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Vigorous-Intensity Physical Activities Are Associated with High Brown Adipose Tissue Density in Humans

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Abstract: Brown adipose tissue (BAT) plays a role in adaptive thermogenesis in response to cold environments and dietary intake via sympathetic nervous system (SNS) activation. It is unclear whether physical activity increases BAT density (BAT-d). Two-hundred ninety-eight participants (age: 41.2 ± 12.1 (mean ± standard deviation), height: 163.6 ± 8.3 cm, weight: 60.2 ± 11.0 kg, body mass index (BMI): 22.4 ± 3.0 kg/m^2, body fat percentage: 25.4 ± 7.5%) without smoking habits were categorized based on their physical activity levels (a group performing physical activities including walking and moderate physical activity (WM) and a group performing WM + vigorous-intensity physical activities (VWM)). We measured the total hemoglobin concentration ([Total-Hb]) in the supraclavicular region, an index of BAT-d, and anthropometric parameters. [Total-Hb] was significantly higher in VWM than WM for all participant groups presumably owing to SNS activation during vigorous-intensity physical activities, and unrelated to the amount of total physical activity levels. Furthermore, multiple regression analysis revealed that BAT-d was related to visceral fat area and VWM in men and related to body fat percentage in women. We conclude that vigorous-intensity physical activities are associated with high BAT-d in humans, especially in men.

Keywords: brown adipose tissue (BAT); vigorous-intensity physical activities (VPA); near-infrared time-resolved spectroscopy (NIR_{TRS}); exercise; sympathetic nervous system (SNS)

1. Introduction

Human brown adipose tissue (BAT) is present in the supraclavicular, cervical, and paravertebral regions [1,2] and plays a role in adaptive thermogenesis in response to cold environments and dietary intake [3–9], thereby increasing systemic energy output [10]. In particular, the mechanism for cold-induced thermogenesis in BAT is achieved through activation of the sympathetic nervous system (SNS) via the transient receptor potential (TRP) channel in the skin [11–16]. BAT is prominent in newborns and infants, deteriorating with growth, and disappearing with aging [17]. In addition to aging and cold stimulation, specific drugs, dietary ingredients, circadian rhythms, and exercise have been reported as factors affecting BAT activity [3–5,18–25]. However, many aspects are unclear regarding the relationship between exercise and BAT [19]. In animal models investigating the relationship between BAT and exercise, endurance exercise training elicited increased BAT activity and white adipose tissue (WAT) browning [26–28]. In human studies, men and women who performed endurance exercises in cross-sectional studies have reported significantly lower cold-induced ^{18}F-fluorodeoxyglucose

(^{18}F-FDG) uptake, an indicator of BAT activity, than sedentary men and non-athlete women [23,24]. In contrast, it has been reported that ^{18}F-FDG uptake does not change after two weeks of high intensity interval training or moderate training in humans [25].

BAT has been evaluated in the supraclavicular region using ^{18}F-FDG–positron emission tomography (PET) with computed tomography (CT) (^{18}FDG-PET/CT) in humans [29–31]. This method has several limitations such as radiation exposure, considerable instrumentation costs, and acute cold exposure [32–34], which make repeated evaluation of BAT in healthy individuals difficult. Therefore, a noninvasive, simple method, which does not require exposure to cold or radiation, is desirable [35]. Near-infrared time-resolved spectroscopy (NIR_{TRS}) can be used to measure total hemoglobin concentration ([Total-Hb]) and oxygenation in biological tissues [36–38]. The abundant capillaries of BAT allow NIR_{TRS} to distinguish the characteristics of BAT from WAT [36–38]. BAT evaluation using NIR_{TRS} does not require cold exposure or radiation exposure, so it can be evaluated safely and non-invasively [39]. [Total-Hb] evaluated by NIR_{TRS} positively correlated with BAT parameters evaluated by ^{18}FDG–PET/CT only in the supraclavicular region, which is the location of BAT [39]. Longitudinal studies revealed that an increase in BAT activity induced by repeated ingestion of thermogenic food ingredients can be detected by an increase in [Total-Hb] [40]. Collectively, [Total-Hb] in the supraclavicular region evaluated by NIR_{TRS} is expected to be suitable for evaluating BAT density (BAT-d) and equivalent to the determination of BAT activity or BAT volume by ^{18}FDG-PET/CT using cold exposure [39–42].

A question arises whether exercise can modulate BAT-d, and if so, which intensity can effectively increase BAT-d in humans. SNS plays an important role in activating BAT and increasing BAT volume [11–16]. The response of SNS during exercise is reported to increase from around 50–70% of the maximal oxygen uptake (VO_{2max}) [43–47]. However, previous exercise studies did not focus on SNS, which makes previous research results regarding the correlation of exercise with BAT unclear [27,48–55]. We hypothesized that vigorous-intensity physical activity (VPA) activates SNS, yielding an increase in BAT-d in the supraclavicular region. The purpose of this study was to clarify the effect of VPA on BAT-d.

2. Materials and Methods

2.1. Participants and Study Design

The study was conducted using a cross-sectional design. Participants were recruited using advertisements or direct contact. Among 319 healthy men and women, aged 20 years or older, who participated in the study from December to March, 298 participants without smoking habits were categorized based on their physical activity levels. The participants responded to a questionnaire and were evaluated in the laboratory for BAT-d, body composition, blood pressure, and heart rate. This study was conducted in accordance with the Declaration of Helsinki, and the protocol was approved by the Tokyo Medical University Medical Ethics Review Board (approval number: SH3957). All participants had given a written consent before participating in the study.

2.2. Brown Adipose Tissue Density

BAT-d was evaluated by NIR_{TRS} (Hamamatsu Photonics K.K., Hamamatsu, Japan) according to previous studies [39,56]. A probe with an optode distance of 3 cm was used. The light could reach a mean depth of 2 cm [57], a depth at which BAT is expected [58]. [Total-Hb] in the supraclavicular region, an index of BAT-d, was calculated as the sum of oxygenated Hb and deoxygenated Hb. Reduced scattering coefficient (μ'_s), which is one of the tissue optical characteristics, was also measured. The [Total-Hb] in the supraclavicular region is adjusted according to the thickness of the subcutaneous adipose tissue layer (1.00 ± 0.48 mm) [59]. NIR_{TRS} data were extracted every 10 seconds. The coefficient of variation within an individual when evaluated repeatedly is 4.9% [39].

2.3. Physical Activity Level

We evaluated the total amount of physical activity using the international physical activity questionnaire (IPAQ, long version) during a representative week for the time of physical activity, time spent on each activity [walking (W), moderate-intensity physical activity (MPA), and VPA, and energy expenditure. IPAQ evaluates physical activity lasting longer than 10 minutes. The total amount of physical activity was calculated by integrating the duration of the physical activity (hours) and the physical activity intensity (METs). Based on the IPAQ analysis guidelines, we assigned 3.3 METs for W, 4.0 METs for MPA, and 8.0 METs for VPA [60]. According to the reference value for Japanese men by the Ministry of Health, Labor and Welfare, the level of 8 METs corresponds to approximately 80% VO_{2max} in participants of the average age in this study (41.3 years) [61]. Thus, the intensity of VPA categorized in this study is considered sufficient to activate SNS [47].

2.4. Measurement of Anthropometric and Circulatory Parameters

We measured body height, weight, body fat mass, body fat percentage, skeletal muscle mass, skeletal muscle percentage, waist circumference, and visceral fat area. Body weight, body fat mass, body fat percentage, and skeletal muscle mass were measured by bioelectric impedance (Inbody 720 Body Composition Analyzer; InBody Japan, Tokyo, Japan) [62,63]. The subcutaneous adipose thickness of the supraclavicular, deltoid, and abdominal regions was monitored using B-mode ultrasonography (Vscan Dual Probe; GE Vingmed Ultrasound AS, Horten, Norway). Waist circumference and visceral fat area were measured in an upright position using an impedance method (Bioelectrical impedance analysis EW-FA90; Panasonic, Osaka, Japan). Systolic blood pressure, diastolic blood pressure, and heart rate were measured using an automated sphygmomanometer (HEM-1025; Omron Healthcare, Kyoto, Japan). Body mass index (BMI) was calculated by weight (kg) per height squared (m^2), and skeletal muscle percentage was calculated by skeletal muscle mass (kg) per body weight (kg).

