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Original Research

Effect of Acute High-intensity Interval Exercise on Whole-body Fat Oxidation and Subcutaneous Adipose Tissue Cell Signaling in Overweight Women

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ABSTRACT

International Journal of Exercise Science 13(2): 554-566, 2020. Exercise-induced alterations in adipose tissue insulin and/or β-adrenergic signaling may contribute to increases in whole-body fat oxidation following acute exercise. Thus, we examined changes in insulin (Akt, AS160) and β-adrenergic (PKA) signaling proteins in subcutaneous adipose tissue and whole-body fat oxidation in overweight women following acute high-intensity interval exercise (HIIE). Overweight females completed two experimental sessions in a randomized order: 1) control (bed rest) and 2) HIIE (10 x 4 min running intervals at 90% HR_{max}, 2-min recovery). Subcutaneous abdominal adipose tissue biopsies were obtained from 10 participants before (pre-), immediately (0hr) after (post-), 2hr post-, and 4hr post-exercise. Plasma glucose and insulin levels were assessed in venous blood samples obtained at each biopsy time-point from a different group of 5 participants (BMI-matched to biopsy group). Fat oxidation rates were estimated using the respiratory exchange ratio (RER) in all participants using indirect calorimetry pre-, 2hr post-, and 4hr post-exercise. RER was decreased ($p < 0.05$) at 2hr post-exercise after HIIE (0.77 ± 0.04) compared to control (0.84 ± 0.04). Despite higher plasma glucose ($p < 0.01$) and insulin ($p < 0.05$) levels at 0hr post-exercise versus control, no significant interaction effects were observed for Akt or AS160 phosphorylation ($p > 0.05$). Phosphorylation of PKA substrates was unaltered in both conditions ($p > 0.05$). Collectively, altered β-adrenergic and insulin signaling in subcutaneous adnominal adipose tissue does not appear to explain increased whole-body fat oxidation following acute HIIE.

KEY WORDS: Adipose tissue, insulin signaling, fat oxidation, high-intensity interval exercise

INTRODUCTION

β-adrenergic (β-AR) and insulin signaling regulate the balance between non-esterified fatty acid (NEFA) release and triglyceride (TG) synthesis in adipose tissue (31). The stimulation of β-AR receptors on adipose tissue initiates lipolysis by increasing the production of cyclic

adenosine monophosphate (cAMP), which activates protein kinase A (PKA) (13) resulting in the subsequent activation of lipases and the release of NEFAs from adipocytes (23, 35). In contrast, insulin phosphorylates and activates protein kinase B (PKB/Akt), which reduces intracellular cAMP, thereby decreasing PKA activity and ultimately reducing PKA-mediated stimulation of lipolysis (18). Moreover, increases in whole body fat oxidation (1, 4, 15, 16) and elevated NEFA release from adipose tissue (2, 11, 26) have been observed during the post-exercise period in humans. However, while the impact of β-AR signaling on adipose tissue during exercise is well known, it seems unlikely that this signaling cascade is fully responsible for prolonged increases in lipolysis given the rapid decrease in circulating catecholamines that occurs following cessation of exercise (9, 43). Altered insulin signaling therefore represents a potential alternative explanation for the elevated rates of adipocyte lipolysis observed during the post-exercise period. Presently, it remains unclear how insulin signaling in subcutaneous abdominal adipose tissue is altered in the post-exercise period in humans.

