Differences in Sympathetic Nervous Stimulation of Brown Adipose Tissue between the young and old, and the lean and obese.

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Running Title: Sympathetic activation and BAT.
ABSTRACT

Brown adipose tissue (BAT) could facilitate weight loss by increasing energy expenditure. Cold is a potent stimulator of BAT, activating BAT primarily through the sympathetic nervous system (SNS). Older or overweight individuals have less metabolic BAT activity than the lean and young, but the role of the SNS in this decline is unknown. We aimed to determine whether this lower metabolic BAT activity in older or overweight individuals can be explained by a lower SNS response to cold.

Methods: This was a prospective observational study. We included 10 young obese, 11 old lean and 14 young lean healthy males. All subjects underwent a 18F-Fluorodeoxyglucose (18F-FDG) PET-CT and a 123I-meta-iodobenzylguanidine (123I-mIBG) SPECT-CT after an overnight fast and two hours of cold exposure. Metabolic BAT activity was expressed as volume and as maximal standardised uptake value (SUV\text{max}) of 18F-FDG. BAT SNS activity was expressed as volume and as the ratio between 123I-mIBG uptake in BAT and a reference region (SQUV\text{max} of 123I-mIBG).

Results: SUV\text{max}, BAT volume and SQUV\text{max} were significantly different between young and old (SUV\text{max} 7.9[4.2-17.3] vs. 2.9[0.0-4.0], volume 124.8[10.9-338.8] vs 3.4 [0.0-10.9] and SQUV\text{max} 2.7[1.9-4.7] vs 0.0[0.0-2.2] all p<0.01) but not between lean and obese (SUV\text{max} 7.9[4.2-17.3] vs 4.0[0.0-13.5] p=0.69; volume 124.8[10.9-338.8] vs 11.8 [0.0-190.2] p=0.64 and SQUV\text{max} 2.7[1.9-4.7]vs 1.7[0-3.5] p=0.69). We found a strong positive correlation between BAT activity measured with 18F-FDG and 123I-mIBG in the whole group of BAT positive subjects (p=0.82, p<0.01).
Conclusion: We conclude that both sympathetic drive and BAT activity are lower in older but not in obese males.

Key words: Brown adipose tissue, $^{18}$F-FDG PET-CT, $^{123}$I-mIBG SPECT-CT, age, obesity
INTRODUCTION

The increasing prevalence of obesity in the young heralds a period where we will be confronted with an older and obese population with an abundance of adverse consequences and associated health costs.\(^{(1,2)}\) So far most solutions for this problem have been unsatisfactory since attempts to lose weight fail in most cases.\(^{(3,4)}\)

Metabolically active brown adipose tissue (BAT) increases energy expenditure (EE) and could play a role in the battle against obesity by facilitating weight loss.\(^{(5,6)}\) If we could identify factors that contribute to stimulation or recruitment of metabolically active BAT, it would be possible to use BAT as a means to lose weight. Though cold exposure is the strongest activator of BAT known so far,\(^{(7)}\) people will not be exposed to cold for most of the day. Factors activating BAT during thermoneutrality have to be found. An important target in this respect is the sympathetic nervous system (SNS), which is thought to be the primary activator of BAT.\(^{(8)}\)

In both older and/or overweight people, metabolic activity of BAT is generally found to be much lower compared to younger and/or leaner controls.\(^{(9-12)}\) The reason for this decreased metabolic BAT activity remains unknown and the role of the SNS in BAT activation in the older and the obese has not been studied.

The common method to demonstrate metabolically active BAT is by performing \(^{18}\text{F-Fluorodeoxyglucose}\) (FDG) Positron Emission Tomography (PET)-CT. Recently, our group showed that the sympathetic nervous stimulation of BAT can be visualised by \(^{123}\text{I-meta-iodobenzylguanidine}\) (\(^{123}\text{I-mIBG}\)) Single Photon Emission
Computed Tomography (SPECT). We showed a strong, positive correlation between $^{18}$F-FDG and $^{123}$I-mIBG in BAT in lean young males.