2.5. Statistical Analysis

Twenty-one participants with smoking habits were excluded from the analysis. The remaining participants were classified according to whether they performed VPA with potential to increase SNS activity. All 232 participants (77 individuals who performed VWM and 155 persons who performed WM group) (men: 87; women: 145) were included in the analysis. The values are shown in the mean ± standard deviation. To determine the difference between the WM and VWM groups, an independent t-test was used. The Mann–Whitney test was used to analyze the energy expenditure, time of VPA, time of moderate physical activity, and time of physical activity related to walking according to the guidelines [60]. The BAT-d level, level of physical activity intensity (with or without vigorous activity), total amount of physical activity, and gender were analyzed by two-way analysis of variance. To evaluate factors correlating with BAT-d, we used stepwise multiple regression analysis with BAT-d as the independent variable and age, body fat percentage, visceral fat area, and with (1)/without (0) VPA, as the dependent variables.

All analyses were performed using the SPSS software (IBM SPSS Statistics 25 and/or 26, IBM Japan, Tokyo, Japan), and $p < 0.05$ was considered statistically significant.

3. Results

3.1. Participant Characteristics

The participants of this study were 298 healthy non-smokers (age: 41.2 ± 12.1, height: 163.6 ± 8.3 cm, weight: 60.2 ± 11.0 kg, BMI: 22.4 ± 3.0 kg/m^2, body fat percentage: 25.4 ± 7.5%). Figure 1 shows the inclusion/exclusion criteria. We analyzed 232 healthy men and women after excluding 66 individuals; the 232 participants were categorized into 2 groups: a group performing WM and a group performing VWM.

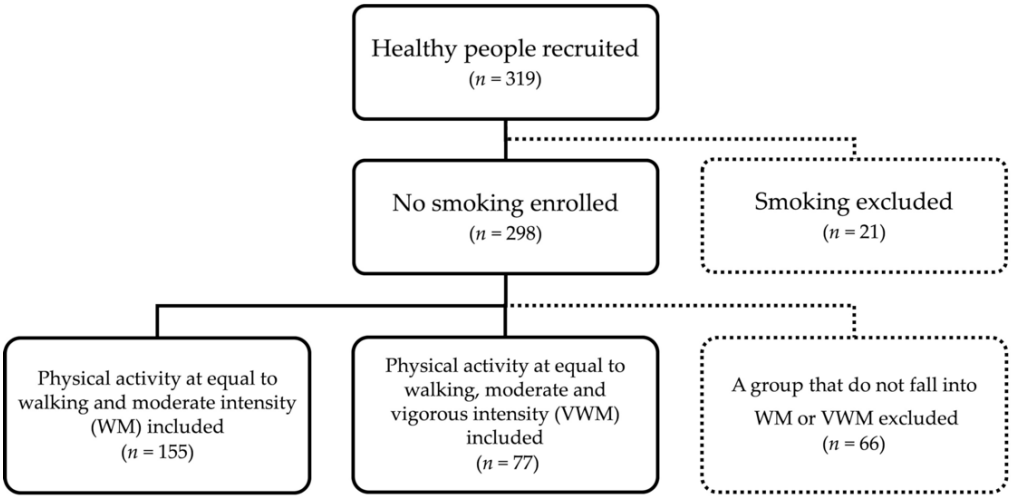


Figure 1. Inclusion/exclusion criteria for the participants. Two-hundred ninety-eight people without smoking habits were extracted. Among them, we analyzed 232 healthy men and women after excluding 66 participants who were not categorized in a group performing all physical activities (including walking, moderate physical activity, and vigorous-intensity physical activity) or a group performing some physical activities (including walking and moderate-intensity physical activity).

The μ'_s in the supraclavicular region was found to be 8.3 (7.3, 9.4) (medians (the first quartile, the third quartile)) cm^{-1} and (total-Hb), 65.1 (50.4, 85.0) μM. Compared to the WM group, the VWM group had a significantly higher [Total-Hb] in the supraclavicular region but similar height, weight, skeletal muscle mass, skeletal muscle percentage, lean body mass, systolic blood pressure, energy expenditure by physical activity, and the time of physical activity at equal to walking. However, the body fat mass and body fat percentage were significantly lower in the VWM group than in the WM group (Table 1).

In women, there was no significant difference in [Total-Hb] in the supraclavicular region between the VWM and WM groups. The skeletal muscle percentage and energy expenditure by physical activity were significantly higher in the VWM group compared to the WM group; only body fat mass was significantly lower in the VWM group (Table 2).

Table 1. Group comparisons for each parameter in men and women.

All	WM (n = 155)	VWM (n = 77)	p-Value
[Total-Hb] (μM)	66.6 ± 20.5	75.3 ± 26.1	<0.05
Age (years)	40.8 ± 11.7	42.2 ± 13.6	0.40
Height (cm)	161.9 ± 8.2	166.0 ± 8.4	<0.01
Weight (kg)	58.7 ± 11.0	62.0 ± 10.6	<0.05
BMI (kg/m^2)	22.3 ± 3.1	22.4 ± 2.5	0.85
Skeletal muscle mass (kg)	23.5 ± 5.0	27.0 ± 6.0	<0.01
Skeletal muscle percentage (%)	40.1 ± 4.2	43.3 ± 4.8	<0.01
Body fat mass (kg)	15.7 ± 5.9	13.5 ± 4.8	<0.01
Fat percentage (%)	26.5 ± 7.0	22.0 ± 7.3	<0.01
Waist circumference (cm)	43.0 ± 8.0	48.5 ± 9.8	<0.01
Visceral fat area (cm^2)	79.3 ± 8.9	79.9 ± 7.3	0.60
Fat-free mass (kg)	61.1 ± 37.6	62.1 ± 37.2	0.86
Body temperature (°C)	36.3 ± 0.4	36.3 ± 0.4	0.50
Heart rate (bpm)	71.7 ± 9.7	69.4 ± 11.9	0.14
Systolic blood pressure (mmHg)	111.7 ± 14.7	116.7 ± 14.9	<0.05
Diastolic blood pressure (mmHg)	71.0 ± 11.2	73.3 ± 10.3	0.14
Energy expenditure by physical activity (kcal/week) [1]	1950 (1231–3651)	4420 (2183–7883)	<0.01
Physical activity time at vigorous-intensity (min/week) [1]	0	180 (60–360)	<0.01

Table 1. *Cont.*

All	WM (n = 155)	VWM (n = 77)	p-Value
Physical activity time at moderate-intensity min/week) [1]	210 (100–510)	180 (93–470)	0.49
Physical activity time at intensity of equal to walking (min/week) [1]	250 (120–480)	350 (188–660)	<0.05

Results are presented as the mean ± standard deviation. An independent t-test was performed to determine the significance of the group differences. [1] Indicated by the median according to guidelines [60]; the Mann–Whitney test was used to determine the significance of group differences. VWM—a group performing all physical activities including walking, moderate physical activity, and vigorous-intensity physical activity; WM—a group performing physical activities including walking and moderate physical activity; [Total-Hb]—total hemoglobin concentration in the supraclavicular region adjusted according to the thickness of the subcutaneous adipose tissue layer; BMI—body mass index.

Table 2. Group comparison of each parameter in women.