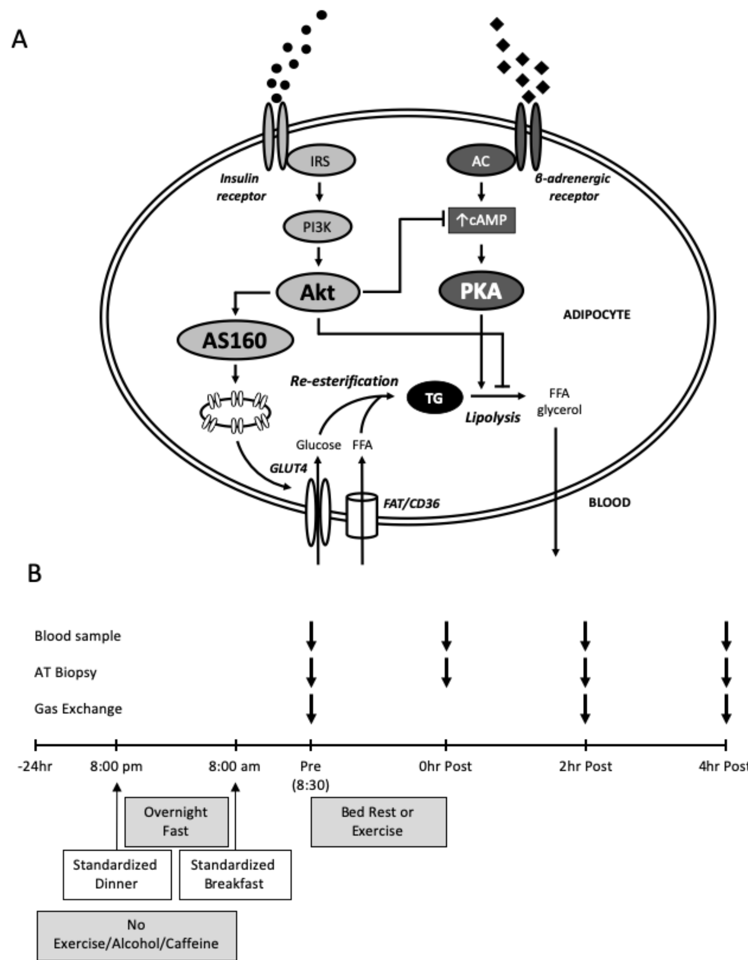

Figure 1. Overview of intracellular signaling pathways examined (A) and the experimental protocol used (B). *Note:* Components of each pathway measured in the current study are shown in large font. AC: adenylate cyclase; Akt; protein kinase B; AS160: Akt substrate of 160 kDa; cAMP: cyclic adenosine monophosphate; FFA: free fatty acid; IRS: insulin receptor substrate; PI3K: phosphoinositide 3-kinase; PKA: protein kinase A; TG: triglyceride.

The primary purpose of the present study was to examine the effect of acute high-intensity interval exercise (HIIE) on insulin (Akt and AS160) signaling proteins in human subcutaneous adipose tissue (Figure 1A) in order to examine the association between changes in adipose tissue insulin signaling and whole-body fat oxidation. We hypothesized that HIIE-induced increases in whole body fat oxidation would be associated with decreased insulin signaling (i.e. decreased phosphorylation of Akt and/or AS160). In addition, we also examined changes in PKA substrate phosphorylation to approximate changes in β-AR signaling pathways in adipose tissue. Findings from the current study may provide important mechanistic insight into the regulation of subcutaneous adipose tissue signaling pathways in relation to changes in substrate oxidation during the post-exercise period.

METHODS

Participants

Sedentary and overweight (waist circumference > 80cm) female participants volunteered to take part in this study. Overweight females were recruited in order to ensure that an adequate amount of subcutaneous adipose tissue could be extracted. All participants were insulin sensitive with normal fasting and postprandial blood glucose levels and were free from pre-existing cardiometabolic disease (e.g. hypertension, type 2 diabetes, dyslipidemia, etc.). Participants were instructed to maintain their usual exercise and nutritional habits throughout the study and were asked to refrain from exercise, alcohol, and caffeine for a minimum of 24hr prior to each experimental visit. Ethical approval was granted by the Health Sciences Human Research Ethics Board at Queen's University and all participants provided written informed consent prior to participation in the study. This research was carried out fully in accordance to the ethical standards of the International Journal of Exercise Science (24).

Protocol

All participants completed a baseline testing session and two experimental visits: (i) control and (ii) acute high-intensity interval exercise (HIIE). Adipose tissue biopsies were obtained from 10 participants (age: 22 ± 4 y; BMI: 30.6 ± 6.0 $kg \cdot m^{-2}$; VO_2peak: 30.3 ± 5.4 $mL \cdot kg^{-1} \cdot min^{-1}$) to determine the impact of HIIE on insulin (Akt, AS160) and β-AR (PKA) signaling proteins. Blood samples were obtained from a separate group of 5 BMI-matched participants (age: 22 ± 3 y; BMI: 30.0 ± 5.76 $kg \cdot m^{-2}$; VO_2peak: 40.3 ± 8.7 $mL \cdot kg^{-1} \cdot min^{-1}$) to examine changes in plasma glucose and insulin concentrations. Gas measurements were obtained from all participants (n = 15) and were used to determine the effects of acute HIIE on whole-body fat oxidation. Experimental visits were administered in a randomized crossover design, with each visit separated by a minimum 14-day recovery period. The experimental protocol is shown in Figure 1B.

