($\beta = -137.5$ kcal/day per 10 μm -difference in peak diameter, CI: -278.0 to 2.9 , $p=0.054$, Fig. 4D). There was no association with residual fat intake ($p=0.1$, Fig. 4F). Results were unchanged in sensitivity analysis using fat-free mass index and fat mass index in place of FFM and FM, respectively. In contrast to women, no associations between adipocyte cell size and DEI or macronutrient intake were observed in men (all $p>0.5$, Fig. 4A–C–E–G). In either sex, neither leptin nor adiponectin were associated with residual DEI (all $p>0.30$). The independent effects of thigh adipocyte size in women and adipokines on DEI were not discernable as the multivariable model including these two variables was not overall significant ($p>0.30$).

Neither thigh circumference nor body fat percentage in the leg were associated with residuals of DEI and macronutrient intake in either men or women (all $p>0.6$). Furthermore, in women, the association between peak diameter of thigh adipocytes on residuals of DEI remained independent of leg fat mass percentage ($p=0.04$) and the ratio of leg fat mass to total body fat mass ($p=0.02$).

3.2.2 Abdominal Depot—The abdominal adipocyte size measurements (peak diameter, nadir, and percent small cells) were not associated with DEI, carbohydrate intake, fat intake or protein intake either in unadjusted analyses or after adjustment for their known determinants in either sex (all $p>0.7$).

3.3 Relationship between adipocyte size and metabolic chamber measurements

3.3.1 Metabolic fuel selection in the thigh—In men, thigh peak diameter was not associated with residual 24-h RQ ($p=0.2$, Fig. 5A), CARBOX ($p=0.1$, Fig. 5C), LIPOX ($p=0.2$, Fig. 5E), and PROTOX ($p=0.3$). Conversely, in women, peak diameter in the thigh was negatively associated with lower residual 24-h RQ ($\beta = -0.022$ per 10 μm -difference in peak diameter, CI: -0.04 to -0.001 , $p=0.04$, Fig 5B, Supplementary Table 2), lower non-protein RQ ($\beta = -0.027$ per 10 μm -difference in peak diameter, CI: -0.054 to -0.00029 , $p=0.02$), lower 24-h RQ sleep ($\beta = -0.031$ per 10 μm -difference in peak diameter, CI: -0.054 to -0.007 , $p=0.004$), lower residual 24-h CARBOX ($\beta = -15.3$ kcal/day, CI: -28.5 to -2.1 , $p=0.02$, Fig. 5D), and higher residual 24-h LIPOX ($\beta = +16.3$ kcal/day, CI: 0.56 to 32.0 , $p=0.04$, Fig. 5F). The association between peak diameter in the thigh and residual 24-h RQ was independent of leg fat mass percentage ($p=0.01$) and the ratio of leg fat mass to total body fat mass.

Furthermore, a positive association between adjusted 24-h RQ and DEI in women was observed ($r=0.47$, $p=0.04$). In a multivariable model including both 24-h RQ and peak diameter of the thigh, the peak diameter was inversely associated with residual daily energy intake ($\beta = -447.1$ per 10 μm -difference in peak diameter, CI: -845.9 to -48.2 , $p=0.03$) independent of 24-h RQ ($p=0.89$) which was no longer a determinant of residual daily energy intake. Neither nadir diameter nor percent small cell in the thigh region was associated with 24-h RQ, CARBOX, LIPOX, or PROTOX (all $p>0.08$).

3.3.2 Metabolic fuel selection in the abdomen—The abdominal adipocyte size measurements (peak diameter, nadir, and percent small cells) were not associated with 24-h RQ, CARBOX, LIPOX, and PROTOX in unadjusted analyses nor after adjustment for their known determinants in either sex (all $p>0.2$).

3.3.3 Energy expenditure—Adipocyte size measurements (peak diameter, nadir, or percent small cell size) were not associated with 24-h EE, SMR, or SPA in either sex or depot (all $p>0.07$).

4. Discussion

In the current study, we evaluated whether adipocyte size from two different subcutaneous depots (abdomen and thigh) were determinants of *ad libitum* daily energy and macronutrient intake in a cohort of 70 healthy adults. To our knowledge, this relationship has not been previously established. We identified a sexual dimorphism and a depot difference in the relationship between *ad libitum* food intake and adipocyte size. Adipocyte size (i.e. peak diameter of the large cell population) of the thigh in women was associated with reduced daily energy intake, carbohydrate intake, and protein intake independent of FM, FFM, and demographic variables. Adipocyte size from the abdomen of women or from both depots in men were not determinants of energy intake. Moreover, we found that in women, thigh adipocytes were larger than those from the abdomen, whereas there were no observed regional differences in men. We found that, in contrast to men, the thigh adipocyte size of women was associated with reduced 24-h RQ, reduced CARBOX, and greater LIPOX after

adjustment of known determinants. Furthermore, thigh adipocyte size in women was inversely associated with *ad libitum* energy intake independent of 24-h RQ.

