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Research article

Plasma Apelin Unchanged With Acute Exercise Insulin Sensitization

Justin D. Waller, Emily H. McNeill, Frank Zhong, Lauren S. Vervaecke and Allan H. Goldfarb ✉
Department of Kinesiology, University of North Carolina at Greensboro, Greensboro, NC, USA

Abstract

Blood glucose and insulin responses to aerobic exercise are well defined yet the mechanisms effecting post-exercise insulin sensitization remain incomplete. Apelin has been reported to enhance glucose uptake and insulin sensitivity *in vivo*, but its role as a regulator of insulin sensitivity following acute aerobic exercise has not been investigated. Therefore, the purpose of this study was to investigate apelin's response to acute bouts of maximal and submaximal aerobic exercise and to elucidate apelin's influence on insulin sensitivity. Twelve (22.8 ± 2.9 yrs) healthy male (n = 7) and female (n = 5) subjects completed a graded to maximal (VO_2max) and submaximal (70-75% VO_2max) treadmill running bouts, as well as a 50g glucose challenge (GC). Blood was obtained at four time points (pre, post, 1hr post and 24hrs post) and assessed for glucose, insulin and apelin. Hepatic insulin sensitivity was assessed at rest and at 1hr and 24hrs via HOMA-IR and QUICKI indices. Results demonstrated that plasma apelin did not significantly change by condition (p = 0.324) or time (p = 0.633). Blood glucose and plasma insulin were significantly elevated immediately after VO_2max and GC, but remained stable after submaximal exercise. Insulin sensitivity was significantly improved 1hr post-submaximal exercise, per HOMA-IR (p = 0.034) and QUICKI (p = 0.018) indices. Plasma apelin was significantly correlated with plasma insulin (r = 0.699, p = 0.011), HOMA-IR (r = 0.626, p = 0.029) and QUICKI (r = 0.660, p = 0.019) at rest. We conclude that, although hepatic insulin sensitivity was improved 1hr post-submaximal exercise, this exercise-induced insulin sensitization occurred independent of plasma apelin changes.

Key words: Aerobic exercise, QUICKI, HOMA-IR, glucose challenge.

Introduction

Apelin is a peptide secreted from various tissues and has been classically characterized as an adipokine, though it has recently been described as a myokine (Besse-Patin et al., 2014; Kleinz and Davenport, 2005). Apelin is purported to assist in regulating glucose homeostasis (Dray et al., 2010; Dray et al., 2008) and plays an integral role in insulin sensitivity in mice (Yue et al., 2010). Additionally, apelin was noted to improve peripheral glucose uptake in normal and insulin-resistant mice (Dray et al., 2008) and apelin injections during a hyperinsulinemic-euglycemic clamp enhanced glucose disposal (Dray et al., 2010). Apelin-stimulated glucose uptake was confirmed in isolated adipocytes from healthy (Attane et al., 2011; Than et al., 2014) and type 2 diabetic (T2D) subjects (Dray et al., 2010). These results support apelin's role as an exogenous insulin sensitizer under hyperinsulinemic conditions. However, apelin's effect under normal insulinemia and its role as an endogenous glucose regulator in healthy individuals during and following exercise has not been determined and requires further clarity (Alexiadou et al., 2012).

Apelin is chiefly influenced by insulin, hypoxia and adiposity, among other factors (Kleinz and Davenport, 2005). Insulin is considered the prime regulator of apelin stimulating its synthesis and release (Boucher et al., 2005). Conversely, apelin exerts a depressive effect on insulin, acting via G proteins in islet cells (Ringström et al., 2010). Acute aerobic exercise, as a result of repeated muscular contractions, stimulates insulin-independent glucose uptake via GLUT translocation in skeletal muscle, and subsequently confers both peripheral and hepatic sensitization to insulin that typically lasts hours to days (Henriksen, 2002; Holloszy, 2005). This improved sensitization to insulin after acute aerobic exercise may be partially attributable to apelin. Previous studies concerning exercise and apelin have focused on apelin's response to training in obese and T2Ds (Besse-Patin et al., 2014; Krist et al., 2013), populations known to exhibit considerable variability in basal apelin concentration due to a variety of contributing factors (Alexiadou et al., 2012, Castan-Laurell et al., 2012, Castan-Laurell et al., 2008, Cavallo et al., 2012, Dray et al., 2010, Krist et al., 2013).