Participants reported to the laboratory > 72hr prior to the first experimental session. During this visit, anthropometric measures were obtained, and participants were instructed to complete a PAR-Q. Additionally, participants completed an incremental ramp test on a treadmill (Sport Art Fitness 6300) to determine peak O_2 uptake (VO_2peak). The VO_2peak ramp protocol consisted of a 3-minute warm-up at a speed of 2 mph and an incline of 2%, followed by a step increase in speed to 4.5mph for 2-minutes and subsequent increases in incline at a

rate of 1% every 2-minutes until volitional exhaustion. Gas exchange was measured continuously using a metabolic cart (Moxus AEI Technologies, Pittsburgh, PA) and heart rate was collected using an integrated heart rate monitor (Polar, QC). Relative VO_2peak and peak heart rate were calculated as the highest 30-second average obtained for each variable.

Participants completed a resting control session and an acute HIIE session separated by two weeks in randomized order. After consuming a standardized dinner on the evening before each experimental visit (Stouffer Sauté Sensation Mediterranean chicken dinner [260 calories; 37g carbohydrate, 5g fat, 16g protein], 500mL of 1% chocolate milk [340 calories; 56g carbohydrate, 5g fat, 18g protein] participants underwent an overnight fast (~12hr) and were provided a standardized breakfast (whole wheat bagel [240 calories; 45g carbohydrate, 2.5g fat, 9g protein], 2 tbsp peanut butter [180 calories; 8g carbohydrate, 16g fat, 6g protein] and 200 ml apple juice [90 calories; 26g carbohydrate, 0g fat, 0g protein]) upon arrival to the laboratory. After 15-minutes of sitting, baseline gas (VO_2 and VCO_2) measures were collected (Moxus AEI Technologies, Pittsburgh, PA) for 15-minutes while participants remained seated. Hereafter, a baseline adipose biopsy or blood sample (Pre) was obtained. Following the first biopsy or blood sample, the participants either completed the acute HIIE protocol, which consisted of a 5-minute warm-up at a speed of 2 mph and incline of 2% followed by 10 intervals of 4-minutes at 90% heart rate max interspersed with 2-minutes at a speed of 2 mph and an incline of 2%, or 65-minutes of bed rest (control) (29, 36). Immediately following HIIE or bed rest a second adipose biopsy or blood sample (0hr) was obtained. Additional adipose biopsies or blood samples were taken 2 and 4hr post-exercise or bed rest with 15-minutes of resting gas exchange collected beforehand as described above.

Adipose tissue biopsies were obtained using a Bergstrom needle with the addition of manual suction following local anaesthetization (2% lidocaine). Biopsies were obtained from the abdominal region with incisions made approximately 5cm lateral to each side of the umbilicus with the needle inserted laterally, as done previously (42). All biopsies were taken from these 2 incision points with the first two taken from the right-side and the last two taken from the left-side. Biopsies sampled from the same incision were separated by a minimum of 5cm by altering the angle of needle insertion. In the subsequent visit, the same two incision locations were used to reduce scarring, while the side first sampled (right or left) was switched in an attempt to minimize the potential influence of adipocyte sampling location. Adipose tissue biopsies were immediately blotted, snap-frozen in liquid nitrogen, and stored at -80˚C for further analysis. Blood samples were obtained via venipuncture from an antecubital vein while participants lay in a supine position. Blood was collected into sterile EDTA-coated Vacutainer tubes and centrifuged at 3000g for 10-min at 4°C. The plasma supernatant was dispensed into Eppendorf tubes and stored at -80˚C for analysis.