The results indicate that energy intake is linked to signals from the thigh depot and emphasizes the putative differences in adipose tissue as a function of sex and depot. The findings may be related to the differences in adipose tissue between men and women. Body fat percentage, at the same BMI, is higher in women compared to men [31, 32]. Furthermore, women preferentially store more adipose tissue in the thigh and gluteal regions (i.e. gynoid fat distribution) whereas men accumulate adipose tissue in the central region (i.e. android fat distribution) [31, 33]. It has been proposed that the gynoid fat distribution has a specialized function in women, supported by evidence that gluteofemoral fat is selectively mobilized for pregnancy and lactation [34]. The gluteofemoral depot in women is the primary source for long chain polyunsaturated fatty acids (LC-PUFAs), such as omega-3 docosahexaenoic acid (DHA), which is vital for fetal/infant brain development [35]. Compared to abdominal adipose tissue, outer thigh fat has been shown to be enriched in essential LC-PUFAs [36]. With each cycle of pregnancy and lactation, gluteofemoral fat is either reduced in absolute terms in poorly nourished population or in relative terms in well-nourished populations [37]. In the current study, women with larger adipocytes in the thigh had reduced *ad libitum* DEI, indicating that brain centers involved in food intake regulation may be sensing the adequacy of these fat reserves from this depot through a negative feedback signal linked to size that is vital to female reproduction and breast-feeding. In contrast to previous reports [10–14], we found no association with circulating levels of adipokines (i.e. leptin and adiponectin) and adipocyte size. This suggests that there may be other appetite-regulating signals linked to adipocyte size. The independent effects of adipocyte size and adipokines on DEI were not able to be assessed due to limited sample size (n=18 women).

Similar to prior evidence [34], our study confirmed that adipocyte size in the thigh is greater than in the abdomen of women but was of similar size in men, further indicating that these depots may be differentially regulated in women. The sex- and depot-specific differences in adipose tissue appear to extend to whole-body metabolic fuel selection. Women with greater thigh adipocyte diameter had reduced 24-h RQ, greater LIPOX, and reduced CARBOX, indicating that the femoral depot influences substrate oxidation in women as opposed to the abdominal depot. Since it is known that 24-h RQ is positively associated with energy intake in humans [9, 38], this study also examined 24-h RQ and adipocyte size in the same statistical model and found that thigh adipocyte diameter in women was a determinant of *ad libitum* DEI independent of 24-h RQ.

A strength of the study is the application of the Coulter counter method which has the advantage of being able to measure a large number of cells (> 10,000) and allows for the quantification of the small adipocyte population not as apparent using other methods (e.g. collagenase digestion or histology) [39]. In addition, an objective measure of food intake was used in contrast to less accurate methods based on questionnaires or self-report [40].

Our study has several limitations. First, although the study included both sexes and more than one fat depot, the sample size was relatively small. Second, some individuals did not

have valid metabolic chamber measurements, reducing the sample size for the analysis of adipocyte size and metabolic fuel selection. Third, since the women were mostly obese, it is uncertain if these results are generalizable to lean women. Fourth, we did not have measurements of hormones in the hypothalamic-pituitary-gonadal axis that coordinate the female reproductive system (e.g. estradiol) which might affect the food intake regulation in women. This precludes assessment of how these reproductive hormones might influence the relationship between adipocyte size and energy intake [41, 42]. Lastly, we did not collect biomarkers related to fat oxidation which might have been helpful to further characterize the relationship of adipose tissue and metabolic fuel selection.

In summary, the thigh adipocyte size of healthy adult women was greater than the size in the abdomen, but size did not differ between the depots in men, indicating that this is privileged depot in women. Importantly, it was this depot (thigh) in women that was also found to be a determinant of *ad libitum* total energy intake and metabolic fuel selection (reduced 24-h RQ, higher 24-h LIPOX) independent of body composition. Moreover, thigh adipocyte size was inversely associated with DEI independent of 24-h RQ. These findings highlight sex- and depot-specific differences in adipose tissue and indicate a special role for thigh fat in women for energy-intake regulation.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Abbreviations:

24-h EE	24-hour energy expenditure
ENBAL	24-hour energy balance
24-h RQ	24-hour respiratory quotient
24-h CARBOX	24-hour carbohydrate oxidation
24-h LIPOX	24-hour lipid oxidation
24-h PROTOX	24-hour protein oxidation
DEI	daily energy intake
FFM	fat-free mass
FM	fat mass
SPA	spontaneous physical activity

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Study Importance Questions

What is already known about this subject?

- Adipose-derived hormones, linked to adipocyte size, influence energy intake.
- Osmium-fixation and high-throughput electronic sizing reveals a bimodal distribution of subcutaneous adipocyte size suggesting distinct large cell (mature) and small cell populations.
- Sex-dependent depot differences are important mediators of both adipocyte development and function.

What are the new findings in your manuscript?