Women	WM (n = 109)	VWM (n = 36)	p-Value
[Total-Hb] (μM)	68.1 ± 19.9	70.9 ± 21.8	0.48
Age (years)	41.2 ± 12.8	45.4 ± 15.7	0.15
Height (cm)	158.2 ± 5.9	158.9 ± 5.4	0.51
Weight (kg)	54.2 ± 8.1	53.3 ± 4.5	0.42
BMI (kg/m^2)	21.6 ± 2.9	21.1 ± 1.8	0.21
Skeletal muscle mass (kg)	20.8 ± 2.3	21.5 ± 2.8	0.10
Skeletal muscle percentage (%)	38.7 ± 3.6	40.4 ± 4.0	<0.05
Body fat mass (kg)	15.6 ± 5.7	13.9 ± 3.6	<0.05
Fat percentage (%)	28.2 ± 6.5	26.0 ± 6.0	0.08
Waist circumference (cm)	38.5 ± 3.9	39.4 ± 4.2	0.26
Visceral fat area (cm^2)	76.7 ± 8.0	76.0 ± 5.9	0.59
Fat-free mass (kg)	47.7 ± 26.7	42.4 ± 18.5	0.27
Body temperature (°C)	36.3 ± 0.4	36.3 ± 0.4	0.35
Heart rate (bpm)	72.2 ± 9.4	68.5 ± 10.7	0.06
Systolic blood pressure (mmHg)	108.3 ± 14.4	112.8 ± 16.9	0.13
Diastolic blood pressure (mmHg)	68.2 ± 10.9	70.4 ± 10.1	0.30
Energy expenditure by physical activity (kcal/week) [1]	2073 (1292–3725)	4288 (2086–7052)	<0.01
Physical activity time at vigorous-intensity (min/week) [1]	0	160 (60–345)	<0.01
Physical activity time at moderate-intensity min/week) [1]	240 (95–630)	255 (120–638)	0.49
Physical activity time at intensity of equal to walking (min/week) [1]	280 (128–555)	350 (165–743)	<0.05

Results are presented as the mean ± standard deviation. An independent t-test was performed to determine the significance of the group differences. [1] Indicated by the median according to guidelines [60]; the Mann–Whitney test was used to determine the significance of group differences. VWM—a group performing all physical activities including walking, moderate physical activity, and vigorous-intensity physical activity; WM—a group performing physical activities including walking and moderate physical activity; [Total-Hb]—total hemoglobin concentration in the supraclavicular region adjusted according to the thickness of the subcutaneous adipose tissue layer; BMI—body mass index.

In men, compared with the WM group, the VWM group showed significantly higher [Total-Hb] in the supraclavicular region and increased skeletal muscle mass, skeletal muscle percentage, lean body mass, energy expenditure by physical activity, and the time of physical activity at equal to walking. Body fat mass and body fat percentage were significantly lower in the VWM group than in the WM group (Table 3).

Table 3. Group comparison of each parameter in men.

Men	WM (n = 46)	VWM (n = 41)	p-Value
[Total-Hb] (μM)	63.3 ±21.8	79.2 ±29.1	< 0.01
Age (years)	39.9 ±8.6	39.4 ±10.9	0.83
Height (cm)	170.8 ±5.5	172.3 ±4.8	0.18
Weight (kg)	69.5 ±9.2	69.7 ±8.2	0.92
BMI (kg/m^2)	23.8 ±3.1	23.4 ±2.5	0.53
Skeletal muscle mass (kg)	30.0 ±3.1	31.8 ±3.1	<0.01
Skeletal muscle percentage (%)	43.5 ±3.7	45.9 ±3.9	<0.01
Body fat mass (kg)	16.0 ±6.3	13.2 ±5.7	<0.05
Fat percentage (%)	22.5 ±6.4	18.5 ±6.6	<0.01
Waist circumference (cm)	53.5 ±5.0	56.5 ±5.1	0.83
Visceral fat area (cm^2)	85.5 ±7.8	83.3 ±6.8	0.18
Fat-free mass (kg)	93.5 ±40.6	79.3 ±40.9	0.92
Body temperature (°C)	36.2 ±0.4	36.3 ±0.4	0.53
Heart rate (bpm)	70.5 ±10.4	70.1 ±12.9	<0.01
Systolic blood pressure (mmHg)	120.1 ±11.6	120.2 ±12.0	<0.01
Diastolic blood pressure (mmHg)	77.8 ±9.1	75.9 ±9.8	<0.05
Energy expenditure by physical activity (kcal/week) [1]	1806 (1061–3539)	4876 (2530–8629)	<0.01
Physical activity time at vigorous-intensity (min/week) [1]	0	180 (60–360)	<0.01
Physical activity time at moderate-intensity min/week) [1]	155 (93–308)	130 (60–300)	0.93
Physical activity time at intensity of equal to walking (min/week) [1]	213 (104–360)	340 (200–585)	<0.01

Results are presented as the mean ± standard deviation. An independent t-test was performed to determine the significance of the group differences. [1] Indicated by the median according to guidelines [60]; the Mann–Whitney test was used to determine the significance of group differences. VWM, a group performing all physical activities including walking, moderate physical activity, and vigorous-intensity physical activity; WM, a group performing physical activities including walking and moderate physical activity; [Total-Hb], total hemoglobin concentration in the supraclavicular region adjusted according to the thickness of the subcutaneous adipose tissue layer; BMI, body mass index.

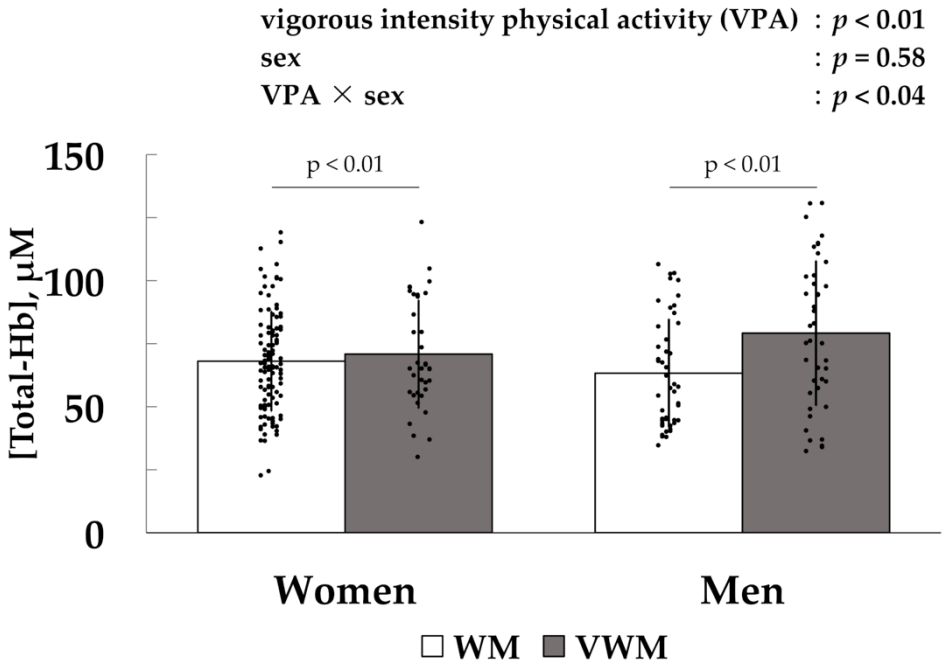
3.2. Association between Vigorous-Intensity Physical Activity and [Total-Hb] in the Supraclavicular Region, an Index of Brown Adipose Tissue Density

Two-way analysis of variance showed a significant interaction with a main effect in the group (Figure 2a). There was no significant relationship between BAT-d and the time spent performing VPA and between BAT-d and the frequency each week performing VPA. There was no significant difference in the amount of total physical activity between groups (Figure 2b).