Fat oxidation rates were estimated using the respiratory exchange ratio (RER). RER was determined from recorded gas exchange (VO_2 and VCO_2) values at baseline (pre) as well as 2 and 4hr post-exercise. A 10-min average was taken from 2.5 to 12.5 min of each 15-min gas measurement period to minimize the impact of any adjustment period following putting on the mask and/or participant anticipation of the mask being removed.

Frozen adipose tissue (~80-100mg) was homogenized in 300 µL of pre-chilled (4°C) lysis buffer supplemented with protease and phosphatase inhibitors (42). Samples were homogenized for 3-5 seconds at 15000 RPM (Polytron PT10/35 GT Benchtop Homogenizer, Kinematic, Luzern, Switzerland). Protein concentrations were determined for all homogenates using a commercially available protein assay (Pierce, Rockford, IL). Samples were diluted to equivalent concentrations with a mixture of 4x Laemmli sample buffer and H_2O and then denatured by heating to 95°C for 5-min. Proteins were separated by SDS-PAGE using 10% (phospho-AS160, phospho-PKA), and 15% (phospho-AKT Ser473) polyacrylamide gels and were subsequently transferred to a polyvinylidene difluoride membrane. For the detection of proteins, commercially available antibodies were used for GAPDH (Millipore, Billerica, MA), phospho-AKT Ser473, and phospho-PKA substrate (Cell Signaling Technologies, Danvers, MA). Membranes were blocked with 5% BSA in TBS-T (0.1%) and immunoblotted with primary antibody. Proteins were visualized by chemiluminescence detection according to the manufacturer's instructions (Millipore, Billerica, MA). Blots were imaged using the FluorChem Cell Biosciences imaging system (ProteinSimple, Santa Clara, CA) and quantified using AlphaView technology (ProteinSimple, Santa Clara, CA). Equal protein loading for all western blots were confirmed using GAPDH. Representative blots for analysis of all proteins of interest are provided in Figure 2.

Plasma was analyzed for glucose concentrations using a glucose calorimetric assay kit (cat no. 10009582, Cayman Chemicals, Ann Arbor, MI) and for insulin concentrations using a commercially available ELISA kit (cat no. DINS00, R&D systems, Minneapolis, MN). All samples were assayed in duplicate and batch-analyzed according to the manufacturer's instructions. The average intra-assay coefficients of variation for insulin and glucose were 4.8 ± 6.2% and 3.5 ± 3.4%, respectively.

Statistical Analysis

A two-way repeated measure analysis of variance (ANOVA) was used to compare the effect of intervention (control and exercise) and time (pre, 0hr, 2hr, 4hr) for adipose biopsy data, substrate oxidation and plasma glucose/insulin concentrations. Bonferroni post hoc tests were used where a significant main effect or interaction was observed. Data analysis was completed with GraphPad Prism v 5.01 (GraphPad Software Inc., La Jolla, CA). Statistical significance was accepted at $p < 0.05$. All data are presented as mean ± standard deviation (SD).

RESULTS

RER demonstrated a significant main effect of time ($p < 0.01$), exercise ($p < 0.05$), and a significant interaction effect ($p < 0.05$). Post hoc analysis revealed that RER values were significantly lower ($p < 0.05$) 2hr post-exercise (0.77 ± 0.04) compared to control (0.84 ± 0.04) (Figure 2).

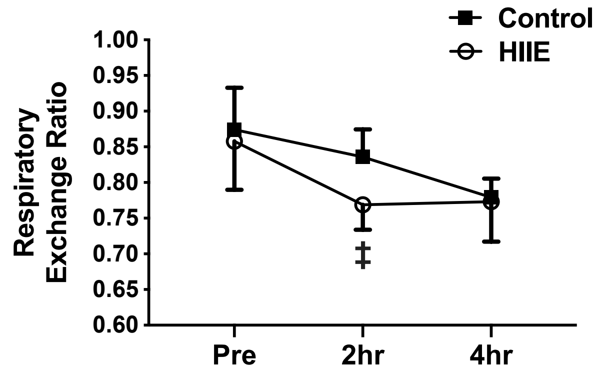

Figure 2. Changes in resting respiratory exchange ratio (RER) at all time-points during each experimental session (n = 15). *Note:* HIIE: high-intensity interval exercise; Pre: pre-exercise; 2hr: 2-hours post-exercise; 4hr: 4-hours post-exercise. ‡Significantly different from control ($p < 0.05$). Values are mean ± SD.