- The diameter of the large adipocytes from the thigh of healthy adult women was greater compared to the abdomen.
- In women, large cell adipocyte diameter from the thigh was independently associated with reduced *ad libitum* daily energy intake and reduced 24-hour respiratory quotient.

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

- Thigh adipose tissue may be a privileged depot in women important for energy-intake regulation and warrants further investigation.
- The present study emphasizes the need for detailed adipocyte sizing methods in metabolic research.

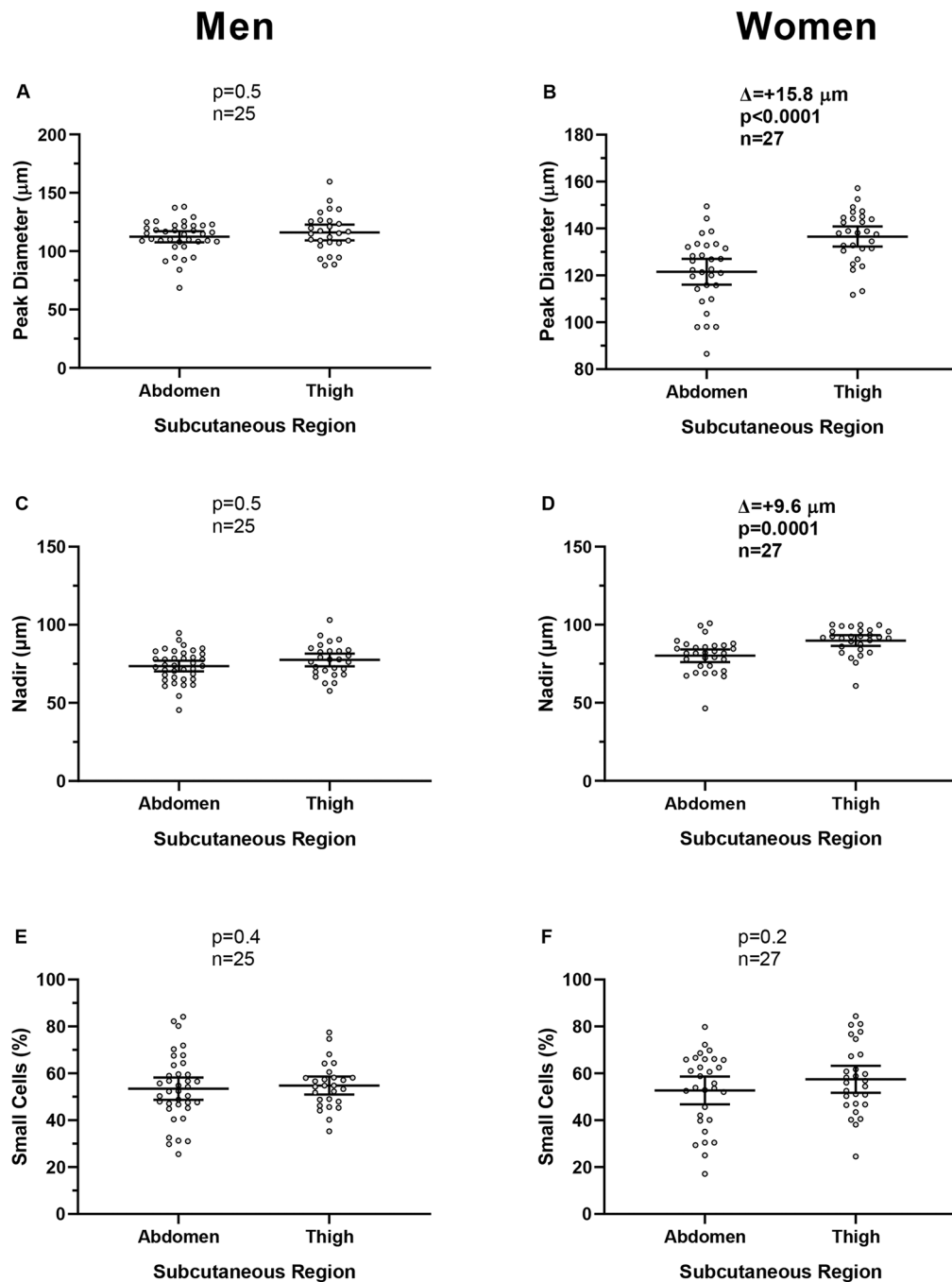


Fig. 1. Adipocyte size by sex

Panel A-C-E. Average difference () between abdominal and thigh regions for peak diameter (panel A), nadir diameter (panel C), and percent small cells (panel E) in men.

Panel B-D-F. Average difference () between abdominal and thigh regions for peak diameter (panel B), nadir diameter (panel D) and percent small cells (panel F) in women.

Paired *t*-test was used to assess differences between regions.

