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Keywords: Caffeine; Brown adipose tissue; Infrared thermography; Aerobic exercise; Metabolism

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Acute Effects of Caffeine Supplementation on Metabolism in Individuals with High or Low Brown Adipose Tissue Activation During and After High-Intensity Interval Training

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Abstract: Aims: to measure whether subjects with high (HBAT) and low activation of brown adipose tissue (LBAT) showed acute differences in energy expenditure during and after 30-minutes of high intense interval training. **Methods:** Forty professional soccer athletes were invited to participate in the study, of which 37 started the protocol and 35 completed it. This was an acute, double-blind, case-control, quasi-experimental study design. Activation of BAT was estimated to be using infrared thermography. All participants were divided into four groups and compared by condition: (a) HBAT-CAF; (b) HBAT-PLA; (c) LBAT-CAF; and (d) LBAT-PLA. They performed 30 min of high-intensity interval training (HIIT) which included four sets of 4-minute of running in a speed corresponding at 90–95% of HRmax, each followed by a 3-minute active recovery interval at 60–70% of HRmax and 30 min of passive recovery. During exercise and recovery, breath-to-breath spirometry was performed and energy expenditure (EE), carbohydrates (g/day), fats (g/day) and proteins (g/day) were measured. **Results:** HBAT showed higher EE at all measurement times ($p \leq 0.01$) in the CAF condition and at all measurement times in the PLA condition ($p \leq 0.001$). For fat catabolism, the CAF condition showed a significant difference between conditions, with CAF showing higher catabolism at 60 min [36.5 g/day (0.8; 137.2); $p=0.047$] compared to PLA. For PTN catabolism, the HBAT-CAF condition, minutes 40, 50, and 60 showed a significant difference compared to the other measurement times ($p \leq 0.023$ for all comparisons). **Conclusion:** After high-intensity interval exercise with CAF supplementation, both HBAT and LBAT subjects experience a significant increase in EE during exercise and recovery compared to baseline. Basal BAT activity did not affect the rate of CHO and FAT oxidation, although a higher rate of PTN utilization was observed in the HBAT-CAF group.

Keywords: caffeine; brown adipose tissue; infrared thermography; aerobic exercise; metabolism

1. Introduction

Overweight and obesity are chronic conditions marked by inflammatory processes driven by excess adipose tissue accumulation relative to height, sex, and age [1]. Emerging research highlights brown adipose tissue (BAT) as a metabolically active organ with thermogenic and endocrine functions. BAT secretes signaling molecules termed "batokines," which modulate systemic

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metabolism by influencing skeletal muscle, liver, pancreatic, and neural activity [2,3]. These interactions enhance glucose and fatty acid uptake in muscle, suppress hepatic lipogenesis, improve beta-cell function, and mitigate cardiovascular strain, collectively promoting metabolic health and aiding in the management of obesity, dyslipidemia, and hypertension [3–5]. Despite advances in understanding obesity pathogenesis, gaps persist in translating BAT's therapeutic potential. It has been probed that activating as little as 50g of BAT may elevate metabolic rate by up to 25%, offering a promising anti-obesity strategy [6].

Brown adipose tissue, once thought exclusive to infants, is now known to persist in adults, as demonstrated by 18F-fludeoxyglucose positron emission tomography (18F-FDG PET/CT) imaging [7]. While 18F-FDG PET/CT remains the gold standard for identifying metabolically active BAT through glucose analog uptake, its clinical utility is limited by radiation exposure and cost [8]. For the other side, Infrared thermography (IRT) presents a non-invasive, low-cost alternative by measuring skin temperature in BAT-rich regions, such as the supraclavicular area, where superficial deposits are abundant [9,10]. IRT quantifies heat emission (a proxy for BAT thermogenesis) before, during, or after cold or pharmacological stimulation, with validation against PET/CT confirming its reliability [11,12].

BAT thermogenesis is driven by mitochondrial uncoupling protein 1 (UCP-1), which dissipates energy as heat [13]. Ergogenic compounds like capsaicin analogs and capsinoids enhance UCP-1 activity, boosting energy expenditure [14,15]. Similarly, CAF (a widely consumed adenosine receptor antagonist) stimulates BAT via β-adrenergic pathways, upregulating UCP-1, PPARγ, and PGC-1α to promote mitochondrial biogenesis and thermogenesis [6,16]. These mechanisms align with caffeine's observed effects on weight loss and metabolic rate increase [16].