A significant main effect of exercise ($p < 0.05$) but no time ($p = 0.78$) or interaction ($p = 0.21$) effect was observed for phosphorylation of Akt (Figure 3A). While there was a significant main effect of time ($p < 0.05$) on the phosphorylation of AS160, no significant effect of exercise ($p = 0.53$) or an interaction effect ($p = 0.78$) were observed and post-hoc analysis revealed no difference between exercise and control at any time point (Figure 3B). There were no significant main effects of condition ($p = 0.87$) or time ($p = 0.11$) or an interaction effect ($p = 0.45$) observed for the phosphorylation of PKA substrates (Figure 4). However, when individual bands were analyzed, there was a significant main effect of time on the phosphorylation of band 1 ($p < 0.05$) (Figure 4B). Equal loading was confirmed using GAPDH protein content. No significant time or interaction effects were detected from GAPDH protein content analysis for any of the gels examined ($p < 0.05$).

There was a significant main effect of time ($p < 0.05$) and a significant interaction effect ($p < 0.05$) for blood glucose (Figure 5A) and insulin concentrations (Figure 5B). Post hoc analysis revealed significantly ($p < 0.01$) higher blood glucose concentrations 0hr after exercise (4.45 ± 0.24 mmol $\cdot L^{-1}$) relative to control (3.71 ± 0.53 mmol $\cdot L^{-1}$). Similarly, insulin concentrations were significantly ($p < 0.05$) higher at 0hr after exercise (298.92 ± 215.46 pmol $\cdot L^{-1}$) compared to control (192.24 ± 182.23 pmol $\cdot L^{-1}$).

