Impact of Age on the Relationships of Brown Adipose Tissue with Sex and Adiposity in Humans

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**Objective:** Brown adipose tissue (BAT) regulates energy homeostasis and fat mass in mammals and newborns and, most probably, in adult humans. Because BAT activity and BAT mass decline with age in humans, the impact of BAT on adiposity may decrease with aging. In the present study we addressed this hypothesis, and further investigated the effect of age on the sex differences in BAT activity and BAT mass.

**Materials and Methods:** Data from 260 subjects (98 with BAT and 162 study date-matched controls) who underwent $^{18}$F-fluorodeoxyglucose positron-emission tomography/ computed tomography ($^{18}$F-FDG PET/CT) under thermoneutral conditions were analyzed. BAT activity and BAT mass were determined in the upper body.

**Results:** BAT activity and BAT mass were higher in females (1.59±0.10 and 32±5 g vs 1.02±0.10 and 18±4 g, both $p \leq 0.0006$) compared to males. In multivariate analyses, sex ($p<0.0001$), age ($p<0.0001$) and BMI ($p=0.0018$) associated independently with BAT activity. Interestingly, only in males there was an interaction between BMI and age in determining BAT activity ($p=0.008$) and BAT mass ($p=0.0002$); BMI decreased with increasing BAT activity and BAT mass in the lowest ($r_s=-0.38$, $p=0.015$ and $r_s=-0.37$, $p=0.017$, respectively) but not in the higher age tertiles. Furthermore, BAT activity and mass differed between females and males only in the upper two age tertiles (all $p \leq 0.09$).

**Conclusions:** Our data corroborate that, in general, BAT activity and BAT mass are elevated in females and in younger people. Importantly, we provide novel evidence that only in males the impact of BAT activity and BAT mass on adiposity appears to decline with aging. Furthermore, while with increasing age BAT activity and BAT mass merely moderately decline in females, a much stronger effect is found in males.
Brown adipose tissue (BAT) is involved in the dissipation of chemical energy as heat, thereby, maintaining core temperature in small mammals and in newborn humans, particularly during cold exposure without shivering (1). This cold-, but also diet-induced heat production, is considered as a target in the prevention and treatment of obesity (2-4). However, because BAT mass and BAT activity in humans, e.g. in contrast to rodents, is rapidly lost within the first years of life, it was thought for a long time that it has only a minor impact on energy homeostasis after infancy. In 2007 Nedergaard, Bengtsson and Cannon (5) summarized and reviewed several studies showing that BAT is present to a considerable amount in adult humans, that human BAT activity is acutely cold induced, and that human BAT is stimulated via the sympathetic nervous system. Importantly, in 2009 areas identified as possible BAT in human scans, were found to truly represent BAT, as they were positive for the presence of BAT-unique UCP1 expression (6). In the same year studies using the combined $^{18}$F-fluorodeoxyglucose positron-emission-tomography/computed tomography (FDG-PET/CT) technology provided strong and convincing evidence for a major role of BAT in the regulation of body weight and energy homeostasis in adult humans (7-14). Thus, the presence of BAT may help explaining the large interindividual differences in energy efficiency in regard to weight gain in humans (15-17).

Several points have now emerged concerning the regulation of BAT mass and BAT activity and their relationships with age, sex, adiposity and glucose and lipid metabolism in humans. Among them, is the question whether BAT mass and BAT activity constantly declines during childhood, adolescence and adulthood? While BAT mass was found to strongly decline with age in some of the recent (7,12) as well as in our study (11), a weaker trend was found in another study (13) and no decline with increasing age was reported in the study by van Marken et al. (9). Furthermore, it is unclear whether such a decline in BAT mass and BAT activity may help explain the observed overall increase in adiposity with aging. In addition, it is unknown, which factors are responsible for the often observed higher BAT activity in females compared to males of similar age, and whether age affects this relationship.