We hypothesized that SNS activation is diminished in both older and obese subjects as compared to young, lean subjects as a cause for the diminished BAT activity. Therefore we investigated whether SNS activation of BAT ($^{123}$I-mIBG) as a mediator of BAT activity ($^{18}$F-FDG) is diminished in young obese or lean older males when compared to the lean, young. In addition, we investigated whether the strong correlation between $^{18}$F-FDG and $^{123}$I-mIBG in lean men is also present in the obese and the older males. Finally, we investigated whether energy expenditure correlated with BAT activity.
MATERIALS AND METHODS

This study was approved by the Medical Ethics Committee of the Academic Medical Center of the University of Amsterdam (Amsterdam, The Netherlands) and was conducted according to the Declaration of Helsinki. Written informed consent was obtained from all subjects after oral and written explanation of the study procedures. The registration numbers of this article are NCT02173834 and NCT02130154 (www.clinicaltrials.gov).

Participants

This study consists of two separate protocols which we have combined into one manuscript. Power calculations were performed using nQuery Advisor© and they were based on previous studies. (J3) Assuming an alpha of 5%, 10 subjects per group were needed to be included in the study in order to obtain a power of 80% to detect a significant difference between the young obese and young lean subjects. To detect a significant difference between the young lean and old lean subjects, assuming an alpha of 5%, 11 subjects per group were needed to be included in the study in order to obtain a power of 80% to detect a significant difference between the young lean and old lean subjects.

We decided to combine the two studies after inclusion of 14 young lean subjects. So in all, we included a group of 35 healthy, Caucasian male volunteers. We studied 10 young obese (inclusion criteria 18-30 years, BMI >28 kg/m²; later referred to as the obese), 11 old lean (inclusion criteria >40 years, BMI 19-25 kg/m²; later referred to as
the older males) and 14 lean young (inclusion criteria 18-30 years, BMI 19-25 kg/m²; later referred to as lean young) males. Subjects were included when they met the age and BMI criteria of the specified group and did not use any medication or drugs. Baseline characteristics were obtained during the first visit. Anthropometric data were measured with the subjects wearing only underwear. Weight was measured on the same calibrated mechanical scale (SECA, Germany) to the nearest 100 g, height and waist circumference were recorded to the nearest 0.01 m.

18F-FDG PET CT and 123I-mIBG SPECT-CT imaging protocol

18F-FDG PET-CT and 123I-mIBG SPECT-CT were performed on 2 separate days within an interval of 2 weeks between April 2013 and March 2014 according to the previously established protocol.(14) In short, all subjects arrived in the morning after an overnight fast, after which they were exposed to mild cold (~16-17 °C) in an air-cooled room for 2 hours. During the cold exposure, subjects were wearing underwear only. After one hour of cold-exposure the radioactive tracer was administered (the 18F-FDG was adjusted for BMI leading to dosages of approximately 200 MBq, for 123I-mIBG a fixed dose of 185 MBq was used). The 18F-FDG PET-CT was performed directly after the 2 hours cold exposure (i.e. one hour after 18F-FDG administration), the 123I-mIBG SPECT-CT was performed approximately 24 hours after the 123I-mIBG administration (according to the previously established protocol).(13) After every two subjects the sequence of 18F-FDG PET-CT and 123I-mIBG SPECT was changed to overcome order effects.
The scans were assessed by LB (PhD student), trained in analysing the scans, and HJV, a nuclear physician. To compare areas with active BAT on both scans, the $^{18}$F-FDG PET-CT and $^{123}$I-$m$IBG SPECT-CT were aligned. Volumes of interest established on $^{18}$F-FDG PET-CT (i.e. uptake of $^{18}$F-FDG identified by PET and the presence of fat identified by CT) were copied, to the aligned $^{123}$I-$m$IBG SPECT-CT images. PET and SPECT images were aligned using an automated rigid method. We measured maximal uptake, mean uptake and volume of BAT on both $^{18}$F-FDG PET-CT and $^{123}$I-$m$IBG SPECT-CT.

BAT volume was measured using a PET or SPECT threshold based delineation. Therefore, volumes measured reflect activated BAT volume. All visually identified areas with active BAT were included in the analysis.