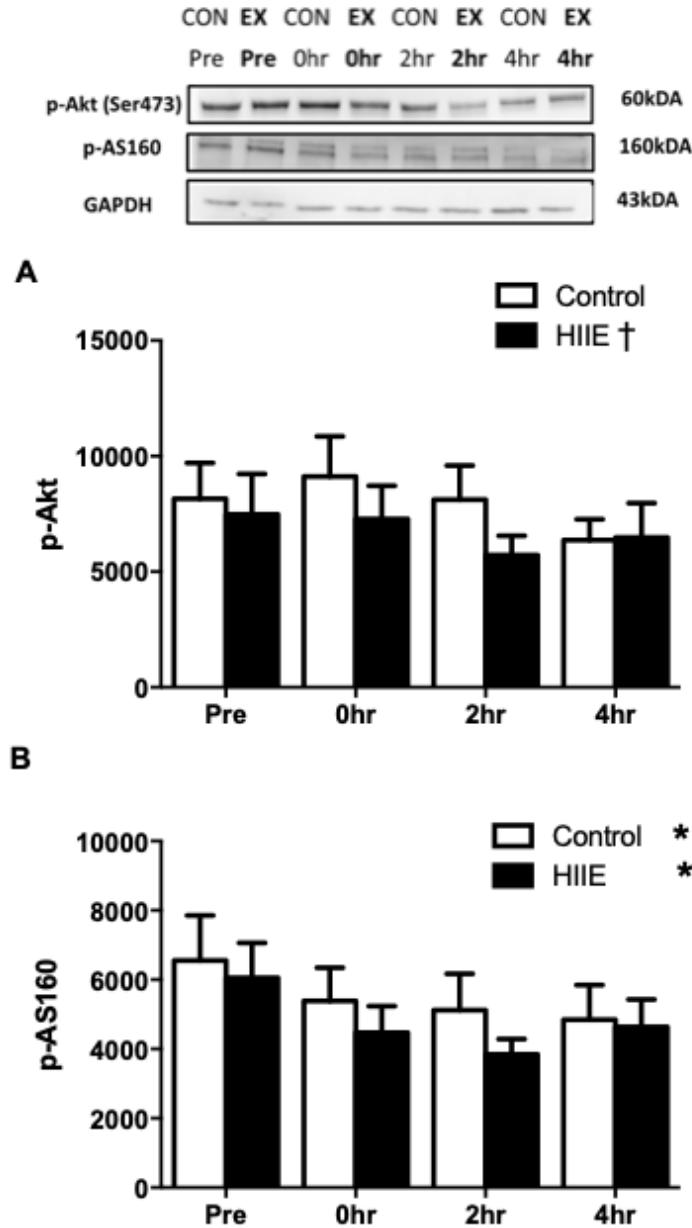

Figure 3. Change in phosphorylated protein content of Akt (A) and AS160 (B), at all time-points during each experimental session (n = 10). *Note:* HIIE: high-intensity interval exercise; Pre: pre-exercise; 0hr: 0-hours post-exercise; 2hr: 2-hours post-exercise; 4hr: 4-hours post-exercise. * Significant main effect of time; † significant main effect of HIIE ($p < 0.05$). Values are mean ± SD.