Beside caffeine, exercise stimulate energy expenditure through sympathetic nervous system (SNS) activation, which enhances skeletal muscle metabolism and BAT thermogenesis [5,15]. SNS-mediated norepinephrine release binds β-adrenergic receptors, upregulating UCP-1 and PGC-1α via the p38MAPK pathway [17]. Exercise also elevates adipocyte triglyceride lipase and hormone-sensitive lipase activity, promoting lipid mobilization and white adipose tissue browning [18]. Concurrently, exercise-induced irisin secretion stimulates WAT thermogenesis through mammalian p38 mitogen-activated protein kinase signaling, further linking physical activity to metabolic health [17]. Despite growing interest in BAT activation strategies, limited research explores exercise's acute effects on BAT-mediated energy expenditure, particularly in combination with ergogenic supplements. This study investigates whether individuals with high (HBAT) versus low BAT activity (LBAT) exhibit differential energy expenditure during and after 28 minutes of HIIT with or without CAF supplementation. We hypothesized that individuals HBAT-CAF would exhibit greater EE and fat catabolism compared to HBAT-PLA, LBAT-CAF and LBAT-PLA.

2. Materials and Methods

Experimental Approach

This study employed a quasi-experimental acute protocol. On the first day of data collection, participants were categorized into either the HBAT or LBAT group based on the thermographic protocol established by Nirengi, *et al.* [19]. On the next day, a researcher not involved in data collection randomly assigned participants to either the CAF or PLA conditions. Participants then underwent a HIIT protocol lasting 30 minutes, followed by 30 minutes of passive recovery. On the third day (seven days after the second session) the conditions were reversed (crossover design), ensuring that all participants experienced both CAF and PLA conditions. This approach ensured that the study was conducted in a double-blind manner, minimizing bias. Throughout both exercise and recovery periods, participants' physiological responses were monitored using a spirometry protocol to collect relevant data.

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Participants

To achieve the objectives of the present study, the researchers established the following inclusion criteria: (a) male; (b) aged 18 years or older; (c) physically active, with a training frequency of at least four times per week; (d) free from any injuries that could impair test performance; and (e) not using stimulant or vasodilator medications. From the initial pool of participants, those who (a) did not complete all stages of the study for any reason, or (b) had errors during the collection or processing of spirometry data were excluded. Forty professional soccer players were invited to participate in the study, of whom 37 began the protocol and 35 completed it. The groups exhibited the following anthropometric characteristics: (a) HBAT: 26.5±4.3 years; 1.7±0.1 m; 77.4±7.2 kg; 25.5±1.8 kg/m²; 15.7±2.0% body fat; and (b) LBAT: 27.0±4.1 years; 1.7±0.1 m; 79.0±8.1 kg; 26.0±1.7 kg/m²; 15.9±4.5% body fat. No significant differences were observed between the two groups for these variables ($p \geq 0.05$).

BAT Protocol and Classification

The classification of participants regarding BAT activity was estimated using a thermographic protocol validated by positron emission tomography – PET-CT (gold-standard) [19]. In this protocol, participants (wearing only shorts) remained seated in a climate-controlled room at 19°C and 48% of humidity for 60 minutes. After this period, two thermal images of the upper trunk region were captured following the thermographic imaging protocol proposed by Moreira, *et al.* [20]. The highest-quality image was selected for analysis. BAT activity was calculated based on the temperature difference between the mean of the supraclavicular region and a reference external region (control). Participants with a temperature difference of ≥1.03°C were classified into the HBAT group. This cut-off demonstrated a specificity of 84.6%, sensitivity of 85.7%, and accuracy of 85.4% [19].

A thermal imaging camera (FLIR T335, FLIR Systems, Sweden) with a resolution of 320x240 pixels, a spectral range of 7.5 to 13.0 μm, an image frequency of 30 Hz, a thermal sensitivity of 50 mK at 30°C, and an accuracy of ±2% was used for registering the thermograms. The emissivity values were set at 0.98, corresponding to the emissivity of human skin. A thermographic image (thermogram) was captured, including the anterior cervical and supraclavicular regions, at 0, 10, 20, 30, 40, 50, and 60 minutes after starting the exercise protocol. Figure 1 illustrates an example of the regions of interest selected for the BAT activity estimation by infrared radiation.

To obtain Tsk data from the recorded thermograms, FLIR Tool+ analysis software (FLIR, Sweden) was used. With this software and following the procedure described by Yoneshiro, *et al.* [21], regions of interest (ROI) were delimited by selecting two 21 x 21 pixel circles, one above the left median clavicular region (1) and the other in the upper thorax (2). The ROIs were selected on the left side of the subject because previous studies indicate that the pulmonary vascular bundle influences the results on the right side of the subject.