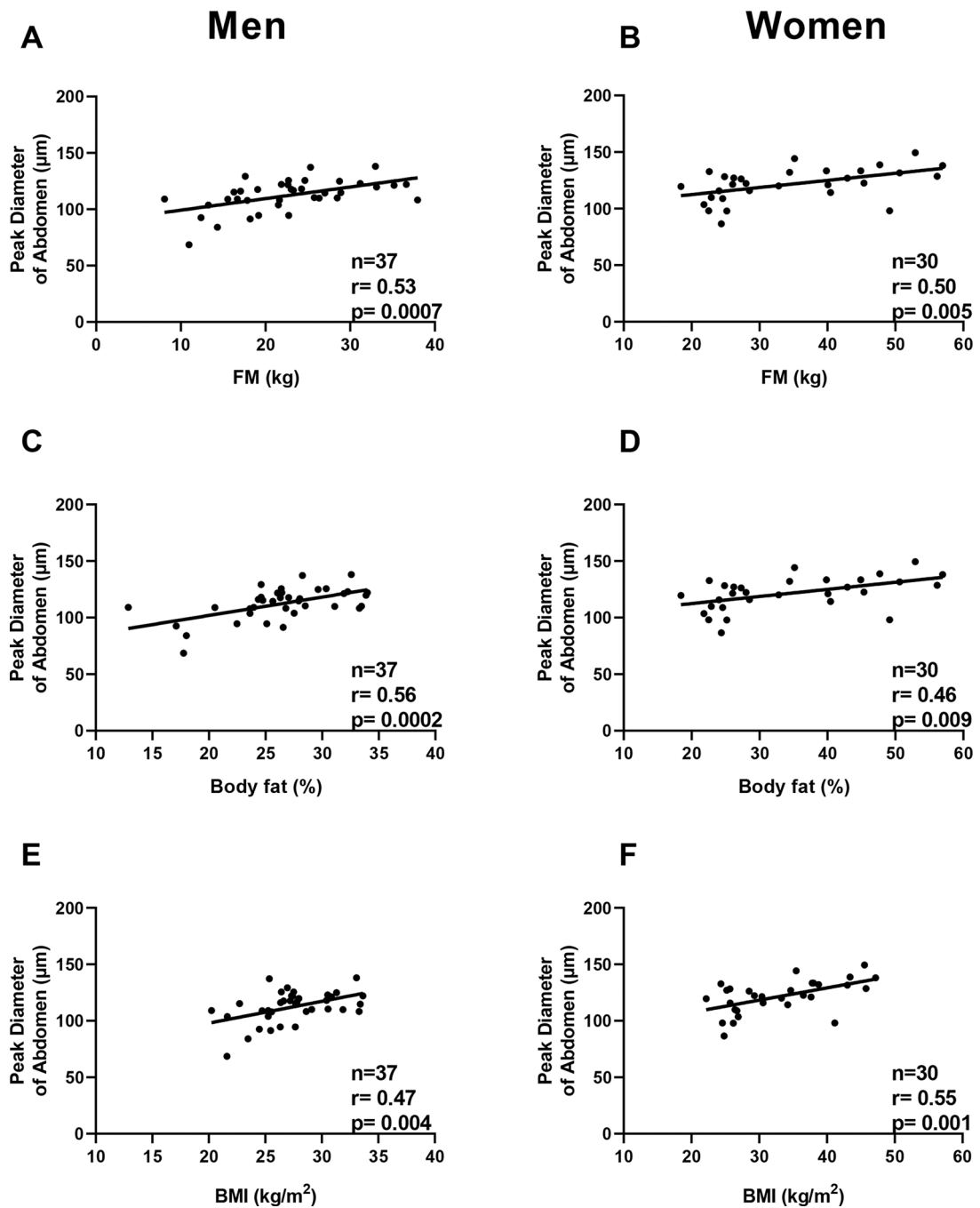


Fig. 2. Relationships between peak diameter of adipocytes (abdomen) and adiposity measures in men and women.

Panel A-C-E. Relationships between peak diameter of adipocyte size in the abdomen and fat mass (panel A), body fat percentage (panel C), and body mass index (panel E) in men.

Panel B-D-F. Relationships between peak diameter of the thigh adipocyte size and fat mass (panel B), body fat percentage (panel D), and body mass index (panel F) in women.

Pearson's correlation coefficient (r) is reported along with its significance (p).