Enhanced contraction-induced glucose uptake lasts up to two hours post-exercise, however, improved insulin sensitivity may last longer (Borghouts and Keizer, 2000; Holloszy, 2005). The benefits of insulin sensitivity improvements are transient and return to normal 12-48 hours post-exercise (Borghouts and Keizer, 2000; Christiansen et al., 2010; Fontana et al., 2010; Holloszy, 2005; Magkos et al., 2010). Endurance training studies have reported insulin sensitivity reversal after cessation of acute exercise (Heath et al., 1983; LeBlanc et al., 1981). Thus, insulin sensitivity improvements appear to be facilitated primarily by single exercise bouts (Goodyear and Kahn, 1998). Given this, acute aerobic exercise represents an excellent model by which the effect of apelin upon insulin sensitivity can be assessed. High-intensity aerobic exercise (e.g. VO_2max) demonstrably elevates blood glucose and insulin, while moderate-intensity aerobic exercise maintains blood glucose and insulin (Marliss and Vranic, 2002); thus, these represent two exercise conditions with readily reproducible, disparate outcomes. Therefore, the primary aim of this study was to determine if plasma apelin concentration is altered by either bout of acute aerobic exercise or whether this is related to insulin-mediated sensitization in a healthy adult population.

Methods

Subjects

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The IRB of UNC Greensboro approved this study and all participants signed informed consent on their first visit. Twelve (n = 7 male; n = 5 female) apparently healthy subjects (22.8 ± 2.9 years), non-tobacco users and not taking medications or supplements for at least 6 months that could alter metabolism, oxidation status, blood glucose and/or insulin, were recruited. Subjects were instructed to maintain their normal diet throughout the study and dietary logs were reviewed to ensure compliance.

Pre-screening procedures

Volunteers (18-35 yrs) were screened for medical, metabolic, cardiovascular and activity factors, per American Heart Association (AHA) and American College of Sports Medicine (ACSM) guidelines (Pescatello, 2014). Obese, hypertensive or pregnant participants were excluded. Resting heart rate (HR), blood pressures (BP), % body fat (%BF) (7-site determination via Siri equation, Jackson and Pollock, 1978), weight and height were determined. Once all criteria were satisfied participants became enrolled subjects. Subjects were provided food logs to record diet intake three days prior to each visit, and for the 24-hour period post-treatment prior to the fourth blood draw time point.

Maximal and submaximal exercise and negative control condition procedures

Subjects reported to the laboratory (6-9 A.M.) on three different days to complete two different exercise conditions (graded to maximal [VO_2max] and steady state [70-75% VO_2max] aerobic exercise on treadmill) and a negative control glucose challenge (GC) of 50g, in a post-absorptive state (10-12 hrs) and after having not exercised for ≥24 hrs. Each condition was separated by at least three days, but not more than 14 days and subjects returned 24 hrs after each condition. The first visit was randomized, either VO_2max or GC, with the 30-minute bout (70-75% VO_2max) necessarily performed after VO_2max (2^{nd} or 3^{rd} visit).

Maximal treadmill graded exercise

Subjects completed a warm-up (5 min) on the treadmill until HR rose to 130 bpm. The test starting speed ranged between 5.5 and 8 mph amongst all subjects. Thereafter, both grade (by 2.5%) and speed (by 0.5 mph) increased each minute until reaching VO_2max or volitional fatigue. Expired gases were collected and analyzed by a Parvo Medics True One 2400 analyzer system (Parvo Medics, Sandy, UT, USA) calibrated to known gases. Ratings of perceived exertion (RPE) were recorded each minute (Noble et al., 1983). All subjects satisfied at least four of five required criteria for a true VO_2max (Midgley et al., 2007; Pescatello, 2014). Subjects were given water before and after the graded to maximal exercise trial and encouraged to drink water ad libitum (up to 1 L) in the 1-hr recovery period.

Submaximal treadmill exercise

Subjects completed a warm-up (3-5 min) on the treadmill. HR was continually monitored during the 70-75% VO_2max exercise (Polar Electro Inc., Bethpage, NY, USA) and recorded every 5 minutes. VO_2 was confirmed in the initial 5 minutes and for 1 minute every 5 minutes during the test to ensure proper intensity. Subject RPE was obtained every 5 minutes to further ensure appropriate subjective intensity was maintained. Subjects were given water every 5 minutes during the exercise and encouraged to drink water ad libitum (up to 1 L) in the 1 hour recovery period.