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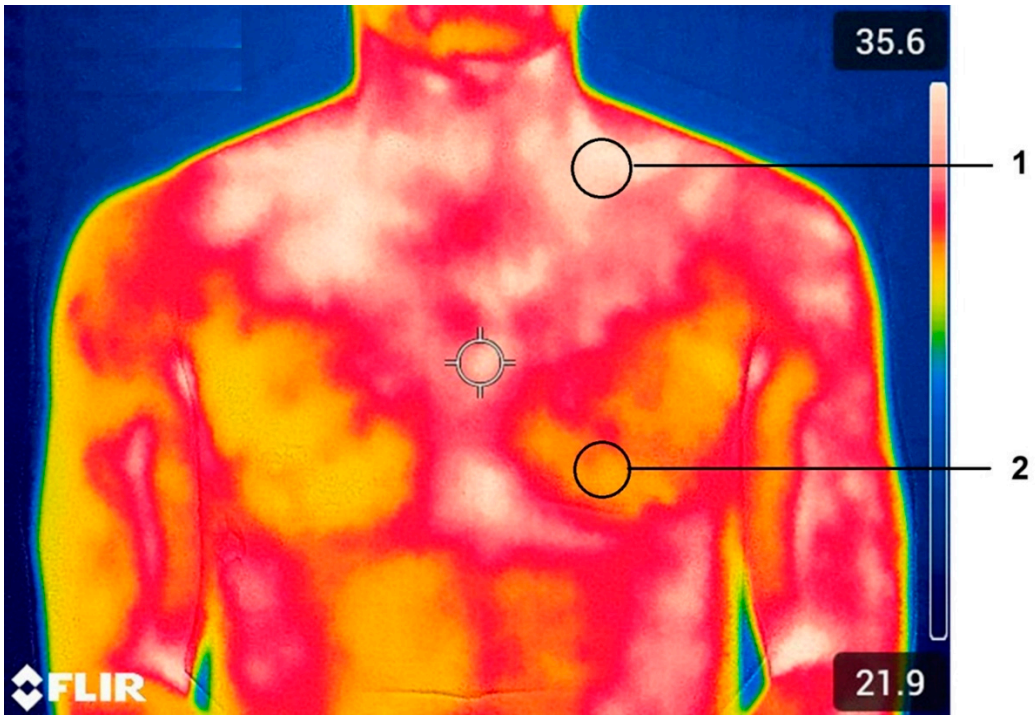

Figure 1. Example of BAT activity estimation. 1: supraclavicular region of interest and 2: external region of interest.

Pre-Exercise Breakfast and Supplement

Data collection was conducted always in the early morning to minimize variability and control for potential circadian influences. Participants were instructed to arrive at the laboratory at 07:00 a.m., where they were provided with a standardized breakfast totaling approximately 320 kcal. The meal included a medium banana (≈90 kcal), two slices of whole-wheat bread (140 kcal) with peanut butter (≈90 kcal), and lemon-flavored water (0 kcal). Concurrently with breakfast, participants were administered a capsule containing either (a) CAF (375 mg, ≈4.8 mg/kg body mass) or (b) PLA (maltodextrin), depending on their assigned experimental condition. This protocol ensured that all participants began the study under consistent nutritional and physiological conditions.

High Intensity Interval Training, Recovery Protocol and Spirometric Measurements

One hour after consuming breakfast and the randomized capsule intake, participants performed a HIIT protocol adapted from Tjønna, *et al.* [22]. The protocol comprised four 4-minute sprints at 90–95% of maximum heart rate (HRmax), each followed by a 3-minute active recovery interval at 60–70% of HRmax. While the original protocol spanned 28 minutes, the final active recovery interval was extended by 2 minutes in this study to standardize the total measurement duration at 30 minutes. HRmax was estimated using the formula established by Roy and Mccrory [23]: [HRmax = 208 − (0.7 × age)].

Following the HIIT session, participants rested in a supine position for 30 minutes while recovery metabolism was assessed via indirect calorimetry. A portable breath-by-breath gas analyzer (Metalyzer 3BR3®, Cortex, Leipzig, Germany) was used to collect metabolic data at seven time points: 0, 10, 20, 30, 40, 50, and 60 minutes. Measurements from 0–30 minutes corresponded to the effort phase, while the subsequent 30 minutes represented the recovery phase. Prior to each session, the device was calibrated in accordance with the manufacturer's specifications. Throughout the experiment, oxygen consumption and carbon dioxide production were continuously monitored to estimate total EE (kcal) and substrate utilization rates (g/day) for CHO, FAT and PTN. CHO and FAT oxidation rates were derived using Frayn's stoichiometric equations Alcantara, *et al.* [24], whereas

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PTN catabolism was calculated via the analyzer's proprietary software (Metasoft® Studio 5.5.1, Cortex, Leipzig, Germany).