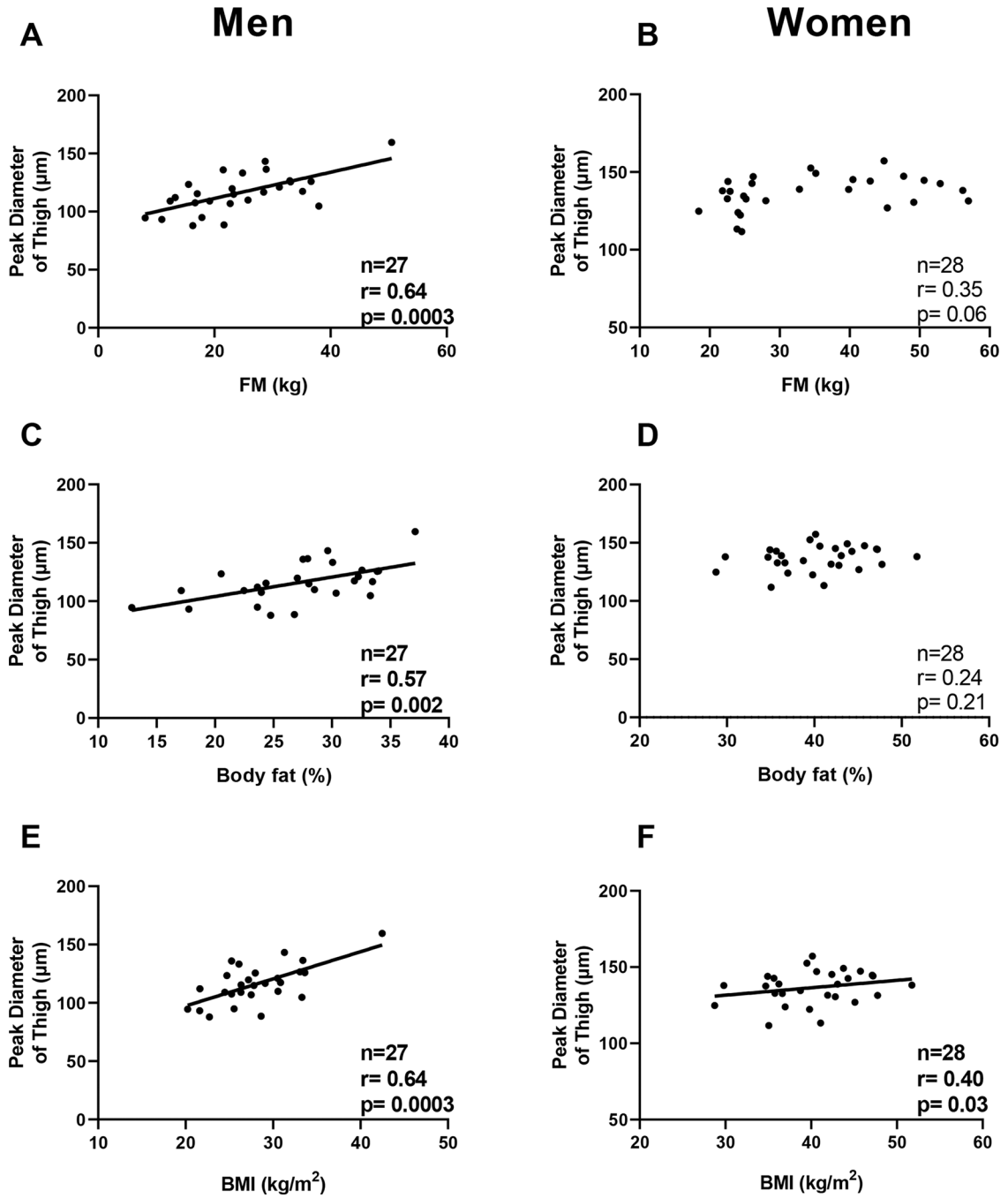


Fig. 3. Relationships between peak diameter of adipocytes (thigh) and adiposity measures in men and women

Panel A-C-E. Relationships between peak diameter of adipocyte size in the thigh and fat mass (panel A), body fat percentage (panel C), and body mass index (panel E) in men. **Panel B-D-F.** Relationships between peak diameter of the thigh adipocyte size and fat mass (panel B), body fat percentage (panel D), and body mass index (panel F) in women. Pearson’s correlation coefficient (r) is reported along with its significance (p).