To be able to integrate the influence of both the metabolic and sympathetic BAT activity within subjects across the three groups we also calculated the $SQUV_{max}/SUV_{max}$ ratio.

**Other measurements**

Energy expenditure was recorded every minute using Cosmed Quark RMR® during 30 minutes via indirect calorimetry after the 2 hours of cold exposure in the waiting period after $^{123}$I-$m$IBG administration and the SPECT-CT acquisition (e.g. 1 hour after the $^{123}$I-$m$IBG administration). Oral Glucose Tolerance Test and Laboratory measurements were performed according to standard procedures (for detailed methods see supplementary data).
Statistical analysis

The characteristics of the study participants are reported as medians with interquartile range (IQR). The p-values for differences between the lean young males and the older males and the obese were determined using the Mann–Whitney U test. Differences between the presence of BAT activity between the groups were calculated with the Fishers’s Exact test. Correlations were determined with the Spearman’s correlation. Data analysis was performed using SPSS software 20.0 (Chicago, IL, USA). A p-value <0.05 was considered to indicate statistical significance.

RESULTS

The study was performed between March 2013 and March 2014. Baseline characteristics of the subjects are shown in table 1 (a more detailed table of baseline characteristics can be found in the supplementary data). As expected, the obese and the older males showed a more adverse metabolic profile as compared to the lean and young subjects i.e. older subjects had a significant higher HbA1c, total- and LDL cholesterol while obese subjects were significantly less insulin sensitive and had higher HDL cholesterol values and triglycerides (Suppl.Table 1).

18F-FDG uptake was visually seen in 7/10 subjects in the obese males, 6/11 in the older males and 13/14 in the lean young males. The presence of BAT activity on 18F-FDG PET-CT was not significantly different between the obese and lean males (p
= 0.27) and borderline significant between the old and young males (p = 0.056). Figure 1 shows three typical cases of metabolically active BAT in our subjects. Metabolic BAT activity (maximal and mean) and volume measured on 18F-FDG PET-CT were different between the lean older males and lean young (p<0.01) but not between the obese and lean young (Fig. 2).

Uptake of 123I-mIBG was seen in 7/10 subjects in the obese males, 5/11 in the older males and 13/14 in the lean young males. Again, the presence of BAT activity on 123I-mIBG SPECT was not significantly different between the obese and lean males. The maximal sympathetic nervous stimulation of BAT (SQUV<sub>max</sub>) and volume measured on 123I-mIBG SPECT were significantly diminished in the older males as compared to the lean young, but did not differ between the obese and the lean young. Mean sympathetic nervous stimulation of BAT (SQUV<sub>mean</sub>) was not significantly different between any of the groups (Fig 2).

All the subjects with 123I-mIBG uptake in BAT also showed 18F-FDG uptake in BAT. Vice versa, there was one older subject who showed 18F-FDG uptake in BAT but no 123I-mIBG uptake.

To assess the relative influence of the sympathetic drive, we calculated the maximal and mean SQUV/SUV ratio in all subjects who were BAT positive on both scans. While both the SQUV<sub>max</sub> and the SUV<sub>max</sub> were lower in the older males, the SQUV<sub>max</sub>/SUV<sub>max</sub> ratio was higher in the older males as compared to the lean young. There was no difference in the ratio between the obese and the lean young. The SQUV<sub>mean</sub>/SUV<sub>mean</sub> ratio did not differ between the groups (Fig 3).
There was a negative correlation between age and maximal metabolic and sympathetic BAT activity. Although the correlation remained significant for age and mean metabolic BAT activity, the correlation disappeared for age and the mean sympathetic BAT activity (table 2). There was no correlation between BMI and maximal or mean metabolic and maximal sympathetic BAT activity or BMI and BAT volume (table 2). There was a strong and positive correlation in the whole group for both maximal and mean BAT activity measured with $^{18}$F-FDG and $^{123}$I-IBG (Fig. 4). Also after removal of the BAT negative subjects this correlation remained strong and positive for maximal BAT activity but not for mean BAT activity (Fig 4).