**MATERIAL AND METHODS**

**Patients.** A total of 3604 patients underwent 5776 consecutive $^{18}$F-FDG-PET/CT examinations between January 2005 and July 2009 at the Department of Radiology of the University of Tübingen, Germany, for a variety of diagnostic reasons. In 110 scans BAT was documented in the radiologist’s report. In a previous analysis a total of 198 scans from patients without documented BAT, who had a PET/CT examination on the same day, were matched to these scans (11). For analyses with continuous parameters, only one PET/CT scan from each patient was included in the present evaluation, resulting in a total of 260 scans. The study was approved by the ethics committee of the University of Tübingen.

**PET/CT Imaging.** Most patients used a car, and few used public transportation to get to the Tübingen University Hospital. From the time of entry in the hospital until the PET/CT measurement patients spent about 2 hours in an air-conditioned environment at about 22°C. Patients fasted overnight for at least 6 hours. All patients underwent PET/CT measurements on the Hi-Rez Biograph 16 (Siemens Medical Solutions, Knoxville, TN), consisting of a high-resolution three-dimensional LSO PET and a 16-row multidetector CT.

**Image Analysis.** FDG distribution was evaluated visually and semiquantitatively using average and maximum standard uptake values ($\text{SUV}_{\text{mean}}$, $\text{SUV}_{\text{max}}$). BAT was suspected if 1) focal FDG uptake exceeded normal regional tracer accumulation and exceeded $\text{SUV}_{\text{max}}$. 
values of 2.0 in 2) tissue with a density between -250 and -50 Hounsfield Units (HU) and fat-like appearance in CT, and in 3) characteristic localization bilaterally in the neck, supra- and infraclavicular regions and paravertebrally (lobster sign), unrelated to muscle, joints, and pathologic findings (18). For the assessment of BAT volume and activity, we used three-dimensional (3D) isocountour regions of interest (ROIs) with a SUV isocountour threshold of 2.0 (7,18). In patients without BAT (matched controls), ellipsoid 3D ROIs were manually placed over nuchal adipose tissue.

In the present study, mean SUVs were automatically derived from a large 3D ROI comprising the whole head and neck region as previously described (7,8,18) (supplemental figure A, B in the online appendix available at http://diabetes.diabetesjournals.org), and are referred to as ‘single’ ROI measurements (SUVsing). In addition, each manifestation of BAT was individually assessed resulting in multiple 3D isocountour ROIs (SUVmult), without overlap (supplemental figure C, D). Outdoor temperature of the study dates in the city of Tübingen was obtained from the local weather station.

**Statistical analyses.** Differences between males and females were determined using the non-parametric Wilcoxon rank sum test. Data that were not normally distributed (Shapiro-Wilk W test) were logarithmically transformed to approximate a normal distribution. Patients with documented BAT activity in the radiologist’s report were matched to patients without BAT. This resulted in that skewed distributions for BAT activity and BAT mass were apparent even after logarithmical transformation of the data. Therefore, for correlation analyses involving BAT mass and BAT activity, Spearman’s rank correlation coefficient ($r_s$) was calculated. To adjust the effects of covariates and identify independent relationships, we performed multiple regression analyses.

**RESULTS**
Age, anthropometrics and metabolic characteristics of the patients covered a wide range (table 1). First we tested whether outdoor temperature was associated with BAT activity. No significant relationship was found with BAT activity determined as SUVsing ($r_s$=-0.05, $p=0.43$), or SUVmult ($r_s$=-0.04, $p=0.49$). Thus, no correction for outdoor temperature was necessary for the following analyses. Next we tested the relationships of BAT activity determined as SUVsing, or SUVmult with each other and with age, sex and BMI. The BAT activity measures were closely correlated with each other ($r_s$=0.73, $p<0.0001$). However, SUVmult consistently correlated stronger with age, sex and BMI than SUVsing (data not shown), suggesting that BAT activity individually assessed with multiple 3D isocountour ROIs may be superior to the single ROI measurements. Therefore, for the further analyses, SUVmult was used as an estimate of BAT activity.