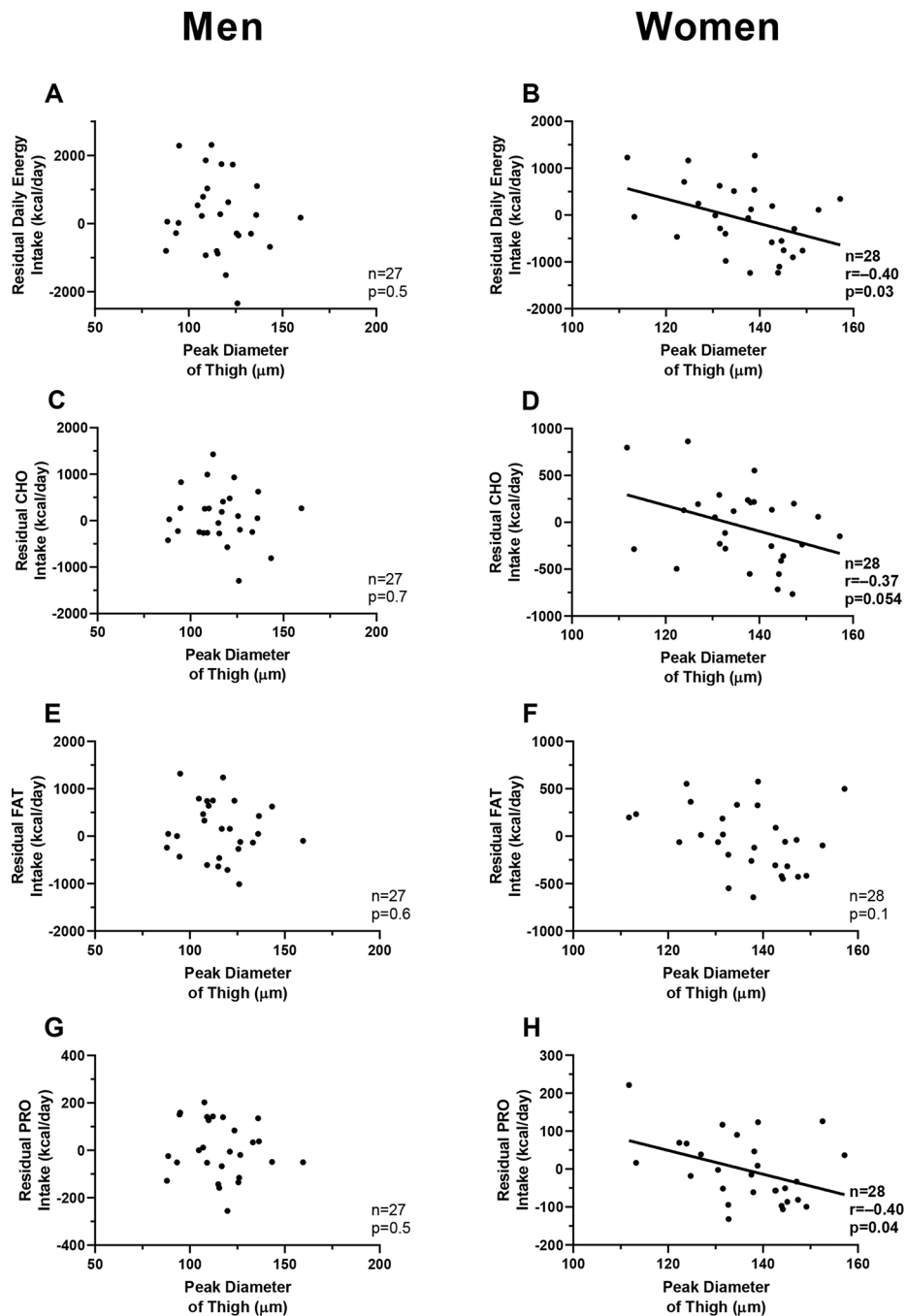


Fig. 4. Relationships between peak diameter of thigh adipocytes and *ad libitum* food intake, by sex

Panel A-C-E-G. Relationships between peak diameter of the thigh adipocytes and residuals of daily energy intake (**panel A**), carbohydrate intake (**panel C**), fat intake (**panel E**), and protein intake (**panel G**) in men.

Panel B-D-F-H. Relationships between peak diameter of the thigh adipocytes and residuals of daily energy intake (**panel B**), carbohydrate intake (**panel D**), fat intake (**panel F**) and protein intake (**panel H**) in women.

Pearson's correlation coefficient (r) is reported along with its significance (p). Residuals of food intake were calculated using linear regression adjusting for age, race, fat mass, and fat-free mass.

Linear regression analysis was used to calculate residuals of CARBOX and LIPOX after adjustment for age, sex, race, fat mass, and fat-free mass.

RQ: respiratory quotient; CARBOX: carbohydrate oxidation; LIPOX: lipid oxidation

The results in panels 4B, 4D, and 4H were not significant when considering a Bonferroni-corrected threshold for significance.