After stratification, the strong positive correlation between maximal BAT activity measured with $^{18}$F-FDG and $^{123}$I-IBG was still apparent in the lean young and the obese males but not in the older males (table 3). After removal of the BAT negative subjects, the correlation was still significant in the lean young males (spearman correlation $\rho=0.74$, $p<0.01$) but not for the obese males (spearman correlation $\rho=0.12$, $p=0.64$). There was no correlation between mean BAT activity measured with $^{18}$F-FDG and $^{123}$I-IBG for any of the groups (table 3).

Energy expenditure during cold did not differ significantly between the three groups (table 1). There was a positive relation between energy expenditure in cold and maximal metabolic and sympathetic BAT activity in the whole group (table 2).

**DISCUSSION**
We found a lower SNS activity in the older males as compared to the lean, young, which may be the explanation for the lower metabolic BAT activity. In line with our earlier data we found a strong and positive correlation for detecting BAT on $^{18}$F-FDG PET-CT and $^{123}$I-$m$IBG SPECT-CT in the lean young.\(^{(13)}\) This correlation disappeared in the older males.

The higher $\text{SQUV}_{\text{max}}/\text{SUV}_{\text{max}}$ ratio in the older males, as compared to the lean young, may suggest a dissociation between the stimulus and effect. This dissociation might also explain the absence of a correlation for detecting BAT between $^{18}$F-FDG PET-CT and $^{123}$I-$m$IBG SPECT-CT.

As the SNS drive is supposed to be the main determinant of BAT activity, an increasing ratio of SNS activity over metabolic BAT activity (for our purposes the ratio of $^{123}$I-$m$IBG uptake over $^{18}$F-FDG uptake), might imply that BAT becomes less sensitive to SNS signals upon aging. After all, the SNS signal, relative to metabolic activity (i.e. the $\text{SQUV}_{\text{max}}/\text{SUV}_{\text{max}}$ ratio) was higher in the older males as compared to the lean young, though just short of statistical significance.

The combination of both a lower absolute SNS signal as well as a possible declined sensitivity of BAT for SNS stimulation may cause the lower ability to activate and recruit BAT in the older males. However, the relative role of the SNS should be confirmed in trials with larger subject numbers.

Strikingly, older humans have an inability to appropriately regulate their temperature when exposed to cold.\(^{(15-17)}\) At least some part of this inability is due to an increased heat loss caused by inefficient vasoconstriction responses or changes in
peripheral blood flow during cold exposure. (17-19) Nonetheless, there is also evidence that upon ageing, the ability to produce metabolic heat decreases. (20) Hypothetically, this might at least in part be caused by the lower absolute SNS signal to BAT in elderly causing a lower ability to activate and recruit BAT in the older males.

These findings support the idea that the SNS has an important role in the activation process of BAT.

Studies investigating the effects of sympathicomimetics (e.g. ephedrine, isoprenaline) on BAT activation did not result in unequivocal evidence. (21-24) However, recently Cypess et all showed an intense BAT activation after administration of mirabegron (a β3 adrenergic receptor agonist). (25) Similar to our previous study, (13) we also clearly show involvement of SNS in BAT. The SNS activity to BAT can be visualised and quantified by performing $^{123}$I-mIBG-SPECT. The involvement of the SNS in BAT activation is clearly demonstrated by the strongly positive correlation for detecting BAT on $^{123}$I-mIBG-SPECT and $^{18}$F-FDG-PET-CT in lean, young, healthy males. (13) The lower metabolic BAT activity in the older males as compared to the lean, young, is in line with previous studies, though less pronounced. (10,12) We found a fairly high metabolic BAT activity in the older males as compared to the literature, this might be explained by the fact that the older males in our study were in a very good physical condition as illustrated by a relative low BMI and the fact that none of our subjects used any medication, despite the median age of 54 (IQR [50-60]) years. We found a borderline significant difference in the presence of BAT activity on $^{18}$F-FDG PET-CT between the young and older males. Though the
metabolic BAT activity in the older males was relatively high, the borderline significant difference is probably a type 2 error, caused by the small sample size.