3.3. Factors Associated with [Total-Hb] in the Supraclavicular Region, an Index of Brown Adipose Tissue Density

In all participants, the body fat percentage and visceral fat area were significantly related to [Total-Hb]. In women, the body fat percentage was significantly related to [Total-Hb]. In men, visceral fat area and VPA were significantly related to [Total-Hb] (Table 4).

(a)



(b)

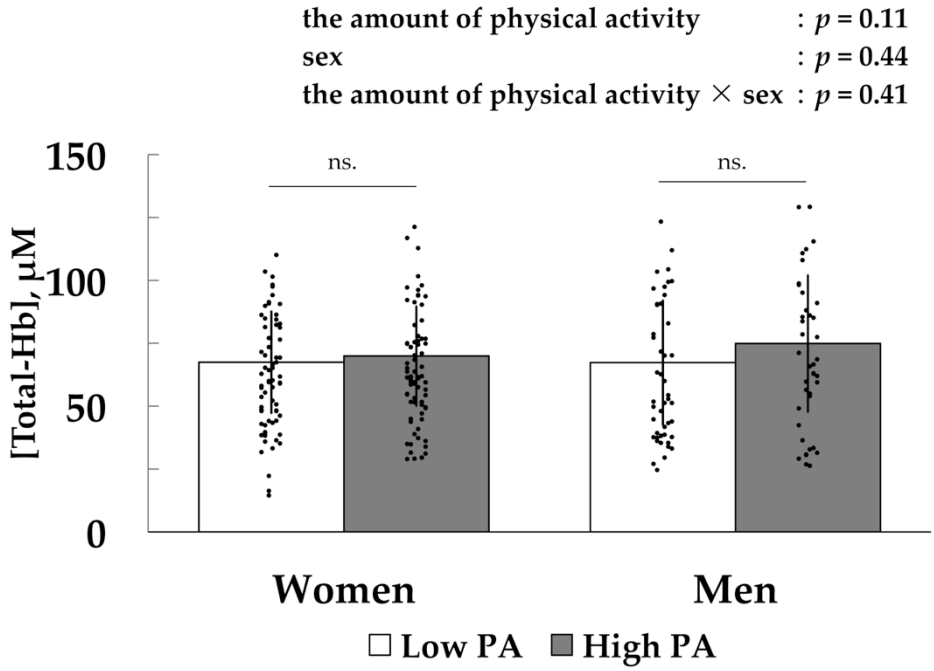


Figure 2. Comparison of [Total-Hb] in the supraclavicular region, an index of brown adipose tissue density (**a**) between a group performing all physical activities including walking, moderate physical activity, and vigorous-intensity physical activity (VWM) and a group performing physical activities including walking and moderate physical activity (WM); (**b**) between the higher physical activity (High PA) group and the lower physical activity (Low PA) group. Results are presented as the mean ± standard deviation. Two-way analysis of variance was performed to determine the significance of group and gender differences; ns., not significant.

Table 4. Multiple regression analysis with [Total-Hb] in the supraclavicular region as an independent variable.

[Total-Hb]	Univariate Regression		Multivariate Regression	
All	r	*p*	Standardized β	*p*
Age (years)	−0.07	0.16	-	-
BF (%)	−0.50	<0.01	−0.39	<0.01
VFA (cm^2)	−0.46	<0.01	−0.33	<0.01
WM-VWM	0.18	<0.01	-	-
			$R^2 = 0.34$	
Women				
Age (years)	−0.03	0.34	-	-
BF (%)	−0.47	<0.01	−0.47	<0.01
VFA (cm^2)	−0.44	<0.01	-	-
WM-VWM	0.06	0.24	-	-
			$R^2 = 0.22$	
Men				
Age (years)	−0.12	0.13	-	-
BF (%)	−0.64	<0.01	-	-
VFA (cm^2)	−0.66	<0.01	−0.62	<0.01
WM-VWM	0.32	<0.01	0.21	<0.05
			$R^2 = 0.47$	

The categorical variables were set at "0" for the group that only walked and performed moderate-intensity physical activity (WM) and "1" for the group that performed all intensity physical activities (walking, moderate, and vigorous intensity—VWM). Abbreviations: BF—body fat; VFA—visceral fat area.

4. Discussion

In this study, participants, especially men, performing VPA, showed high BAT-d in the supraclavicular region. Information is sparse from human studies investigating the relationship between exercise and BAT; previous studies have focused on aerobic exercise [19,50], vigorous-intensity exercise [25], and muscle strength [64]. This study was the first to observe a relationship between VPA and BAT-d. Furthermore, multiple regression analysis of BAT-d as the independent variable and age, body adiposity, and VPA as the dependent variables revealed that BAT-d is related to visceral fat area and VPA only in men. The reason for this observation is not clear but may be related to androgens [65]. Differentiated association of BAT-d and fat distribution between men and women observed in the study may explained by an evidence that estrogens increase the sympathetic tone differentially to the adipose tissue depots favoring lipid accumulation in the subcutaneous fat in women and visceral fat deposition in men [66].

The mechanism of cold-induced BAT activation is well known [67–70]. When the TRP channel in the skin receives a cold stimulus, the generated afferent nerve impulse is transmitted to the dorsal horn of the spinal cord and further to the hypothalamic preoptic area (POA), the thermoregulatory center. When the nerve impulse is received by POA, disinhibition of the thermogenic neuron in the dorsomedial hypothalamus and sympathetic and somatic premotor neuron in the rostral medullary raphe region occurs to prevent hypothermia. Eventually, a nerve impulse from the excited SNS is transmitted to BAT via sympathetic preganglionic neurons (SPNs) located in the spinal intermediolateral nucleus (IML) of the spinal ventral horn (SVH). When released noradrenaline from the sympathetic nerve ends binds to β_3 adrenergic receptors on brown adipocyte membranes, uncoupling protein 1 (UCP-1) on the inner mitochondrial membrane is activated, and BAT thermogenesis is induced [67–70].

It is postulated that exercise has an analogous mechanism to cold-induced enhancement of BAT via SNS activation. Exercise-induced mechanical and metabolic stimuli in the periphery are integrated in the circulatory center of the medulla oblongata via afferent fibers (group III, IV). The integrated information is transmitted to SPNs located in the SVH IML, which activate cardiac sympathetic nerves, muscle vasoconstrictor nerves, and presumably also BAT [71]. In humans, a 6-minute rowing

ergometer exercise resulted in a 2-fold increase in the blood levels of the inflammatory cytokine IL-6, at rest [72]. Inflammatory cytokines such as IL-6 induce the expression of prostaglandin synthases such as cyclooxygenase-2 in cerebral vascular endothelial cells, leading to prostaglandin E2 (PGE2) production [73]. When PGE2 binds to the POA receptor EP3, disinhibition of the sympathetic nerve drive is suppressed, leading to an increase in SNS through a pathway similar to cold stimulation, which activates BAT thermogenesis [74].

In previous cross-sectional studies investigating the relationship between exercise and BAT, the ^{18}F-FDG uptake in men and women performing endurance exercise was significantly lower than in sedentary men and non-athlete women [23,24]. On the other hand, two weeks of high intensity interval training and moderate training interventions did not alter ^{18}F-FDG uptake [25]. A cross-sectional report on the relationship between muscle strength and BAT activity showed a positive correlation between grip strength and BAT activity [64]. The concentration of blood myokine, a marker for promoting WAT browning, has been reported to be higher in an 8-week resistance training group than in control and endurance training groups [75,76]. Moreover, 12 weeks of moderate- and vigorous-intensity bicycle training increased UCP-1 mRNA expression in human abdominal WAT [77]. From these previous studies, VPA and/or related physiological modifications were expected to affect BAT activity and WAT browning. Therefore, in this study, we investigated the relationship between VPA accompanied with increased SNS activity and BAT-d in the supraclavicular region. We found that men performing VPA showed high BAT-d in the supraclavicular region. The results of the association between BAT-d and VPA in men are consistent with a previous report that androgen has the potential to promote browning of WAT in animal models [65]. Men have larger muscle mass, which may lead enhanced myokine levels and an increase in BAT-d [76]. Furthermore, it has been suggested that female hormones may also promote BAT function in women [78,79]; however, the study did not account for the menstrual cycle. Therefore, these factors may have affected gender differences in BAT-d.