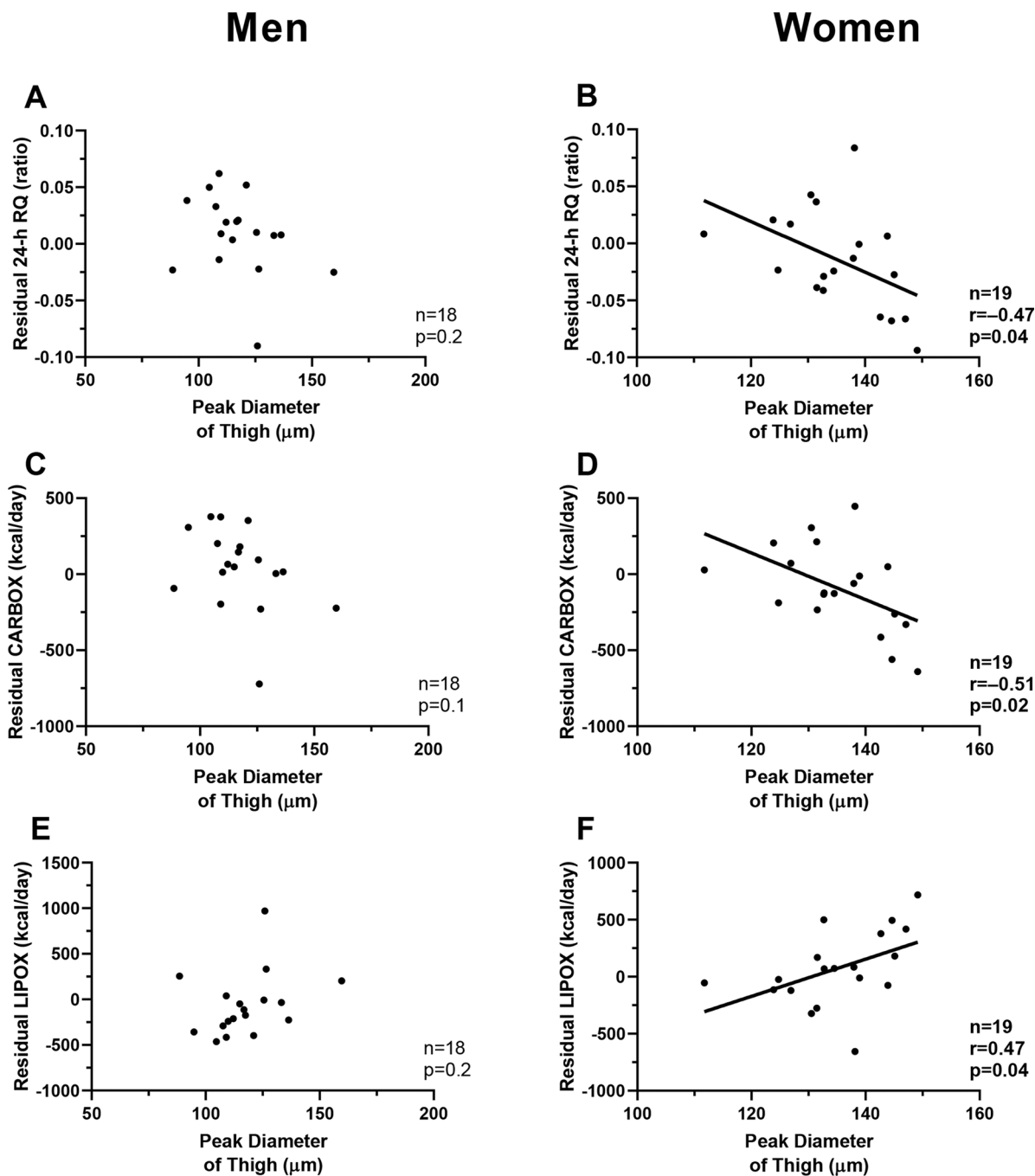


Fig. 5. Relationships between peak diameter of thigh adipocytes and metabolic fuel selection near energy balance, by sex

Panel A-C-E. Relationships between peak diameter of the thigh adipocytes and residuals of 24-h respiratory quotient (**panel A**), carbohydrate oxidation (**panel C**), and lipid oxidation (**panel E**) in men.

Panel B-D-F. Relationships between peak diameter of the thigh adipocytes and residuals of 24-h respiratory quotient (**panel B**), carbohydrate oxidation (**panel D**), and lipid oxidation (**panel F**) in women.

Pearson's correlation coefficient (r) is reported along with its significance (p). Linear regression analysis was used to calculate residuals of 24-h RQ after adjustment for age, race, body fat percentage and energy balance. Linear regression analysis was used to calculate residuals of CARBOX and LIPOX after adjustment for age, race, fat mass, and fat-free mass. RQ: respiratory quotient; CARBOX: carbohydrate oxidation; LIPOX: lipid oxidation. The results in panels 5B, 5D, and 5F were not significant when considering a Bonferroni-corrected threshold for significance.

Table 1.

Demographic and anthropometric characteristics of the study cohort.

	Whole group (n=70)	Men (n=39)	Women (n=31)	p value
Age (years)	39.0 ± 11.9	41.8 ± 11.8	35.5 ± 11.3	0.03
Race	32 NA, 38 O	13 NA, 26 O	19 NA, 12 O	0.02
Height (cm)	168.2 ± 10.8	175.2 ± 8.3	159.4 ± 6.0	<0.0001
Body weight (kg)	84.7 ± 17.8	85.7 ± 16.4	83.3 ± 19.5	0.58
BMI (kg/m ²)	30.0 ± 6.3	27.8 ± 4.1	32.8 ± 7.4	0.0007
FM (kg)	28.3 ± 11.2	23.5 ± 8.4	34.2 ± 11.7	<0.0001
FFM (kg)	56.4 ± 11.1	62.2 ± 9.1	49.1 ± 8.9	<0.0001
Body fat (%)	32.7 ± 8.5	26.8 ± 5.1	40.2 ± 5.3	<0.0001
Trunk fat (%)	31.7 ± 9.9	24.1 ± 5.2	40.9 ± 5.2	<0.0001
Leg fat (%)	36.3 ± 8.2	31.3 ± 6.2	42.5 ± 6.0	<0.0001
Waist circumference (cm)	100.6 ± 15.5	97.0 ± 11.9	104.9 ± 18.5	0.04
Thigh circumference (cm)	62.7 ± 8.1	59.9 ± 6.1	66.0 ± 8.9	0.001
Waist-thigh-ratio	1.6 ± 0.20	1.6 ± 0.16	1.6 ± 0.24	0.62
Fasting glucose (mg/dL)	92.8 ± 6.2	93.6 ± 6.7	91.8 ± 5.3	0.25
2-h glucose (mg/dL)	128.4 ± 27.2	126.1 ± 28.5	131.2 ± 25.6	0.44
Leptin (ng/mL) ^a	19.9 ± 20.5	7.1 ± 6.2	36.1 ± 20.9	<0.0001
Adiponectin (µg/mL) ^b	5.3 ± 4.1	5.8 ± 5.2	4.8 ± 1.9	<0.0001