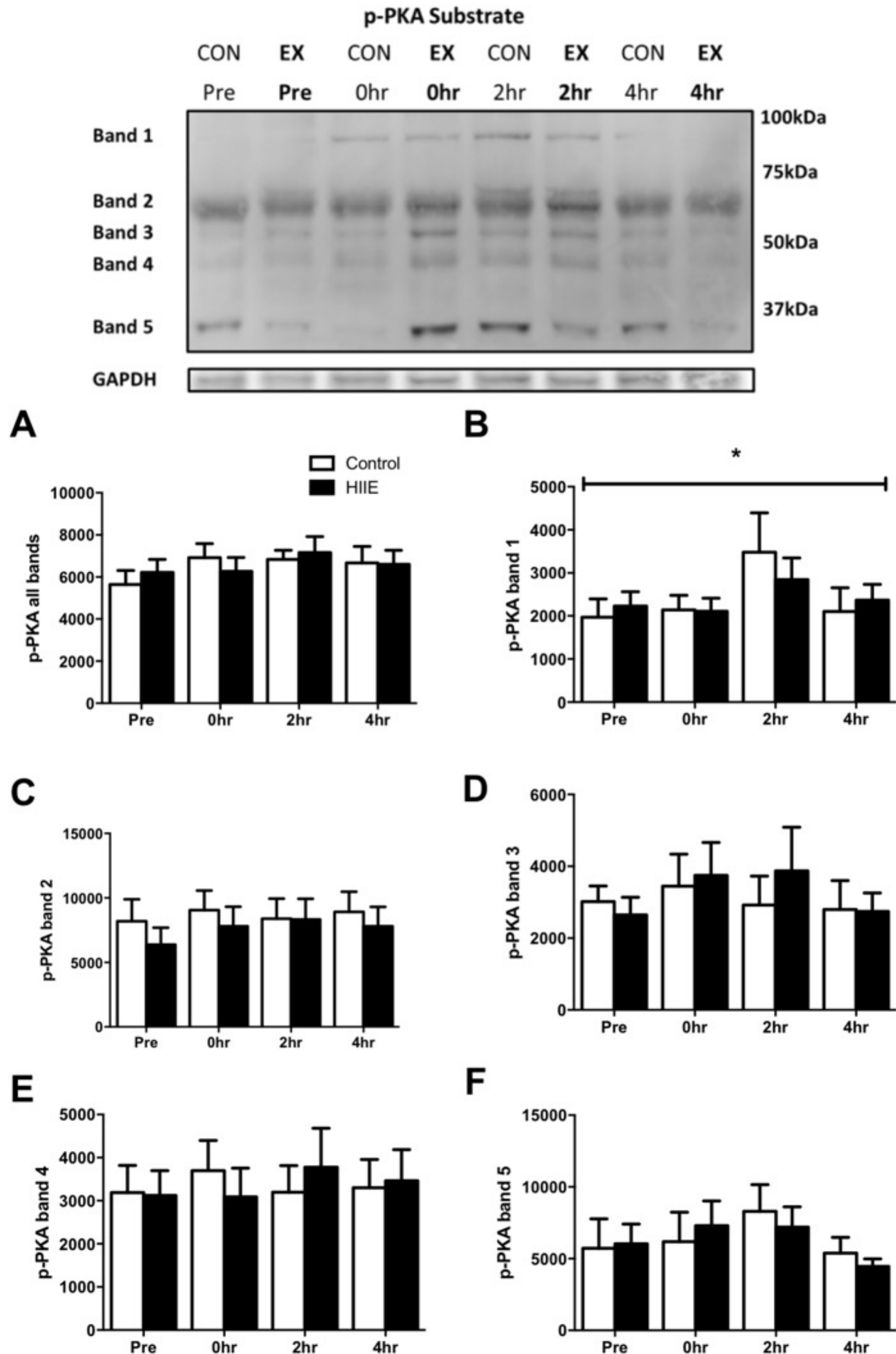

Figure 4. Changes in p-PKA substrate phosphorylation (A: all bands; B-F: individual bands) at all time-points during each experimental session (n = 10). Note: HIIE: high-intensity interval exercise; Pre: pre-exercise; 0hr: 0-hours post-exercise; 2hr: 2-hours post-exercise; 4hr: 4-hours post-exercise. * Significant main effect of time ($p < 0.05$). Values are mean ± SD.

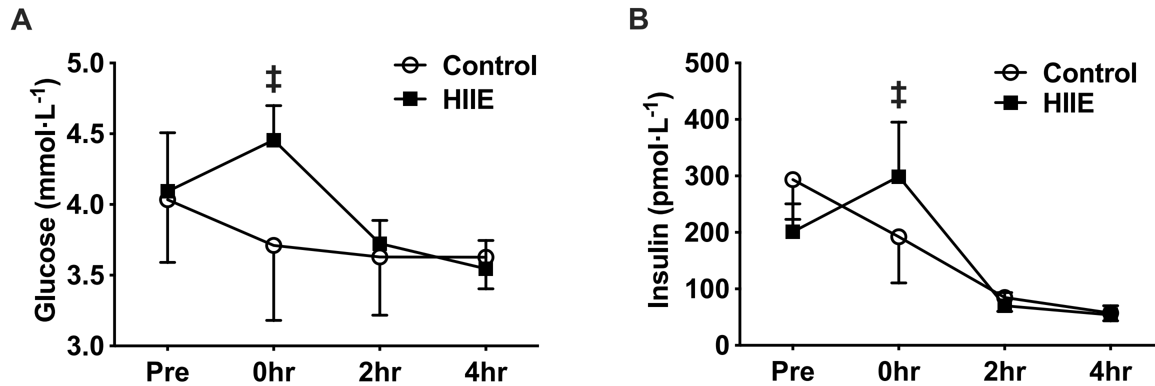

Figure 5. Changes in blood glucose (A) and insulin (B) concentrations during each experimental session (n = 5). *Note:* HIIE: high-intensity interval exercise; Pre: pre-exercise; 0hr: 0-hours post-exercise; 2hr: 2-hours post-exercise; 4hr: 4-hours post-exercise. ‡Significantly different from control ($p < 0.05$). Values are mean ± SD.

DISCUSSION

The current study investigated the impact of acute HIIE on intracellular signaling cascades in subcutaneous abdominal adipose tissue and changes in whole body fat oxidation in overweight females. Our results indicate that an acute bout of HIIE increases whole body fatty acid oxidation 2hr into the post-exercise recovery period. However, this increase in fat oxidation was not accompanied by alterations in in subcutaneous adipose tissue insulin or β-AR signaling despite elevated insulin levels immediately post-exercise, suggesting a reduced responsiveness of adipose tissue to circulating insulin after HIIE. Together, altered β-adrenergic and insulin signaling in subcutaneous adipose tissue does not appear to explain increased whole-body fat oxidation following acute HIIE.

Acute HIIE significantly increased whole body fat oxidation 2hr post-exercise (as reflected by a decrease in RER) in accordance with previous studies demonstrating increases in whole body fat oxidation during the post-exercise period (4, 11, 15, 16, 32). Accordingly, elevated plasma FFA and glycerol levels have been reported for 2-3hr post-exercise in parallel with elevated fatty acid oxidation (2, 11, 26). The increased availability of FFA and glycerol has been suggested to shift substrate utilization towards a greater reliance on fat, thereby decreasing reliance on glycogen such that metabolic priority can be given to its resynthesis (17). Additionally, although β-AR signaling in adipose tissue did not increase in the current study, it is likely that the catecholamine response associated with intense exercise (8, 38, 39, 43) stimulated lipolysis and metabolism in other tissues (i.e. skeletal muscle) (10, 44).