It is well known that the autonomic nervous system is predominantly regulated by reduced parasympathetic activity until the exercise intensity reaches 30–50% VO_{2max} [80,81]. Then, SNS activity begins to elevate at approximately 50% VO_{2max} and progressively increases from around 70% VO_{2max} [43–47]. The classification of physical activity intensity in the international physical activity questionnaire (IPAQ) in this study is W: 3.3 METs, M: 3 METs to 6 METs or less, VPA: 8 METs or higher (MET, metabolic equivalent). According to the reference value for Japanese men by the Ministry of Health, Labor and Welfare, the level of 8 METs corresponds to approximately 80% VO_{2max} in participants of the average age in this study (41.3 years) [61]. Thus, the intensity of VPA categorized in this study is considered sufficient to activate SNS [47]. We failed to find a significant relationship between BAT-d and the time spent performing VPA and between BAT-d and the frequency each week performing VPA, indicating that duration and frequency are not factors for enhancing BAT-d in this study.

The following are the limitations of this research. First, BAT-d was evaluated noninvasively using NIR_{TRS}. Although NIR_{TRS} has been used for evaluating BAT-d in previous studies [39], comparisons of NIR_{TRS} to ^{18}FDG-PET/CT are limited, and further research is required. NIR_{TRS} could be capable of distinguishing BAT from muscle tissue. In a study [82], tissue optical characteristics are reported to be different for deltoid muscle (μ'_s = 9.6 (9.1, 10.4) (medians (the first quartile, the third quartile] cm^{-1})), (total-Hb) = 114.9 (107.0, 127.7) μM) and for the supraclavicular region (μ'_s = 7.9 (7.2, 8.7) cm^{-1}, (total-Hb) = 60.7 (48.9, 74.7) μM,). The values in the supraclavicular region in this study (μ'_s = 8.3 (7.3, 9.4) cm^{-1}, (total-Hb) = 65.1 (50.4, 85.0) μM) are comparable to those reported earlier [82]. However, there is no concrete evidence from human studies to support the claim that NIR_{TRS} only measures BAT characteristics in the supraclavicular region. Although we attempted to avoid a large muscle such as sternocleidomastoid muscle using an ultrasound guidance, the supraclavicular region comprises other thin muscles such as the omohyoid muscle. Thus, we could not exclude a possibility that NIR_{TRS} measured not only BAT but also slightly thin muscles in this study, which, we believe, had a minor impact on the measurements. In a future study, it is necessary to compare data obtained from tissue biopsy and NIR_{TRS} measurement in humans to improve data accuracy. Although previous

studies have reported no association between [Total-Hb] in the deltoid muscle region (negative control) and BAT activity measured using FDG-PET/CT [39], we have not measured [Total-Hb] in the deltoid muscle region (negative control) in this study.

The [Total-Hb] in the supraclavicular region is an index of BAT microvascular density rather than that of BAT metabolic activity. As it is well known that the [Total-Hb] in the supraclavicular region does not change during a 2-h cold exposure [39], we did not regulate the measurement time of the day and eating status. However, the confounding factors such as aging and cold stimulation, in addition to the effect of the measurement time of the day and eating status along with dietary patterns of the individuals and the consumption of spicy foods, on BAT-d, should be confirmed to improve the data accuracy in future studies.

Second, the physical activity of participants was evaluated using the international physical activity questionnaire (IPAQ). The VPA determined using IPAQ is only 8 METs or higher. According to IPAQ, VPA includes activities such as aerobics, running, fast bicycling, or fast swimming. However, it was not possible to accurately calculate the physical activity intensity or specify the type of exercise. Moreover, because IPAQ evaluates physical activity for longer than 10 minutes, it may not be possible to include a short bout (less than 10 minutes) of high intensity physical activity. Also, in the IPAQ evaluation, there may be a response bias, such as selecting a socially desirable response. Since this study does not assess the indicators of SNS activity, for instance neural activity and blood catecholamine levels, it cannot be concluded whether VPA affects BAT-d via SNS activation. In the future, it is necessary to conduct exercise training interventions that control participant characteristics and exercise mode, intensity, duration, and frequency.

Finally, since we did not measure blood myokines, which may potentially be associated with the increase in BAT-d, we would like to investigate this aspect in future studies. It would be of interest to investigate the relationship between BAT-d and thoracic visceral fat area (epicardial or pericardial fat deposits) in the future following validation of the thoracic visceral fat area using the impedance methods.

5. Conclusions

We conclude that vigorous-intensity physical activities are associated with high BAT-d in humans, especially in men. Furthermore, we confirmed that BAT-d in men is related to visceral fat area and VPA. In the future, we need to investigate the effects of longitudinal high-intensity exercise training on BAT-d by adjusting the exercise mode, intensity, frequency, and duration, as well as the characteristics of the participants.

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References

1. Sidossis, L.; Kajimura, S. Brown and beige fat in humans: Thermogenic adipocytes that control energy and glucose homeostasis. *J. Clin. Investig.* **2015**, *125*, 478–486. [CrossRef] [PubMed]
2. Ikeda, K.; Maretich, P.; Kajimura, S. The Common and Distinct Features of Brown and Beige Adipocytes. *Trends Endocrinol. Metab.* **2018**, *29*, 191–200. [CrossRef] [PubMed]
3. Van Marken Lichtenbelt, W.D.; Vanhommerig, J.W.; Smulders, N.M.; Drossaerts, J.M.A.F.L.; Kemerink, G.J.; Bouvy, N.D.; Schrauwen, P.; Teule, G.J.J. Cold-activated brown adipose tissue in healthy men. *N. Engl. J. Med.* **2009**, *360*, 1500–1508. [CrossRef] [PubMed]