Data are presented as mean ± standard deviation.

NA: Native American; O: Other; BMI: Body mass index; FM: Fat mass; FFM: Fat free mass

Difference between men and women determined by unpaired *t*-test or χ^2 test as appropriate.^{a,b}Leptin and adiponectin measurements were available in 28 men had 22 in women.

ClinicalTrials.gov identifier: NCT00342732

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Table 2.

Measurements of adipocyte size

	Whole group n=67	Men n=37	Women n=30	p value
Abdomen				
Peak diameter, (µm)	116.5 ± 15.0	112.3 ± 14.1	121.6 ± 14.7	0.01
Nadir diameter, (µm)	76.5 ± 11.0	73.6 ± 10.3	80.1 ± 11.0	0.02
Small cells, (%)	53.1 ± 14.9	53.4 ± 14.3	52.7 ± 15.9	0.80
Thigh				
Peak diameter, (µm)	126.5 ± 17.5	116.1 ± 17.1	136.6 ± 11.0	<0.0001
Nadir diameter, (µm)	83.8 ± 11.4	77.5 ± 10.4	89.8 ± 8.8	<0.0001
Small cells, (%)	56.1 ± 12.5	54.8 ± 9.7	57.5 ± 14.8	0.40

Data are presented as mean ± standard deviation.

Difference between men and women determined by unpaired *t*-test.

Table 3.

Ad Libitum food intake and metabolic chamber measurements.

	Whole study group		Men	Women	p value
Vending machine system					
Daily energy intake (kcal/day) ^a	3229.4 ± 1103.8	3723.0 ± 1078.0	2608.4 ± 784.3	<0.0001	
WMEN (kcal/day)	2459.2 ± 862.9	2829.7 ± 869.6	1993.0 ± 592.9	<0.0001	
Daily energy intake (% WMEN)	125.4 ± 38.7	139.7 ± 39.8	107.4 ± 29.2	0.002	
CHO intake (kcal/day)	1700.0 ± 585.2	1950.9 ± 580.5	1384.4 ± 419.6	<0.0001	
FAT intake (kcal/day)	1180.7 ± 512.8	1366.0 ± 541.6	947.5 ± 363.9	0.0004	
PRO intake (kcal/day)	379.2 ± 146.2	442.7 ± 143.1	299.6 ± 106.8	<0.0001	
Metabolic chamber measures					
Energy balance (kcal/day)	139.8 ± 194.0	172.1 ± 220.8	91.25 ± 135.1	0.11	
EE in energy balance (kcal/day)	2049.9 ± 341.2	2174.6 ± 338.6	1862.92 ± 251.63	0.0003	
Sleeping metabolic rate (kcal/day)	1589.7 ± 257.4	1680.0 ± 247.4	1454.1 ± 212.0	0.0005	
RQ in energy balance (ratio)	0.86 ± 0.05	0.87 ± 0.04	0.85 ± 0.06	0.08	
Non-protein RQ (ratio)	0.88 ± 0.07	0.89 ± 0.05	0.86 ± 0.08	0.07	
RQ Sleep (ratio)	0.82 ± 0.06	0.83 ± 0.04	0.80 ± 0.08	0.043	
CARBOX, (kcal/day)	999.0 ± 386.3	1118.5 ± 308.1	819.8 ± 427.3	0.003	
LIPOX (kcal/day)	663.8 ± 425.7	631.7 ± 405.3	712.1 ± 459.2	0.48	
PROTOX (kcal/day)	361.5 ± 125.5	396.5 ± 132.0	309.1 ± 95.4	0.007	
SPA (%)	6.7 ± 4.0	7.0 ± 4.1	6.2 ± 3.9	0.48	

Data are presented as mean ± standard deviation.

Difference between men and women determined by unpaired *t*-test.

^a *Ad Libitum* food intake measures are reported as the average of three days on the vending machines.

WMEN: weight maintaining energy needs; CHO: carbohydrate; PRO: protein; EE: energy expenditure; RQ: respiratory quotient;

CARBOX: carbohydrate oxidation; LIPOX: lipid oxidation; PROTOX: protein oxidation; SPA: spontaneous physical activity