Data Analysis

Data were initially organized and managed in spreadsheets using Microsoft Excel 2024 (Microsoft Corporation, Redmond, WA, USA). Statistical analyses were conducted via generalized estimating equations, a method suited for clustered data, as outlined by Ballinger [25]. When significant main or interaction effects were identified in the models, pairwise comparisons were adjusted for multiple testing using the Bonferroni post-hoc procedure. All analyses were performed in IBM SPSS Statistics (Version 25.0, IBM Corporation, Armonk, NY, USA), with statistical significance defined a priori as $p \leq 0.05$.

3. Results

For EE we observed an interactive effect between Group X Supplement X Moment of measurement (W = 12.745; GL = 6; p = 0.047). For moment of measurement comparison HBAT-CAF group showed differences at 0 min vs. 40 min [1,018.3 kcal (632.0; 1,404.6); p≤0.001], 50 min [2,276.0 kcal (1,997.9; 2,554.1); p≤0.001] and 60 min [2,411.5 kcal (2,107.0; 2,715.9); p≤0.001], at 10 min vs. 40 min [1,010.6 kcal (691.2; 1,330.0); p≤0.001], 50 min [2,268.3 kcal (2,039.2; 2,497.3); p≤0.001] and 60 min [2,403.7 kcal (2,171.0; 2,636.4); p≤0.001], at 20 min vs. 30 min [-295.5 kcal (-521.7; -68.7); p=0.011], 40 min [900.2 kcal (549.4; 1,251.0); p≤0.001], 50 min [2,157.9 kcal (1,892.6; 2,423.1); p≤0.001] and 60 min [2,293.4 kcal (2,001.5; 2,585.2); p≤0.001], at 30 min vs. 40 min [1195.4 kcal (857.3; 1,533.5); p≤0.001], 50 min [2,453.1 kcal (2212.1; 2,694.1); p≤0.001] and 60 min [2,588.6 kcal (2,322.6; 2,854.5); p≤0.001], and at 50min vs. 60 min [135.5 kcal (41.7; 229.2); p=0.05].

Comparations of the HBAT-PLA group results by moment showed differences at 0 min vs. 30 min [-433.9 kcal (-810.3; -57.5); p=0.024], 40 min [707.2 kcal (286.0; 1,128.3); p=0.001], 50 min [1,167.1 kcal (1378.1; 1,976.1); p≤0.001] and 60 min [1613.7 kcal (1297.2; 1930.3); p≤0.001], at 10 min vs. 20 min [-359.5 kcal (-642.9; -76.2); p=0.013], 30 min [-584.7 kcal (-920.7; -248.7); p=0.001], 40 min [556.4 kcal (239.5; 873.3); p=0.001], 50 min [1,526.3 kcal (1246.4; 1,806.3); p≤0.001] and 60 min [1463.0 kcal (1206.0; 1720.0); p≤0.001], at 20 min vs. 30 min [-225.1 kcal (-425.5; -24.8); p=0.028], 40 min [915.9 kcal (631.5; 1,200.4); p≤0.001], 50 min [1,885.9 kcal (1,668.7; 2,103.1); p≤0.001] and 60 min [1,822.5 kcal (1,571.7; 2,073.3); p≤0.001], at 30 min vs. 40 min [1141.1 kcal (790.9; 1,491.2); p≤0.001], 50 min [2,111.0 kcal (1,823.2; 2,398.9); p≤0.001] and 60 min [2,047.6 kcal (1782.2; 2,313.1); p≤0.001], and at 40 min vs. 50 min [970.0 kcal (746.8; 1,193.1); p≤0.001] and 60 min [906.6 kcal (624.3; 1,188.9); p≤0.001].

LBAT-CAF group showed differences by moment at 0 min and other moments of measurement (p≤0.043) except 10 min [-200.2 kcal (-567.5; 167.1); p=0.285], at 10 min showed differences vs. the other moments of measurement (p≤0.01) except 30 min [-274.4 kcal (-563.5; 14.7); p=0.063], at 20 min showed difference vs. the other moments of measurement (p≤0.01) except 30 min [62.7 kcal (-201.2; 327.0); p=0.641], at 30 min showed significative differences vs. 40 min [1057.7 kcal (552.1; 1,563.4); p≤0.001], 50 min [2107.1 kcal (1724.0; 2,490.2); p≤0.001] and 60 min [2,150.1 kcal (1,754.8; 2,545.3); p≤0.001], and at 40 min showed significative differences vs. 50 min [1049.3 kcal (798.0; 1,300.7); p≤0.001] and 60 min [1,092.3 kcal (880.9; 1,303.7); p≤0.001].