Although recent work has examined metabolic adaptations in human subcutaneous adipose tissue following exercise training (6, 20, 21, 25), it remains unclear how intracellular pathways that modulate substrate storage and mobilization are altered in this tissue following an acute bout of HIIE. Similar to previous work involving intense exercise, an increase in plasma insulin and glucose levels was observed immediately after the exercise bout in the current study (7, 22, 34, 40). However, the increased whole-body fat oxidation rates were not accompanied by robust alterations in adipose tissue insulin signaling protein during the post-

exercise period, as reflected by the lack of significant interaction effect for Akt phosphorylation. Downstream of Akt, AS160 phosphorylation was also unaltered with HIIE compared to control, which suggests that insulin signaling was largely unaffected by exercise. Interestingly, the preservation of Akt and AS160 phosphorylation was observed despite increases in plasma insulin concentrations following the high-intensity exercise bout, indicating an altered responsiveness of subcutaneous adipose tissue to circulating insulin. This decreased responsiveness of adipose tissue to increased levels of circulating insulin would be expected to reduce lipid storage in adipose tissue (3) and contribute to the increase in plasma NEFAs (11, 26) and elevated whole-body fat oxidation rates following intense exercise (4, 15, 16). Alternatively, it is possible that rise in circulating insulin was simply insufficient to invoke measurable changes in insulin signaling in subcutaneous abdominal adipose tissue, particularly since blood flow to this area may be lower than other fat depots (19, 41).

PKA is activated by β-AR stimulation which leads to the activation of lipases and the subsequent initiation of lipolysis and NEFA release (18, 27). Further, it is well established that high intensity exercise results in high levels of circulating epinephrine (8, 38, 39, 43). Thus, the lack of exercise-induced increase in PKA phosphorylation in our study is unexpected. In this light, there is evidence suggesting that blood flow to subcutaneous abdominal adipose tissue as well as the responsiveness of this region to catecholamine-induced lipolysis may be lower than other fat depots (i.e. visceral) (19, 41). Alternatively, blood flow may have diverted away from subcutaneous abdominal adipose tissue to accommodate the demands of metabolically active tissues, thus delivering inadequate epinephrine to produce a detectable change in PKA phosphorylation. Although low-to-moderate intensity exercise can augment adipose tissue blood flow (37), this effect decreases with increasing exercise intensity and splanchnic blood flow can be substantially reduced during high intensity exercise (5). Finally, the reduced responsiveness of PKA signaling to β-AR stimulation in overweight/obese individuals (14, 33) and/or PKA-independent mechanisms of catecholamine induced lipolysis (30) may have also played a role in the lack of observed effect on PKA phosphorylation in our study.

The findings of the current study should be interpreted with the following limitations in mind. First, as participants' menstrual cycle phase was not controlled in the current study, the potential influence of sex hormones on substrate utilization and/or insulin signaling may have influenced our results. Second, the lack of additional blood (e.g. catecholamines, free fatty acids, glycerol) and tissue (e.g. hormone sensitive lipase activity) measures limits our ability to make inferences regarding the potential sources of substrate that may have contributed to the observed elevations in fat oxidation following exercise. Third, the small sample size included in the current study may have limited our ability to observed between-group differences at the post-exercise time-points examined due to a lack of statistical power. Finally, as blood samples and adipose tissue biopsies were obtained from separate groups of participants, potential differences between the two groups of participants may have influenced our findings. These limitations should be addressed in future work to provide greater mechanistic insight into the regulation of whole-body fat oxidation following HIIE.

Collectively, our findings suggest that acute HIIE does not significantly alter intracellular signaling pathways controlling fat mobilization (e.g. PKA) and/or storage (e.g. Akt, AS160) in

subcutaneous abdominal adipose tissue. However, the preservation of insulin signaling protein phosphorylation despite increased circulating insulin levels in the post-exercise period could indicate a potential altered responsiveness of adipose tissue to this hormone. This phenomenon should be explored further in future work as it may partially account for the increased whole-body fat oxidation rates observed in the post-exercise period. On the other hand, increases in whole-body fat oxidation were not accompanied by altered PKA substrate phosphorylation induced via epinephrine signaling, the reasons for which remain unclear.

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