In addition to the lower metabolic BAT activity in the older males, we now demonstrate a lower SNS activity to BAT, as compared to the lean, young. This might, at least in part, explain the lower metabolic BAT activity in older males. Promising results have been obtained in several studies indicating that BAT can be recruited, even in individuals with decreased BAT activity.\(^{(26,27)}\) However, whether this could also be applied in older humans has to be investigated.

We use \(^{123}\text{I}-\text{mIBG-SPECT}\) to measure sympathetic stimulation in an acute exposure setting. The uptake of \(^{123}\text{I}-\text{mIBG}\) in neuroendocrine cells occurs by an active uptake mechanism via the norepinephrine transporter (NET). There is evidence that acute modulation of the NET trafficking may provide additional modulatory capacity for noradrenergic signalling.\(^{(29)}\) This makes that the \(^{123}\text{I}-\text{mIBG}\) uptake patterns we have observed most likely reflect the fast sympathetic response to the acute cold exposure. After the uptake, \(^{123}\text{I}-\text{mIBG}\) is stored in neurosecretory granules and may subsequently be secreted in the synaptic cleft. \(^{123}\text{I}-\text{mIBG}\) uptake in a specific organ is dependent on catecholamine excretion and/or the adrenergic innervation of the specific organ.\(^{(28)}\)

In contrast to other groups, we use a uniform cooling method in which all subjects are exposed to the same room temperature instead of personalized cooling. Uniform cooling is considered clinically more relevant since humans are exposed to outdoor temperatures which are also uniform. Nonetheless, despite the uniform
cooling, we found no differences in metabolic BAT activity between the lean young and the obese males. This might be explained by the slightly lower age of the obese subjects as compared to the subjects in the literature. Aging processes might be more important as determinants of BAT than BMI. Furthermore, the small number of participants may have caused a lack of power to demonstrate a difference between the obese and the lean young subjects. However, other studies, using the same sample size but a different cooling protocol, found a significant difference in metabolic BAT activity and volume between the lean young and the obese.

Likewise, there was no difference in SNS stimulation between the lean and the obese. The strong and positive correlation for detecting BAT on 18F-FDG PET-CT and 123I-mIBG SPECT-CT we found in the lean young was also found in the obese, confirming that 123I-mIBG SPECT-CT is capable of detecting the SNS BAT activity in both the lean young and the obese subjects.

We measured BAT volume by using a PET/SPECT threshold based delineation. Therefore, BAT volumes included in this study reflect activated BAT volume instead of total BAT volume and total BAT volume might be underestimated. However, by exposing our subjects to cold, BAT is maximally activated. Therefore in this setting the differences between activated BAT and total BAT are probably small.

We found a weak but positive correlation between energy expenditure during cold exposure and BAT activity emphasizing that activated BAT could contribute to weight loss. These findings are in line with previous publications.
CONCLUSION

We conclude that a lower sympathetic drive may explain the lower BAT activity in the older males but not in the obese males. Furthermore, with increasing age there might also be a diminished sensitivity of BAT to SNS stimulation. If the activation of BAT is regarded as a promising anti-obesity strategy, future research has to be directed to identify factors able to (re)activate or recruit BAT in thermoneutrality possibly by increasing the SNS signal to BAT.

Disclosure

The authors declare no conflicts of interest.
REFERENCES


Figure 1. Three typical examples of BAT activity in accordance with their age and BMI. Left subject is a lean and old subject showing minimal BAT activity; middle subject is a lean and young subject showing abundant BAT activity; and right subject is an obese and young subject showing BAT activity but less than the lean and young subject.
Figure 2. A,B,C; Metabolic activity of BAT between the three groups defined as median [IQR] SUV\textsubscript{max}, SUV\textsubscript{mean} and volume of \textsuperscript{18}F-FDG (defined as activity in Becquerel per milliliter within region of interest divided by injected dose in Becquerel per gram of body weight) respectively.