4. Hanssen, M.J.W.; Van Der Lans, A.A.J.J.; Brans, B.; Hoeks, J.; Jardon, K.M.C.; Schaart, G.; Mottaghy, F.M.; Schrauwen, P.; Van Marken Lichtenbelt, W.D. Short-term cold acclimation recruits brown adipose tissue in obese humans. *Diabetes* **2016**, *65*, 1179–1189. [CrossRef] [PubMed]
5. Blondin, D.P.; Daoud, A.; Taylor, T.; Tingelstad, H.C.; Bézaire, V.; Richard, D.; Carpentier, A.C.; Taylor, A.W.; Harper, M.E.; Aguer, C.; et al. Four-week cold acclimation in adult humans shifts uncoupling thermogenesis from skeletal muscles to brown adipose tissue. *J. Physiol.* **2017**, *595*, 2099–2113. [CrossRef]
6. Hibi, M.; Oishi, S.; Matsushita, M.; Yoneshiro, T.; Yamaguchi, T.; Usui, C.; Yasunaga, K.; Katsuragi, Y.; Kubota, K.; Tanaka, S.; et al. Brown adipose tissue is involved in diet-induced thermogenesis and whole-body fat utilization in healthy humans. *Int. J. Obes.* **2016**, *40*, 1655–1661. [CrossRef]
7. Carpentier, A.C.; Blondin, D.P.; Virtanen, K.A.; Richard, D.; Haman, F.; Turcotte, É.E. Brown adipose tissue energy metabolism in humans. *Front. Endocrinol.* **2018**, *9*, 447. [CrossRef]
8. Fedorenko, A.; Lishko, P.V.; Kirichok, Y. Mechanism of fatty-acid-dependent UCP1 uncoupling in brown fat mitochondria. *Cell* **2012**, *151*, 400–413. [CrossRef]
9. Bouillaud, F.; Ricquier, D.; Thibault, J.; Weissenbach, J. Molecular approach to thermogenesis in brown adipose tissue: cDNA cloning of the mitochondrial uncoupling protein. *Proc. Natl. Acad. Sci. USA* **1985**, *82*, 445–448. [CrossRef]
10. Yoneshiro, T.; Aita, S.; Matsushita, M.; Kayahara, T.; Kameya, T.; Kawai, Y.; Iwanaga, T.; Saito, M. Recruited brown adipose tissue as an antiobesity agent in humans. *J. Clin. Investig.* **2013**, *123*, 3404–3408. [CrossRef]
11. Yoneshiro, T.; Matsushita, M.; Saito, M. Translational aspects of brown fat activation by food-derived stimulants. In *Handbook of Experimental Pharmacology*; Springer: Cham, Switzerland, 2019; Volume 251, pp. 359–379.
12. Bartness, T.J.; Vaughan, C.H.; Song, C.K. Sympathetic and sensory innervation of brown adipose tissue. *Int. J. Obes.* **2010**, *34*, S36–S42. [CrossRef] [PubMed]
13. Jimenez, M.; Léger, B.; Canola, K.; Lehr, L.; Arboit, P.; Seydoux, J.; Russell, A.P.; Giacobino, J.P.; Muzzin, P.; Preitner, F. B1/B2/B3-Adrenoceptor Knockout Mice Are Obese and Cold-Sensitive But Have Normal Lipolytic Responses To Fasting. *FEBS Lett.* **2002**, *530*, 37–40. [CrossRef]
14. Lowell, B.B.; Bachman, E.S. β-Adrenergic receptors, diet-induced thermogenesis, and obesity. *J. Biol. Chem.* **2003**, *278*, 29385–29388. [CrossRef] [PubMed]
15. Bachman, E.S.; Dhillon, H.; Zhang, C.Y.; Cinti, S.; Bianco, A.C.; Kobilka, B.K.; Lowell, B.B. βAR signaling required for diet-induced thermogenesis and obesity resistance. *Science* **2002**, *297*, 843–845. [CrossRef]
16. Collins, S.; Cao, W.; Robidoux, J. Learning new tricks from old dogs: β-adrenergic receptors teach new lessons on firing up adipose tissue metabolism. *Mol. Endocrinol.* **2004**, *18*, 2123–2131. [CrossRef]
17. Lean, M. Brown adipose tissue in humans. *Proc. Nutr. Soc.* **1989**, *48*, 243–257. [CrossRef]
18. Osuna-Prieto, F.J.; Martinez-Tellez, B.; Sanchez-Delgado, G.; Aguilera, C.M.; Lozano-Sánchez, J.; Arráez-Román, D.; Segura-Carretero, A.; Ruiz, J.R. Activation of human brown adipose tissue by capsinoids, catechins, ephedrine, and other dietary components: A systematic review. *Adv. Nutr.* **2019**, *10*, 291–302. [CrossRef]
19. Ruiz, J.R.; Martinez-Tellez, B.; Sanchez-Delgado, G.; Osuna-Prieto, F.J.; Rensen, P.C.N.; Boon, M.R. Role of Human Brown Fat in Obesity, Metabolism and Cardiovascular Disease: Strategies to Turn Up the Heat. *Prog. Cardiovasc. Dis.* **2018**, *61*, 232–245. [CrossRef]
20. Yoneshiro, T.; Aita, S.; Matsushita, M.; Okamatsu-Ogura, Y.; Kameya, T.; Kawai, Y.; Miyagawa, M.; Tsujisaki, M.; Saito, M. Age-related decrease in cold-activated brown adipose tissue and accumulation of body fat in healthy humans. *Obesity* **2011**, *19*, 1755–1760. [CrossRef]
21. Froy, O.; Garaulet, M. The circadian clock in white and brown adipose tissue: Mechanistic, endocrine, and clinical aspects. *Endocr. Rev.* **2018**, *39*, 261–273. [CrossRef]
22. Scheele, C.; Nielsen, S. Metabolic regulation and the anti-obesity perspectives of human brown fat. *Redox Biol.* **2017**, *12*, 770–775. [CrossRef] [PubMed]
23. Vosselman, M.J.; Hoeks, J.; Brans, B.; Pallubinsky, H.; Nascimento, E.B.M.; Van Der Lans, A.A.J.J.; Broeders, E.P.M.; Mottaghy, F.M.; Schrauwen, P.; Van Marken Lichtenbelt, W.D. Low brown adipose tissue activity in endurance-trained compared with lean sedentary men. *Int. J. Obes.* **2015**, *39*, 1696–1702. [CrossRef] [PubMed]
24. Singhal, V.; Maffazioli, G.D.; Ackerman, K.E.; Lee, H.; Elia, E.F.; Woolley, R.; Kolodny, G.; Cypess, A.M.; Misra, M. Effect of chronic athletic activity on brown fat in young women. *PLoS ONE* **2016**, *11*, 1–12.