Finally, the LBAT-PLA group showed a significative differences at 0 min vs. all moments of measurement (p≤0.023) except 10 min [69.8 kcal (-272.6; 412.2); p=0.69] and 40 min [266.5 kcal (-123.5; 656.6); p=0.18], at 10 min vs. all moments of measurement except 40 min [196.8 kcal (-378.7; 772.2); p=0.503], at 20 min vs. all moments of measurement except 30 min [52.0 kcal (-326.5; 430.5); p=0.788], at 30 min vs. 40 min [719.2 kcal (74.8; 1,363.6); p=0.029], 50 min [1833.9 kcal (1382.9; 2,285.0); p≤0.001] and 60 min [1977.1 kcal (1,569.3; 2,384.9); p≤0.001], at 40 min showed significative differences vs. 50 min [1114.7 kcal (824.9; 1,404.6); p≤0.001] and 60 min [1,257.9 kcal (843.6; 1,672.1); p≤0.001].

In CAF condition HBAT group showed a significative high EE vs. LBAT group in all moments of measurement except 20 min [273.0 kcal (-86.6; 632.7); p=0.137], 50 min [284.9 kcal (-8.8; 578.6); p=0.057] and 60 min [192.5 kcal (-104.8; 489.7); p=0.204]. In PLA condition HBAT showed a higher EE

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vs. LBAT at 0 min [466.4 kcal (66.7; 866.1); p=0.022] and 60 min [377.1 kcal (162.6; 591.6); p=0.001]. Finally, regardless of group, CAF showed a higher EE in all moments of measurement (p≤0.014). Figure 2 shows the EE for all groups and conditions.