D,E,F; Sympathetic nervous stimulation to BAT between the three groups defined as median [IQR] SQUV\textsubscript{max} and volume of \textsuperscript{123}I-\textit{mIBG} (calculated as maximum count in BAT divided by mean count per voxel in the mediastinum) respectively.
Figure 3. The relative influence of the sympathetic drive, calculated as SQUV/SUV ratio in all subjects who were BAT positive on both scans for maximal values (A) and mean values (B).
Figure 4. Spearman's correlations for metabolic BAT activity measured on $^{18}$F-FDG PET CT (expressed as $SUV_{\text{max}}$ of $^{18}$F-FDG) and sympathetic nervous stimulation to BAT measured on $^{123}$I-mIBG SPECT (expressed as $SQUV_{\text{max}}$ of $^{123}$I-mIBG). A; maximal BAT activity in all subjects (n=35) B; maximal BAT activity in all BAT positive subjects (n=26). C; mean BAT activity in all subjects (n=35) D; mean BAT activity in all BAT positive subjects (n=26), regression line not shown because of non-significance.
<table>
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<tr>
<th></th>
<th>Obese Young</th>
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<th>Lean Young</th>
<th>IQR</th>
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<tr>
<td>N</td>
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<td>14</td>
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<tr>
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<td>BMI (kg/m²)</td>
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<td>30.8-38.9</td>
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<td>Waist circumference (cm)</td>
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<td>110.5-144.5</td>
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<td>82.0-85.0</td>
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<td>HbA1c (mmol/mol)</td>
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<td>31.8-35.0</td>
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<td>0.3-0.8</td>
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<td>Energy Expenditure in Cold (kcal/24h)</td>
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<td>1499-2530</td>
<td>2294</td>
<td>1807-2666</td>
<td>1484</td>
<td>1033-2294</td>
</tr>
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</table>

BMI, body mass index; TSH, thyroid stimulating hormone. *Data are presented as n or median [interquartile range] Significant different as compared to the lean young males * p<0.05, **p<0.01, ***p<0.001.
Table 2. Correlations between BAT parameters and patient characteristics

<table>
<thead>
<tr>
<th></th>
<th>$S_{\text{UV}}\text{ max}$</th>
<th>$S_{\text{UV}}\text{ mean}$</th>
<th>$S_{\text{QUV}}\text{ max}$</th>
<th>$S_{\text{QUV}}\text{ mean}$</th>
<th>BAT volume</th>
<th>BAT volume</th>
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<td>Age (years)</td>
<td>$\rho=-0.48^{**}$</td>
<td>$\rho=-0.44^{**}$</td>
<td>$\rho=-0.38^{*}$</td>
<td>$\rho=-0.27$</td>
<td>$\rho=-0.43^{**}$</td>
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<td>$\rho=-0.28$</td>
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<tr>
<td>Energy Expenditure in Cold (kcal/24h)</td>
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<td>$\rho=0.48^{*}$</td>
<td>$\rho=0.46^{*}$</td>
<td>$\rho=0.41$</td>
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</table>

Spearman's correlations for BAT activity parameters and age, BMI and energy expenditure in cold. * $p<0.05$, **$p<0.01$. 
Table 3. Correlations between BAT parameters after stratification

<table>
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<th>SUV$_{\text{max}}$</th>
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<td><strong>Young, lean</strong></td>
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<tr>
<td>SQUV$_{\text{max}}$</td>
<td>$\rho=0.79^{**}$</td>
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<tr>
<td>SQUV$_{\text{mean}}$</td>
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<td>$\rho=0.17$</td>
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<td><strong>Young, obese</strong></td>
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<tr>
<td>SQUV$_{\text{max}}$</td>
<td>$\rho=0.88^{**}$</td>
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<tr>
<td>SQUV$_{\text{mean}}$</td>
<td></td>
<td>$\rho=0.58$</td>
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<tr>
<td><strong>Old, lean</strong></td>
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<td></td>
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<tr>
<td>SQUV$_{\text{max}}$</td>
<td>$\rho=0.57$</td>
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<tr>
<td>SQUV$_{\text{mean}}$</td>
<td></td>
<td>$\rho=0.56$</td>
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Stratification of the spearman's correlations between maximal and mean BAT metabolic activity (respectively SUV$_{\text{max}}$ and SUV$_{\text{mean}}$) and maximal and mean sympathetic nervous stimulation to BAT (respectively SQUV$_{\text{max}}$ and SQUV$_{\text{mean}}$).