25. Motiani, P.; Virtanen, K.A.; Motiani, K.K.; Eskelinen, J.J.; Middelbeek, R.J.; Goodyear, L.J.; Savolainen, A.M.; Kemppainen, J.; Jensen, J.; Din, M.U.; et al. Decreased insulin-stimulated brown adipose tissue glucose uptake after short-term exercise training in healthy middle-aged men. *Diabetes Obes. Metab.* **2017**, *19*, 1379–1388. [CrossRef] [PubMed]
26. De Matteis, R.; Lucertini, F.; Guescini, M.; Polidori, E.; Zeppa, S.; Stocchi, V.; Cinti, S.; Cuppini, R. Exercise as a new physiological stimulus for brown adipose tissue activity. *Nutr. Metab. Cardiovasc. Dis.* **2013**, *23*, 582–590. [CrossRef]
27. Aldiss, P.; Betts, J.; Sale, C.; Pope, M.; Budge, H.; Symonds, M.E. Exercise-induced 'browning' of adipose tissues. *Metabolism* **2018**, *81*, 63–70. [CrossRef]
28. Peppler, W.T.; Townsend, L.K.; Knuth, C.M.; Foster, M.T.; Wright, D.C. Subcutaneous inguinal white adipose tissue is responsive to, but dispensable for, the metabolic health benefits of exercise. *Am. J. Physiol. Endocrinol. Metab.* **2018**, *314*, E66–E77. [CrossRef]
29. Saito, M.; Okamatsu-Ogura, Y.; Matsushita, M.; Watanabe, K.; Yoneshiro, T.; Nio-Kobayashi, J.; Iwanaga, T.; Miyagawa, M.; Kameya, T.; Nakada, K.; et al. High incidence of metabolically active brown adipose tissue in healthy adult humans: Effects of cold exposure and adiposity. *Diabetes* **2009**, *58*, 1526–1531. [CrossRef]
30. Lee, P.; Smith, S.; Linderman, J.; Courville, A.B.; Brychta, R.J.; Dieckmann, W.; Werner, C.D.; Chen, K.Y.; Celi, F.S. Temperature-acclimated brown adipose tissue modulates insulin sensitivity in humans. *Diabetes* **2014**, *63*, 3686–3698. [CrossRef]
31. Yeung, H.W.D.; Grewal, R.K.; Gonen, M.; Schöder, H.; Larson, S.M. Patterns of 18F-FDG uptake in adipose tissue and muscle: A potential source of false-positives for PET. *J. Nucl. Med.* **2003**, *44*, 1789–1796.
32. Martinez-Tellez, B.; Sanchez-Delgado, G.; Garcia-Rivero, Y.; Alcantara, J.M.A.; Martinez-Avila, W.D.; Muñoz-Hernandez, M.V.; Olza, J.; Boon, M.R.; Rensen, P.C.N.; Llamas-Elvira, J.M.; et al. A new personalized cooling protocol to activate brown adipose tissue in young adults. *Front. Physiol.* **2017**, *8*, 1–10. [CrossRef] [PubMed]
33. Martinez-Tellez, B.; Nahon, K.J.; Sanchez-Delgado, G.; Abreu-Vieira, G.; Llamas-Elvira, J.M.; Van Velden, F.H.P.; Pereira Arias-Bouda, L.M.; Rensen, P.C.N.; Boon, M.R.; Ruiz, J.R. The impact of using BARCIST 1.0 criteria on quantification of BAT volume and activity in three independent cohorts of adults. *Sci. Rep.* **2018**, *8*, 1–8. [CrossRef] [PubMed]
34. Borga, M.; Virtanen, K.A.; Romu, T.; Leinhard, O.D.; Persson, A.; Nuutila, P.; Enerbäck, S. Brown adipose tissue in humans: Detection and functional analysis using PET (positron emission tomography), MRI (magnetic resonance imaging), and DECT (dual energy computed tomography). *Methods Enzymol.* **2014**, *537*, 141–159. [PubMed]
35. Nirengi, S.; Fuse, S.; Amagasa, S.; Homma, T.; Kime, R.; Kuroiwa, M.; Endo, T.; Sakane, N.; Matsushita, M.; Saito, M.; et al. Applicability of supraclavicular oxygenated and total hemoglobin evaluated by near-infrared time-resolved spectroscopy as indicators of brown adipose tissue density in humans. *Int. J. Mol. Sci.* **2019**, *20*, 2214. [CrossRef] [PubMed]
36. Lee, P.; Swarbrick, M.M.; Ho, K.K.Y. Brown adipose tissue in adult humans: A metabolic renaissance. *Endocr. Rev.* **2013**, *34*, 413–438. [CrossRef] [PubMed]
37. Wang, W.; Seale, P. Control of brown and beige fat development. *Nat. Rev. Mol. Cell Biol.* **2016**, *17*, 691–702. [CrossRef]
38. Cinti, S. Symposium on "new perspectives on adipose tissue function": The adipose organ: Morphological perspectives of adipose tissues. *Proc. Nutr. Soc.* **2001**, *60*, 319–328. [CrossRef]
39. Nirengi, S.; Yoneshiro, T.; Sugie, H.; Saito, M.; Hamaoka, T. Human brown adipose tissue assessed by simple, noninvasive near-Infrared time-resolved spectroscopy. *Obesity* **2015**, *23*, 973–980. [CrossRef]
40. Nirengi, S.; Homma, T.; Inoue, N.; Sato, H.; Yoneshiro, T.; Matsushita, M.; Kameya, T.; Sugie, H.; Tsuzaki, K.; Saito, M.; et al. Assessment of human brown adipose tissue density during daily ingestion of thermogenic capsinoids using near-infrared time-resolved spectroscopy. *J. Biomed. Opt.* **2016**, *21*, 091305. [CrossRef]
41. Nirengi, S.; Amagasa, S.; Homma, T.; Yoneshiro, T.; Matsumiya, S.; Kurosawa, Y.; Sakane, N.; Ebi, K.; Saito, M.; Hamaoka, T. Daily ingestion of catechin-rich beverage increases brown adipose tissue density and decreases extramyocellular lipids in healthy young women. *Springerplus* **2016**, *5*, 1363. [CrossRef]
42. Nirengi, S.; Sakane, N.; Amagasa, S.; Wakui, S.; Homma, T.; Kurosawa, Y.; Hamaoka, T. Seasonal differences in brown adipose tissue density and pulse rate variability in a thermoneutral environment. *J. Physiol. Anthropol.* **2018**, *37*, 6. [CrossRef]

43. Katayama, K.; Saito, M. Muscle sympathetic nerve activity during exercise. *J. Physiol. Sci.* **2019**, *69*, 589–598. [CrossRef] [PubMed]
44. Robinson, B.F.; Epstein, S.E.; Beiser, G.D.; Braunwald, E. Control of heart rate by the autonomic nervous system. Studies in man on the interrelation between baroreceptor mechanisms and exercise. *Circ. Res.* **1966**, *19*, 400–411. [CrossRef] [PubMed]
45. Zouhal, H.; Jacob, C.; Delamarche, P.; Gratas-Delamarche, A. Catecholamines and the effects of exercise, training and gender. *Sport. Med.* **2008**, *38*, 401–423. [CrossRef] [PubMed]
46. Christensen, N.J.; Galbo, H.; Hansen, J.F.; Hesse, B.; Richter, E.A.; Trap-Jensen, J. Catecholamines and exercise. *Diabetes* **1979**, *28*, 58–62. [CrossRef] [PubMed]
47. Saito, M.; Tsukanaka, A.; Yanagihara, D.; Mano, T. Muscle sympathetic nerve responses to graded leg cycling. *J. Appl. Physiol.* **1993**, *75*, 663–667. [CrossRef]
48. Sanchez-Delgado, G.; Martinez-Tellez, B.; Olza, J.; Aguilera, C.M.; Gil, Á.; Ruiz, J.R. Role of exercise in the activation of brown adipose tissue. *Ann. Nutr. Metab.* **2015**, *67*, 21–32. [CrossRef]
49. Flouris, A.D.; Dinas, P.C.; Valente, A.; Andrade, C.M.B.; Kawashita, N.H.; Sakellariou, P. Exercise-induced effects on UCP1 expression in classical brown adipose tissue: A systematic review. *Horm. Mol. Biol. Clin. Investig.* **2017**, *31*, 1–13. [CrossRef]
50. Lehnig, A.C.; Stanford, K.I. Exercise-induced adaptations to white and brown adipose tissue. *J. Exp. Biol.* **2018**, *221*, jeb161570. [CrossRef]
51. Pagnotti, G.M.; Styner, M. Exercise regulation of marrow adipose tissue. *Front. Endocrinol.* **2016**, *7*, 94. [CrossRef]
52. Stanford, K.I.; Middelbeek, R.J.W.; Goodyear, L.J. Exercise effects on white adipose tissue: Beiging and metabolic adaptations. *Diabetes* **2015**, *64*, 2361–2368. [CrossRef] [PubMed]
53. Dinas, P.C.; Lahart, I.M.; Timmons, J.A.; Svensson, P.A.; Koutedakis, Y.; Flouris, A.D.; Metsios, G.S. Effects of physical activity on the link between PGC-1a and FNDC5 in muscle, circulating Irisin and UCP1 of white adipocytes in humans: A systematic review. *F1000Research* **2017**, *6*, 286. [CrossRef] [PubMed]
54. Marlatt, K.L.; Ravussin, E. Brown Adipose Tissue: An Update on Recent Findings. *Curr. Obes. Rep.* **2017**, *6*, 389–396. [CrossRef] [PubMed]
55. Dewal, R.S.; Stanford, K.I. Effects of exercise on brown and beige adipocytes. *Biochim. Biophys. Acta Mol. Cell Biol. Lipids* **2019**, *1864*, 71–78. [CrossRef] [PubMed]
56. Fuse, S.; Nirengi, S.; Amagasa, S.; Homma, T.; Kime, R.; Endo, T.; Sakane, N.; Matsushita, M.; Saito, M.; Yoneshiro, T.; et al. Brown adipose tissue density measured by near-infrared time-resolved spectroscopy in Japanese, across a wide age range. *J. Biomed. Opt.* **2018**, *23*, 1. [CrossRef] [PubMed]
57. Gunadi, S.; Leung, T.S.; Elwell, C.E.; Tachtsidis, I. Spatial sensitivity and penetration depth of three cerebral oxygenation monitors. *Biomed. Opt. Express* **2014**, *5*, 2896. [CrossRef]
58. Flynn, A.; Li, Q.; Panagia, M.; Abdelbaky, A.; Macnabb, M.; Samir, A.; Cypess, A.M.; Weyman, A.E.; Tawakol, A.; Scherrer-Crosbie, M. Contrast-Enhanced Ultrasound: A Novel Noninvasive, Nonionizing Method for the Detection of Brown Adipose Tissue in Humans. *J. Am. Soc. Echocardiogr.* **2015**, *28*, 1247–1254. [CrossRef]
59. Niwayama, M.; Hamaoka, T.; Lin, L.; Shao, J.; Kudo, N.; Katoh, C.; Yamamoto, K. Quantitative muscle oxygenation measurement using NIRS with correction for the influence of a fat layer: Comparison of oxygen consumption rates with measurements by other techniques. *Biomed. Diagnostic Guid. Surg. Syst.* **2000**, *3911*, 256–265.
60. IPAQ (2005) Guidelines for Data Processing and Analysis of the International Physical Activity Questionnaire (IPAQ)—Short and Long Forms, revised on November 2005. *Ipaq* **2005**. Available online: https://www.researchgate.net/file.PostFileLoader.html?id=5641f4c36143250eac8b45b7&assetKey=AS%3A294237418606593%401447163075131 (accessed on 17 April 2020).
61. Ministry of Health, Labour and Welfare (Japan). *Report of the review meeting on revision of exercise standards and exercise guidelines*; 2013. Available online: https://www.mhlw.go.jp/content/000306883.pdf (accessed on 17 April 2020).
62. Lee, S.Y.; Ahn, S.; Kim, Y.J.; Ji, M.J.; Kim, K.M.; Choi, S.H.; Jang, H.C.; Lim, S. Comparison between dual-energy x-ray absorptiometry and bioelectrical impedance analyses for accuracy in measuring whole body muscle mass and appendicular skeletal muscle mass. *Nutrients* **2018**, *10*, 738. [CrossRef]
63. Sullivan, P.A.; Still, C.D.; Jamieson, S.T.; Dixon, C.B.; Irving, B.A.; Andreacci, J.L. Evaluation of multi-frequency bioelectrical impedance analysis for the assessment of body composition in individuals with obesity. *Obes. Sci. Pract.* **2019**, *5*, 141–147. [CrossRef] [PubMed]