Blood collection and handling procedures

Subjects rested >15 minutes before blood was obtained from an antecubital vein prior to any intervention. Blood was drawn into vacutainer EDTA tubes and a small sample of whole blood (<10µl) was used to assess blood glucose immediately. Hematocrit (Hct) was determined by taking <25µl blood drawn into micro-hematocrit capillary tubes, sealed and centrifuged (micro centrifuge at 3000 rpm 5 min) and volume ratio determined (in duplicate). Blood was immediately centrifuged (Allegra, Beckman Coulter, Indianapolis, IN, USA) for 10 minutes at 3000 rpm (4°C). Plasma was aliquoted into tubes and placed in an -80°C freezer until analyzed. Assays for plasma apelin and plasma insulin were completed with batched samples.

Assays and insulin sensitivity measures

Blood glucose was determined immediately by a glucose meter (Bayer Contour, Bayer HealthCare, LLC, Whippany, NJ, USA), which was calibrated prior to use (within 0.2 mM). Plasma insulin was assayed using an ELISA immunoassay (#10-1113-01 Mercodia, Winston-Salem, NC, USA). Plasma apelin was assayed using an ELISA immunoassay (EK-057-15 Phoenix Pharmaceuticals, Inc., Burlingame, CA, USA), which was noted to have 100% cross-reactivity with human apelin-36, -13 and -12. Absorption for both assays was read at 450 nm via KC Junior microplate reader (BioTek Instruments, Winooski, VT, USA). Intra-assay variance was <10% for insulin and <8% for apelin. Using batched samples from the same subject on common plates eliminated inter-assay variance. Plasma lactate was determined monitoring absorption change at 340 nm using LDH enzyme (Sigma-Aldrich, Inc., St. Louis, MO, USA) on a Shimadzu (Shimadzu Scientific Instruments, Columbia, MD, USA) 1800 dual beam spectrophotometer and compared to lactate standards. HOMA-IR and QUICKI indices were calculated using the formulas: HOMA-IR = [FPG][FPI] ÷ 405 and QUICKI = 1 / (log (FPI) + log (FPG)), where FPG is fasting plasma glucose, in mg/dl, and FPI is fasting plasma insulin, in µU/ml. Both HOMA-IR and QUICKI indices have been reported to correlate well with glucose clamp methodologies representing hepatic insulin sensitivity measures (Marliss and Vranic, 2002). All assays were conducted using a minimum of duplicates for each sample.

Statistical analysis

A three (3) condition (two exercise conditions [VO_2max and 70-75% VO_2max] and one GC) x four (4) time points (pre-, immediately post-, 1hr post- and 24hrs post-condition) repeated measures analysis of variance (RMANOVA) design was employed. Bonferroni adjustments were utilized to evaluate main time effects if needed. Pearson product moment correlation analysis between apelin and insulin, as well as HOMA-IR and QUICKI, were

Table 1. Descriptive characteristics of participants. Data are presented as mean ± SD.

Variable	Total (n=12)	Males (n=7)	Female (n=5)
Age (yr)	22.75 ± 2.96	22.43 ± 2.88	23.2 ± 3.35
Weight (kg)	68.56 ± 13.08	72.14 ± 15.11	63.54 ± 8.59
Height (m)	1.71 ± 0.08	1.74 ± 0.06	1.67 ± 0.08
BMI ($kg \cdot m^{-2}$)	23.37 ± 3.55	23.66 ± 3.96	23.66 ± 3.96
BF%	15.32 ± 8.09	11.02 ± 6.11*	21.33 ± 6.84*
HR (bpm)	65 ± 9	63 ± 9	67 ± 10
SBP (mmHg)	119.67 ± 9.34	120.29 ± 9.83	118.8 ± 9.65
DBP (mmHg)	77.83 ± 7.29	76.57 ± 8.04	79.6 ± 6.54
MAP (mmHg)	91.78 ± 7.29	91.14 ± 8.51	92.67 ± 6.02
VO_2max ($L \cdot min^{-1}$)	3.49 ± 0.95	4.02 ± 0.86*	2.77 ± 0.47*
VO_2max ($ml \cdot kg^{-1} \cdot min^{-1}$)	51.14 ± 11.07	56.17 ± 10.32	44.09 ± 8.42

* = Significant difference between sex ($p < 0.05$).

computed. Data were analyzed using SPSS 21 software (IBM, Armonk, NY, USA) with significance set at $P \leq 0.05$.