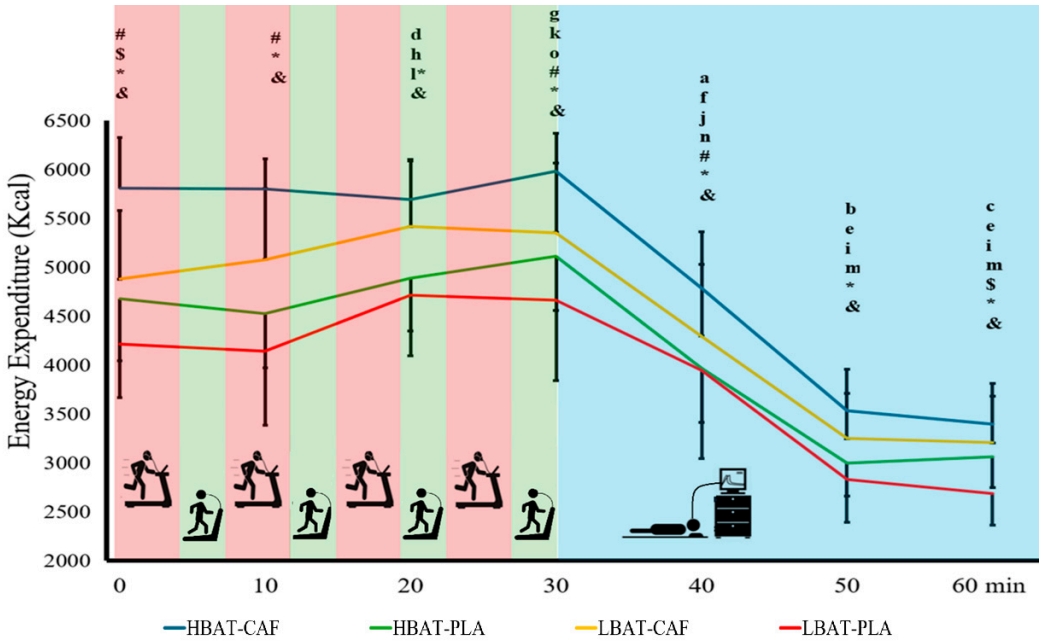

Figure 2. EE along the experimental time. Red zone – sprint (90-95% of maximum heart rate); green zone – active interval (60-70% of maximum heart rate); blue zone – recovery. HBAT-CAF = a p≤0.001 this moment of measurement vs. the others; b p≤0.05 this moment of measurement vs. the others; c p≤0.05 this moment of measurement vs. the others; d p=0.011 this moment of measurement vs. 30 min. HBAT-PLA = e p≤0.001 this moment of measurement vs. the others. f p≤0.001 this moment of measurement vs. the others. g p≤0.028 this moment of measurement vs. the others; h p=0.013 this moment of measurement vs. 10 min. LBAT-CAF = i p≤0.001 this moment of measurement vs. the others; j p≤0.003 this moment of measurement vs. the others; k p=0.043 this moment of measurement vs. 10 min; l p≤0.01 this moment of measurement vs. 10 min and 0 min. LBAT-PLA = m p≤0.001 this moment of measurement vs. the others; n p≤0.029 this moment of measurement vs. the others except 10 min; o p≤0.007 this moment of measurement vs. the others except 20 min; p p≤0.05 this moment of measurement vs. 10 min and 0 min. # p≤0.026 HBAT-CAF vs LBAT-CAF. $ p≤0.022 HBAT-PLA vs LBAT-PLA. * p≤0.001 HBAT-CAF vs HBAT-PLA. & p≤0.014 LBAT-CAF vs LBAT-PLA.

For CHO catabolism, there were significative interaction between Condition X moments of measurement (W = 15,574; GL = 6; p = 0.016). CAF showed significative differences between 50 min vs all moments of measurement except 60 min [46.3 g/day (-3.8; 96.5); p=0.105]. Similarly for PLA we observed significative differences between 50 min vs all moments of measurement except 60 min [-6.0 g/day (-60.0; 48.1); p=1.0]. When compared to PLA, CAF presents a higher catabolism at 0 min [207.3 g/day (86.8; 327.7); p=0.001], 10 min [172.2 g/day (47.5; 296.9); p=0.007], 40 min [136.8 g/day (56.6; 217.0); p=0.001] and 50 min [69.0 g/day (0.8; 137.2); p=0.047].

To FAT catabolism we observed an interaction effect between Condition X moments of measurement (W = 19,403; GL = 6; p = 0.004). For moments of measurement CAF showed a significative difference between 60 min vs. 0 min [63.1 g/day (8.5; 117.7); p=0.009], 10 min [66.8 g/day (12.3; 121.3); p=0.004] and 40 min [104.2 g/day (71.6; 136.7); p≤0.001]. For significative differences between Conditions, CAF showed a higher catabolism at 60 min [36.5 g/day (0.8; 137.2); p=0.047] when compared to PLA.

About PTN catabolism, we find an interactive effect between Group X Supplement X Moment of measurement (W = 14.783; GL = 6; p = 0.022). For HBAT-CAF 40, 50 and 60 min showed a significative difference for the other moments of measurement (p≤0.023 for all comparisons). HBAT-PLA present difference 0 min vs other moments of measurement (p≤0.01) except 10 (p=0.111) and 20

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min (p=0.144). 10 min showed difference vs. other moments (p≤0.002) except 40 min (p=0.082). 50 and 60 min showed difference vs. others (p≤0.001). For LBAT-CAF we observed differences between 40 min and other moments of measurement (p≤0.015). 50 and 60 min showed difference vs. others (p≤0.001). 20 min showed difference vs. 0 [6.9 g/day (2.3; 11.5); p=0.003] and 10 min [4.7 g/day (1.5; 8.0); p=0.004]. For LBAT-PLA 50 and 60 min showed difference vs. others (p≤0.001). 40 min showed difference vs others (p≤0.042) except 10 min (p=0.139). 0 min present difference vs. 20 [-9.3 g/day (-14.3; -4.3); p≤0.001] and 30 min [-17.1 g/day (-32.3; -2.0); p=0.027]. 10 present difference vs. 20 min [-9.0 g/day (-14.4; -3.6); p=0.001]. For Group and Condition comparison, HBAT-CAF showed a significative difference vs HBAT-PLA at all moments of measurement (p≤0.01), except 20 min (p=0.57). LBAT-CAF presents a significant difference in all moments of measurement (p≤0.002) vs. LBAT-PLA, except 20 (p=0.122) and 30 min (p=0.367).

Table 2. Carbohydrates, lipids and protein catabolism along the experiment. Data presented by means ± standard deviation and 95% CI.