Next we found that both, BAT activity (1.59±0.10 vs 1.02±0.10, $p=0.0003$) and BAT mass (32±5 g vs 18±4 g, $p=0.0006$) were higher in females compared to males. In univariate analyses, age and BMI correlated negatively with BAT activity ($r_s$=-0.44, $p<0.0001$ and $r_s$=-0.33, $p<0.0001$) and with BAT mass ($r_s$=-0.34, $p<0.0001$ and $r_s$=-0.21, $p=0.0006$). In multivariate analyses (table 2), sex ($p<0.0001$), age ($p<0.0001$) and BMI ($p=0.0018$) remained independently associated with BAT activity. Similar relationships, although weaker, were found for BAT mass with sex ($p=0.0002$) and age ($p<0.0001$), however, not for BAT mass with BMI ($p=0.37$).

Because age turned out to be the strongest determinant of BAT activity and BAT mass, we studied whether the relationships between BAT activity and BAT mass with BMI and sex are similar among different age categories. Interestingly, there was an interaction between age and BMI in determining BAT activity ($p=0.008$) and BAT mass ($p=0.0002$) in males, but not in females ($p=0.41$ and $p=0.61$). When
males were divided into tertiles, BMI decreased with increasing BAT activity and BAT mass only in the lower age tertile (figure 1, panels A and B, $r_s=-0.38, p=0.015$ and $r_s=-0.37, p=0.017$), but not in the upper two tertiles (figure 1, panels C-F).

Finally, we tested whether age also impacts on the observed differences in BAT activity and BAT mass between males and females. Interestingly, BAT mass and BAT activity were not different in subjects in the lowest age tertile (figure 2, panels A and B), however, BAT mass and BAT activity were higher in females compared to males in the 2nd and 3rd age tertiles (figure 2, panels C-F).

DISCUSSION

In the present study we addressed the questions, whether BAT activity has similarly strong impacts on the development of adiposity in younger and older people, and whether the observed sex differences in BAT activity and BAT mass are apparent among several age categories. First we determined BAT activity on $^{18}$F-FDG-PET/CT scans, either applying the widely used single ROI measurement, or by using multiple 3D isocontour ROIs to assess BAT activity at each manifestation, individually. We found that these measurements were highly correlated with each other. Furthermore, BAT activity estimated from the multiple ROIs turned out to be stronger associated with age, sex and BMI, indicating that this method may be the more accurate one.

Next we confirmed the findings of most studies that BAT activity is higher in females. These findings indicate that yet unknown parameters in humans, possibly sex hormones, may be involved in the regulation of BAT function. A sexual dimorphism in BAT activity and in UCP-1 expression (higher levels in females at 22°C) was found in rats (19).

However, in that study the steroid hormones, beta-estradiol, estrone and progesterone, did not increase but actually reduced norepinephrine-induced UCP-1 synthesis in brown adipocytes which were differentiated in primary culture. In another study, in rodent brown adipocytes differentiated in culture, testosterone treatment dose-dependently inhibited norepinephrine-induced UCP-1 mRNA expression, while 17-beta-estradiol did not have any remarkable effect (20). In addition, because sex hormones modulate 5'-iodothyronine deiodinase activity (21), which is responsible for enzymatic conversion of thyroxine into the bioactive form, triiodothyronine, which regulates the thermogenic function of BAT (22), indirect effects of sex steroids may be involved in the regulation of BAT activity. Because in our study a large decline in BAT activity was observed in 43 to 56 year old males, who have declining testosterone levels, it would be important to specifically study the effect of testosterone levels and/or testosterone supplementation on BAT activity.

We then found strong negative univariate correlations of BAT activity both, with age and BMI. In multivariate analyses BAT activity was independently associated with age, sex and BMI. This supports that, in general, BAT activity is lower in males, declines with aging and most probably, has direct effects on the regulation of adiposity.

Next we investigated whether the relationship of BAT activity with BMI is similarly strong among different age groups. We can provide novel data that, only in younger males, a significant negative relationship between BAT activity and BMI can be found. This suggests that in older males BAT is not an important regulator of adiposity, anymore. In contrast, in females similarly strong negative relationships of BAT activity with BMI were found among all studied age groups. An interaction of BAT with age in determining BMI was found in the study by Cypess et al. (7). In contrast to our data, in that study the relationship between the presence of BAT and BMI became stronger with increasing age. Differ-
ences between both studies may be explained by the fact that males and females were analysed together in the study by Cypess et al., differences in the age of the studied populations and differences in the methodology used for detection of BAT.