Results

Subject characteristics

Each subject completed all treatment conditions, as well as achieved true VO_2max (51.14 ± 11.07 $ml \cdot kg^{-1} \cdot min^{-1}$). VO_2max ($L \cdot min^{-1}$) and %BF were significantly different between genders (Table 1).

VO_2max and 70-75% VO_2max exercise outcomes

Total work completed for the submaximal exercise was not significantly different between genders ($p = 0.118$), though males (1064.5 ± 122.18 W) performed significantly ($p = 0.021$) more work during VO_2max than females (612.97 ± 111.53 W). The mean duration of VO_2max across all subjects was 7.21 ± 0.3 mins. Mean HR_{max} values (187.2 ± 1.8 bpm) were similar between genders. Exercise intensity was maintained (72.71 ± 1.01%) between 70.62-74.33% for the 70-75% VO_2max, with mean HR (166.22 ± 3.36 bpm). Plasma lactate at rest was similar across exercise visits (Figure 1) with post-VO_2max (11.79 ± 0.51 mM) lactate elevated greater than 70-75% VO_2max (2.72 ± 0.45 mM) lactate ($p < 0.01$).

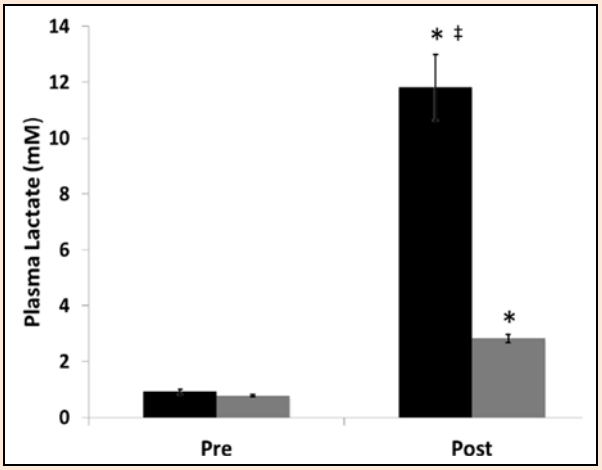

Figure 1. Plasma Lactate Response to Exercise. Plasma lactate (mean ± SEM) response to both VO_2max (black) and 70-75% VO_2max (grey). * Significantly greater than pre ($p < 0.05$). ‡Significantly greater than 70-75% VO_2max post ($p < 0.01$).