	Moments of measurement						
Group-Condition	**0 min**	**10 min**	**20 min**	**30 min**	**40 min**	**50 min**	**60 min**
				CHO (g/day)			
HBAT-CAF	1,021.6±205.5* (917.6; 1,125.6)	1,119.0±123.2* (1,056.6; 1,181.4)	1,052.0±223.9 (938.6; 1,165.3)	1,066.8±190.8 (970.2; 1,163.3)	950.2±233.9* (831.8; 1,068.6)	527.8±184.5a* (434.4; 621.1)	457.1±123.6a (394.5; 519.7)
LBAT-CAF	894.5±217.1* (784.6; 1,004.4)	900.3±250.7* (773.4; 1,027.1)	1,065.1±522.2 (800.8; 1,329.4)	962.8±231.8 (845.5; 1,080.1)	797.9±138.4* (727.9; 867.9)	457.1±165.8a* (373.2; 541.0)	415.4±165.6a (331.6; 499.3)
HBAT-PLA	1,018.1±268.8 (900.3; 1,135.9)	966.5±350.3 (813.0; 1,120.0)	1,019.2±356.5 (863.0; 1,175.4)	1,117.9±722.4 (861.3; 1,494.5)	891.3±222.2 (794.0; 988.7)	402.3±167.2a (329.0; 475.6)	380.3±160.1a (310.1; 450.4)
LBAT-PLA	730.7±368.9 (569.0; 892.3)	840.8±226.3 (741.7; 940.0)	821.3±307.6 (686.6; 956.1)	1,119.5±797.9 (769.8; 1,469.2)	770.1±196.3 (684.1; 856.1)	335.0±170.6a (260.2; 409.7)	364.7±169.2a (290.5; 438.8)
				FAT(g/day)			
HBAT-CAF	133.4±93.6 (86.0; 180.7)	94.3±62.1 (62.8; 125.7)	116.6±85.8 (73.2; 160.0)	139.9±65.1 (107.0; 172.8)	45.3±46.7 (21.7; 68.9)	129.3±67.1 (95.3; 163.2)	146.9±56.3b* (118.4; 175.4)
LBAT-CAF	84.8±92.9 (37.8; 131.8)	62.8±77.6 (23.5; 102.1)	83.6±90.6 (37.8; 129.5)	94.0±81.2 (52.9; 135.1)	64.9±48.8 (40.3; 89.6)	107.4±72.7 (70.6; 144.2)	129.5±71.4b* (93.4; 165.6)
HBAT-PLA	48.4±104.8 (2.5; 94.3)	80.1±105.3 (33.9; 126.2)	101.9±128.8 (45.5; 158.4)	97.4±107.3 (50.4; 144.4)	54.3±51.9 (31.5; 77.0)	155.5±71.4 (124.2; 186.7)	161.1±64.2 (132.9; 189.2)
189.2	72.3±115.1 (21.9; 122.8)	62.9±101.3 (18.5; 107.3)	84.1±102.0 (39.3; 128.8)	85.2±114.4 (35.1; 135.3)	76.2±90.5 (36.5; 115.8)	127.4±92.8 (86.8; 168.1)	105.5±80.9 (70.1; 141.0)
				PTN(g/day)			
HBAT-CAF	64.1±5.4# (61.3; 66.9)	64.4±3.3# (62.7; 66.1)	63.7±4.0 (61.6; 65.7)	64.4±9.4# (59.6; 69.1)	52.1±6.2a# (48.9; 55.2)	39.6±4.7a# (37.2; 42.0)	38.4±4.8a# (36.0; 40.8)
LBAT-CAF	51.8±7.4 (48.1; 55.5)$	48.3±6.1 (45.3; 51.4)$	60.2±21.0 (49.5; 70.8)e	57.6±5.6 (54.7; 60.4)	44.6±6.3a$ (41.4; 47.8)	33.6±3.8a$ (31.7; 35.5)	34.4±3.7a$ (32.5; 36.2)
HBAT-PLA	53.1±8.0c (49.6; 56.6)	55.3±8.9d (51.4; 59.2)	60.0±7.3 (56.8; 63.2)	76.6±50.8 (54.3; 98.9)	48.1±7.8 (44.7; 51.5)	36.7±5.3a (34.4; 39.0)	36.3±5.4a (33.9; 38.6)
LBAT-PLA	46.5±5.8g (44.0; 49.1)	46.8±9.1h (42.8; 50.8)	55.8±9.7 (51.6; 60.1)	63.7±36.0 (47.9; 79.4)	41.4±11.0f (36.6; 46.3)	31.5±5.5a (29.1; 33.9)	29.6±4.1a (27.8; 31.4)

Note. a p≤0.023 for this moment of measurement vs. the others. b p≤0.009 for this moment of measurement vs. 0, 10 and 40 min. c p≤0.01 for this moment of measurement vs. the others, except 10- and 20-min. d p≤0.002 for this moment of measurement vs. the others, except 40 min. e p≤0.004 for this moment of measurement vs. 0- and 10-min. f p≤0.042 for this moment of measurement vs the others, except 10 min. g p≤0.027 for this moment of measurement vs. 20- and 30-min. h p=0.001 for this moment of measurement vs. 20 min. * p≤0.047 vs. PLA. # p≤0.01 vs. HBAT-PLA. $ p≤0.002 vs. LBAT-PLA.