Finally we investigated whether age impacts on the observed differences in BAT activity between males and females. Interestingly, in the lowest age tertile these differences were not apparent. However, while in both, males and females, BAT activity declined with aging, this process appears to be accelerated in males. Thus, a significant higher BAT activity in females can be found, particularly in older people.

Limitations of our study are that our measurements of BAT activity were not done under cold exposure, which further increases BAT activity, and thus, may allow to more precisely determining BAT activity and BAT mass. Nevertheless, because such studies with a large number of subjects are not available yet, findings from our and other studies may help to stimulate and focus such research in this important field in the future.

In conclusion, we provide novel data that only in males the impact of BAT activity on adiposity appears to decline with aging. In addition, while with increasing age BAT activity only moderately declines in females, a much stronger effect is found in males. If confirmed by other studies, these findings may considerably impact on the future research about the role of BAT in humans.

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**Table 1: Subject characteristics**

| Characteristics          | Mean ± SEM | Range   |
|--------------------------|------------|---------|
| Sex (Females/Males)      | 136 / 124  |         |
| Age (years)              | 48 ± 1     | 11 - 82 |
| Height (cm)              | 170 ± 1    | 112 - 200 |
| Weight (kg)              | 71.3 ± 1.0 | 35.0 - 137.0 |
| BMI (kg·m⁻²)             | 24.5 ± 0.3 | 15.5 - 40.8 |
| Fasting glucose (mM)     | 5.17 ± 0.06| 2.50 - 10.22 |
| BAT activity             | 1.32 ± 0.07| 0.16 - 3.84 |
| BAT mass (g)             | 25.24 ± 3.20| 0.02 - 287.90 |

BAT, brown adipose tissue

**Table 2. Relationships of BAT activity and BAT mass with sex, age and BMI in multivariate linear regression analyses.**

| Parameter   | Estimate | SE   | p      |
|-------------|----------|------|--------|
| BAT activity|          |      |        |
| Intercept   | 3.66     | 0.81 | <0.0001|
| Male sex    | -0.20    | 0.05 | <0.0001|
| Age         | -0.02    | 0.05 | <0.0001|
| BMI         | -0.85    | 0.27 | 0.0018 |

| BAT mass    |          |      |        |
|-------------|----------|------|--------|
| Intercept   | 5.96     | 2.61 | 0.023  |
| Male sex    | -0.56    | 0.15 | 0.0002 |
| Age         | -0.06    | 0.01 | <0.0001|
| BMI         | -0.77    | 0.86 | 0.37   |

BAT, brown adipose tissue; SUV, standardized uptake value; intercept, it sets the ‘baseline’ event rate, when all covariate values are set equal to zero; estimates, they are the coefficients of the linear model found by least squares; SE, is the standard error, an estimate of the standard deviation of the distribution of the parameter estimate, p, probabilities less than 0.05 are often considered as significant evidence that the parameter is not zero
Figure legends

Figure 1. Relationships of body mass index (BMI) with brown adipose tissue (BAT) activity and BAT mass in males divided into tertiles by age (Spearman's rank correlation) (1st, 2nd, 3rd age tertiles, N=83/90/87).

Figure 2. Differences between sex in brown adipose tissue (BAT) activity and BAT mass (Wilcoxon rank sum test) (1st, 2nd, 3rd age tertiles, N=males (females), 40 (43)/41(49)/43 (44).
**Figure 2**

**1st Age Tertile**  
(11 – 43 years)

**A**  
BAT activity

- Males
- Females

**B**  
BAT mass (g)

- Males
- Females

**2nd Age Tertile**  
(43 – 56 years)

**C**  
BAT activity

- Males
- Females

**D**  
BAT mass (g)

- Males
- Females

**3rd Age Tertile**  
(56 – 82 years)

**E**  
BAT activity

- Males
- Females

**F**  
BAT mass (g)

- Males
- Females

- p=0.96
- p=0.75
- p=0.0002
- p=0.0002
- p=0.015
- p=0.09