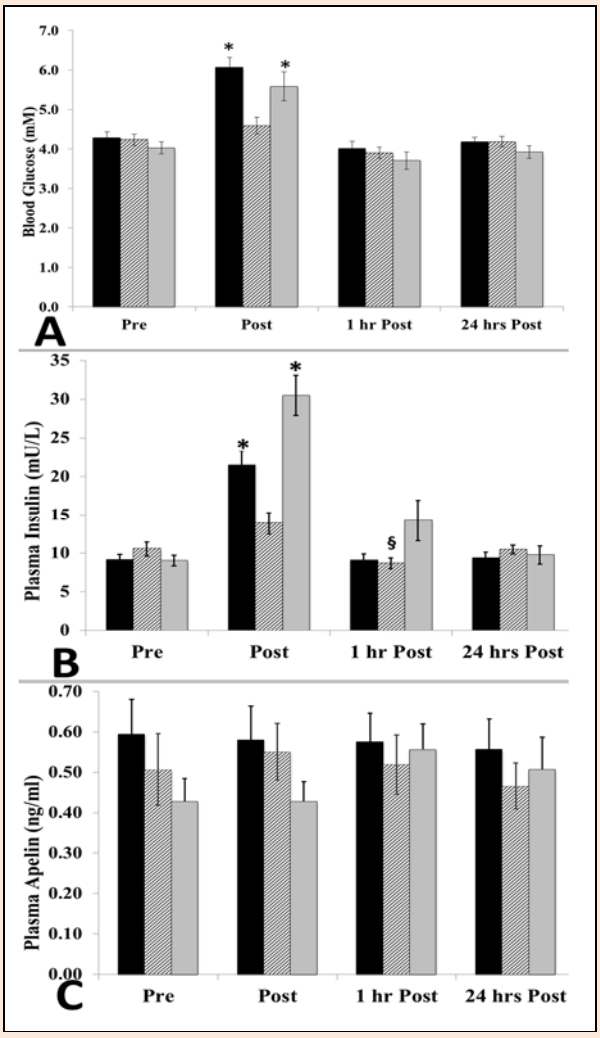

Figure 2. Blood glucose, plasma insulin and apelin. Values are (mean ± SEM) response to VO_2max (black), 70-75% VO_2max (striped) and GC (gray) across each of four time points. Blood glucose (A), Insulin (B) and Apelin (C) * Significantly greater than all other time points ($p < 0.05$). § Significantly lower than pre ($p < 0.05$).

Hematocrit, blood glucose, plasma insulin and plasma apelin

No differences in Hct were observed across time in any condition. Blood glucose analysis revealed a significant time ($p < 0.001$), condition ($p = 0.016$) and condition by time interaction ($p = 0.014$). Plasma insulin had a significant time ($p < 0.001$), condition ($p < 0.001$) and condition by time ($P=0.001$) effect. Post-hoc analysis

revealed that blood glucose was significantly elevated immeditely post-VO_2max and post-GC and returned to normal 1hr post-treatment (Figure 2). Insulin was similar at rest across visits and increased significantly immediately post-VO_2max (21.49 mU/L, $p < 0.001$) and post-GC (30.51 mU/L, $p < 0.001$) and returned to normal 1hr post-treatment. The submaximal exercise resulted in a significant decrease in insulin 1hr post-exercise compared to pre-exercise. Plasma apelin remained unchanged for both time ($p = 0.633$) and condition ($p = 0.324$), though a near-significant interaction effect occurred (P=0.073) (Figure 2).

Plasma apelin and insulin sensitivity

The HOMA-IR and QUICKI index scores over time for each condition are displayed in Figure 3. HOMA-IR and QUICKI scores immediately post-treatment are not shown in Figure 3, as these measures do not reflect steady state, nor do they adequately approximate hepatic insulin sensitivity in the period immediately following exercise. No significant effects by gender were noted for HOMA-IR ($p = 0.736$) or QUICKI ($p = 0.350$). Compared to resting, HOMA-IR significantly decreased ($p = 0.034$) and QUICKI significantly increased ($p = 0.018$) at 1hr post-70-75% VO_2max but was reversed at 24hr post-70-75% VO_2max. Insulin sensitivity remained unchanged for both VO_2max and GC conditions.

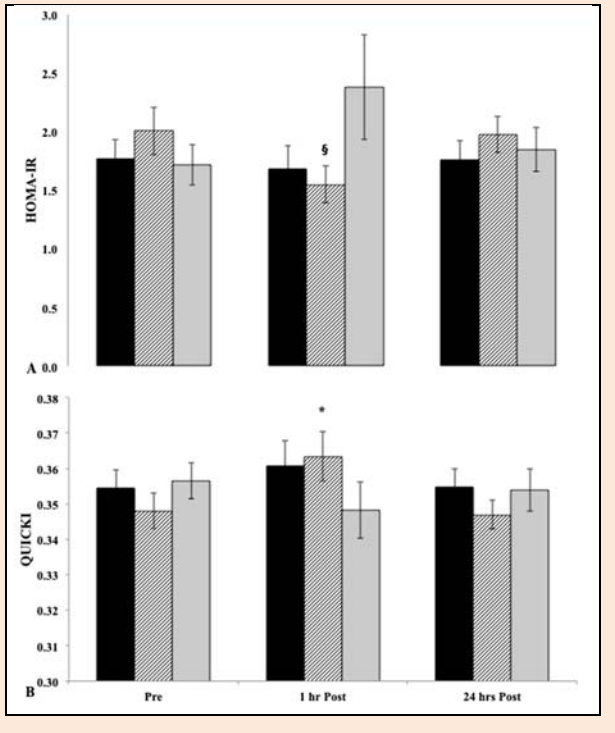

Figure 3. HOMA-IR and QUICKI indices. Values are (mean ± SEM), response to VO_2max (black), 70-75% VO_2max (striped) and GC (gray). HOMA-IR (A) and QUICKI (B) indices. Immediate post measures have been omitted, as these do not reflect the resting requirement of indirect insulin sensitivity measures. § Significantly lower than pre ($p < 0.05$). *Significantly greater than pre ($p < 0.05$).