64. Martinez-Tellez, B.; Sanchez-Delgado, G.; Amaro-Gahete, F.J.; Acosta, F.M.; Ruiz, J.R. Relationships between cardiorespiratory fitness/muscular strength and 18F-fluorodeoxyglucose uptake in brown adipose tissue after exposure to cold in young, sedentary adults. *Sci. Rep.* **2019**, *9*, 11314. [CrossRef]
65. Quarta, C.; Mazza, R.; Pasquali, R.; Pagotto, U. Role of sex hormones in modulation of brown adipose tissue activity. *J. Mol. Endocrinol.* **2012**, *49*, 1–7. [CrossRef]
66. Adler, E.S.; Hollis, J.H.; Clarke, I.J.; Grattan, D.R.; Oldfield, B.J. Neurochemical characterization and sexual dimorphism of projections from the brain to abdominal and subcutaneous white adipose tissue in the rat. *J. Neurosci.* **2012**, *32*, 15913–15921. [CrossRef] [PubMed]
67. Uchida, K.; Sun, W.; Yamazaki, J.; Tominaga, M. Role of thermo-sensitive transient receptor potential channels in brown adipose tissue. *Biol. Pharm. Bull.* **2018**, *41*, 1135–1144. [CrossRef] [PubMed]
68. Morrison, S.F.; Nakamura, K. Central Mechanisms for Thermoregulation. *Annu. Rev. Physiol.* **2019**, *81*, 285–308. [CrossRef] [PubMed]
69. Nakamura, K.; Morrison, S.F. A thermosensory pathway that controls body temperature. *Nat. Neurosci.* **2008**, *11*, 62–71. [CrossRef]
70. Nakamura, K.; Morrison, S.F. A thermosensory pathway mediating heat-defense responses. *Proc. Natl. Acad. Sci. USA* **2010**, *107*, 8848–8853. [CrossRef]
71. Spranger, M.D.; Krishnan, A.C.; Levy, P.D.; O'Leary, D.S.; Smith, S.A. Blood flow restriction training and the exercise pressor reflex: A call for concern. *Am. J. Physiol. Heart Circ. Physiol.* **2015**, *309*, H1440–H1452. [CrossRef]
72. Fischer, C.P. Interleukin-6 in acute exercise and training: What is the biological relevance? *Exerc. Immunol. Rev.* **2006**, *12*, 6–33.
73. Yamagata, K.; Matsumura, K.; Inoue, W.; Shiraki, T.; Suzuki, K.; Yasuda, S.; Sugiura, H.; Cao, C.; Watanabe, Y.; Kobayashi, S. Coexpression of microsomal-type prostaglandin E synthase with cyclooxygenase-2 in brain endothelial cells of rats during endotoxin-induced fever. *J. Neurosci.* **2001**, *21*, 2669–2677. [CrossRef]
74. Nakamura, K. Central circuitries for body temperature regulation and fever. *Am. J. Physiol. Regul. Integr. Comp. Physiol.* **2011**, *301*, R1207–R1228. [CrossRef] [PubMed]
75. Boström, P.; Wu, J.; Jedrychowski, M.P.; Korde, A.; Ye, L.; Lo, J.C.; Rasbach, K.; Boström, E.A.; Choi, J.H.; Long, J.Z.; et al. A PGC1a dependent myokine that derives browning of white fat and thermogenesis. *Nature* **2012**, *481*, 463–468. [CrossRef] [PubMed]
76. Kim, H.J.; Lee, H.J.; So, B.; Son, J.S.; Yoon, D.; Song, W. Effect of aerobic training and resistance training on circulating irisin level and their association with change of body composition in overweight/obese adults: A pilot study. *Physiol. Res.* **2016**, *65*, 271–279. [CrossRef]
77. Otero-Díaz, B.; Rodríguez-Flores, M.; Sánchez-Muñoz, V.; Monraz-Preciado, F.; Ordoñez-Ortega, S.; Becerril-Elias, V.; Baay-Guzmán, G.; Obando-Monge, R.; García-García, E.; Palacios-González, B.; et al. Exercise Induces White Adipose Tissue Browning Across the Weight Spectrum in Humans. *Front. Physiol.* **2018**, *9*, 1781. [CrossRef]
78. Malo, A.; Puerta, M. Oestradiol and progesterone change β3-adrenergic receptor affinity and density in brown adipocytes. *Eur. J. Endocrinol.* **2001**, *145*, 87–91. [CrossRef]
79. Monjo, M.; Rodríguez, A.M.; Palou, A.; Roca, P. Direct Effects of Testosterone, 17β-Estradiol, and Progesterone on Adrenergic Regulation in Cultured Brown Adipocytes: Potential Mechanism for Gender-Dependent Thermogenesis. *Endocrinology* **2003**, *144*, 4923–4930. [CrossRef]
80. Yamamoto, Y.; Hughson, R.L.; Nakamura, Y. Autonomic nervous system responses to exercise in relation to ventilatory threshold. *Chest* **1992**, *101*, 206S–210S. [CrossRef]
81. Nakamura, Y.; Yamamoto, Y.; Muraoka, I. Autonomic control of heart rate during physical exercise and fractal dimension of heart rate variability. *J. Appl. Physiol.* **1993**, *74*, 875–881. [CrossRef]
82. Fuse, S.; Hamaoka, T.; Kuroiwa, M.; Kime, R.; Endo, T.; Tanaka, R.; Amagasa, S.; Kurosawa, Y. Identification of human brown/beige adipose tissue using near-infrared time-resolved spectroscopy. In Proceedings of the SPIE; Available online: https://www.spiedigitallibrary.org/conference-proceedings-of-spie/11237/2545273/Identification-of-human-brown-beige-adipose-tissue-using-near-infrared/10.1117/12.2545273.short?SSO=1 (accessed on 17 April 2020).