4. Discussion

While BAT is a key regulator of energy homeostasis, the combined effects of thermogenic compounds and exercise on BAT activity remain underexplored in humans [26]. This study is the first to investigate the acute interaction between HIIT and CAF intake in individuals stratified by BAT activity. While our hypothesis was partially supported, because participants in the HBAT-CAF exhibited significantly higher EE during and post-exercise compared to other groups (HBAT-PLA,

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LBAT-CAF, LBAT-PLA). However, no significant differences in CHO or fat oxidation rates were observed across BAT activation states. Intriguingly, HBAT-CAF was linked to higher PTN catabolism at all measurement time points except 20 minutes, suggesting a transient metabolic shift. These findings underscore the potential of combining HIIT and CAF to amplify energy expenditure, a critical factor in weight management [27]. Prior studies confirm CAF's capacity to stimulate BAT thermogenesis [6,12], yet the interplay between exercise and thermogenic supplements in modulating BAT activity remains poorly characterized, highlighting the novelty of this work [26].

Our results bridges this gap, revealing that a CAF supplementation (4.8 mg/kg) amplified EE across all groups, irrespective of BAT activation status, consistent with previous studies of Pérez, Soto, Barroso, Dos Santos, Queiroz, Miarka, Brito and Quintana [12]. CAF likely potentiates HIIT-induced sympathetic nervous system (SNS) activation, as it stimulates β-adrenergic receptors to enhance metabolic and cardiovascular activity [6]. This synergy may explain the pronounced EE in HBAT-CAF, where elevated norepinephrine (NE) levels during HIIT [28] and post-exercise cortisol release [29] likely amplified thermogenesis. Notably, peak EE occurred between 10–30 minutes of HIIT, aligning with studies linking exercise intensity to catecholamine surges [29,30]. While HIIT predominantly relies on anaerobic glycolysis, aerobic pathways (potentiated by SNS activation) contribute to sustained lipolysis and EE [31,32]. Despite these effects, BAT activation did not modulate substrate oxidation, contrasting with Mekonen, *et al.* [33], who identified exercise-induced proteolysis as a minor but measurable energy source.

Differently, we observed PTN catabolism in HBAT-CAF may reflect transient metabolic demands, though protein supplementation strategies could mitigate nitrogen imbalance without compromising performance [34]. These results position HIIT combined with caffeine as a viable strategy to combat obesity through elevated EE, consistent with D'Amuri, Sanz, Capatti, Di Vece, Vaccari, Lazzer, Zuliani, Dalla Nora and Passaro [30], who reported HIIT's efficacy in weight loss and cardiovascular improvement. Despite the innovative results, our protocol presents limitations, which include the indirect assessment of supraclavicular BAT via thermography, which may underestimate heat emission in individuals with low BAT volume [19]. Additionally, the 85% accuracy in BAT classification raises potential misgrouping risks. Future studies should explore interactions between moderate-intensity exercise, caffeine, and BAT activity in diverse populations, including females and sedentary individuals, to improve translational applications.

5. Conclusions

Based on our aims, methodology, findings, and limitations, we conclude that CAF supplementation increases EE in both HBAT and LBAT groups during and after a HIIT session. While basal BAT activity did not influence CHO or fat oxidation rates. Furthermore, the HBAT-CAF demonstrated a marked increase in PTN catabolism. These results suggest that combining acute exercise with caffeine may enhance EE, offering a potential strategy to address energy imbalance in overweight and obesity management. Further research is warranted to explore long-term metabolic adaptations and the mechanistic role of BAT in substrate utilization.

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Institutional Review Board Statement: This study was conducted in accordance with the Declaration of Helsinki and approved by the Institutional Review Board (or Ethics Committee) of Santo Tomás University (82.17).

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Data Availability Statement: The database for this study will be made available on demand, just send an email to ciro.brito@ufjf.br.

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Abbreviations

The following abbreviations are used in this manuscript:

BAT	Brown adipose tissue
HBAT	High brown adipose tissue
LBAT	Low brown adipose tissue
CAF	Caffeine
PLA	Placebo
EE	Energy expenditure
CHO	Carbohydrate
FAT	Lipids
PTN	Protein
HIIT	High intensity interval training
HRmax	Maximum heartrate
18F-FDG PET/CT	18F-fludeoxyglucose positron emission tomography
IRT	Infrared thermography
UCP-1	Uncoupling protein 1
PGC-1α	peroxisome proliferator-activated receptor-gamma coactivator
SNS	sympathetic nervous system

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