The relationship between plasma apelin and insulin, as well as with HOMA-IR and QUICKI are shown in Figure 4. Resting plasma apelin significantly correlated with plasma insulin ($r = 0.699$, $p = 0.011$), HOMA-IR ($r = 0.626$, $p = 0.029$) and QUICKI ($r = 0.660$, $p = 0.019$) scores.

Discussion

This study sought to establish resting plasma apelin concentration in a healthy adult cohort, as well as to assess the relationship of plasma apelin to insulin sensitivity, both at rest and following exercise. The current study noted no significant difference in resting plasma apelin concentration by gender ($p = 0.141$), which is supported by previous research (Krist et al., 2013). Additionally, resting plasma apelin concentration amongst all 12 subjects remained stable across visits ($p = 0.990$) at the times measured.

VO_2max, 70-75% VO_2max and GC elicited hypothesized blood glucose and plasma insulin responses; blood glucose and insulin were significantly elevated after VO_2max and GC but not following submaximal exercise. Despite the anticipated elevation in glucose and insulin, plasma apelin was not significantly altered from baseline immediately post-, one hour post- or 24 hours post-treatment following VO_2max and GC. The response of glucose and insulin to GC in our study mirrors the effects observed from a 75g glucose bolus in healthy subjects (Alexiadou et al., 2012). As such, our choice to use this modified glucose load in apparently healthy subjects was supported. Our results also support a previous finding that a glucose load of sufficient dose to increase insulin does not elevate apelin in apparently healthy subjects (Alexiadou et al., 2012). Laboratory and subject time constraints, as well as costs, precluded the performance of a 75g, two-hour OGTT, prompting utilization of the 50g glucose bolus. The observed insulin sensitivity responses following VO_2max and 70-75% VO_2max aerobic exercise conditions confirm the findings of Brestoff et al. (2009), that 45 minutes at 75% VO_2max aerobic cycling on an ergometer, but not supramaximal cycling exercise, improved insulin sensitivity. However, in their study, insulin sensitivity was determined via OGTT 12 hours after exercise cessation, whereas the present study assessed insulin sensitivity at one hour following exercise and glucose bolus ingestion. Our study suggests this enhanced insulin sensitivity, as assessed via hepatic indices, was not related to a change in plasma apelin at the times measured. The present study did not determine insulin sensitivity in peripheral tissues (e.g. skeletal muscle) following exercise. Computation of the Matsuda index, an indirect composite measure of whole body insulin sensitivity that considers both hepatic and peripheral tissue sensitivity, following administration of a 2 hour OGTT is warranted for future study. We assessed insulin sensitivity at one hour and 24 hours post-exercise via HOMA-IR and QUICKI indices; nevertheless it is possible that apelin demonstrated endocrine and/or myokine actions at the skeletal muscle level (Besse-Patin et al., 2014). Future study in this area should adequately assess peripheral glucose disposal via administration of a 12- or 24-hour post-treatment OGTT or through application of the minimal model to stable label intravenous glucose tolerance test findings (Avogaro et al., 1989).

Apelin concentration remained within a narrow range across time after exercise (0.2-1.0 ng/ml) in the

present study. No significant change in plasma apelin was observed, despite VO_2max eliciting blood glucose and plasma insulin elevations. Moreover, a significant correlation was revealed between plasma apelin and plasma insulin ($r = 0.699$, $p = 0.011$) at rest, suggesting that elevated insulin could lead to enhanced plasma apelin. It is possible that the transient insulin elevation might not have met the threshold necessary to significantly alter apelin or the length of time was insufficient. Our study suggests that apelin is unaffected by the transient hyperinsulinemia in the period immediately following an acute graded maximal aerobic exercise test. Additionally, apelin concentration may have peaked outside of our sampling window, as apelin half-life ($t_{1/2}$) was noted previously to be < 5 minutes (Japp et al., 2010). This potential limitation could be mediated with increased sampling frequency over the two-hour window following exercise or GC treatment. Greater sampling frequency, in concert with a glucose tolerance test, as noted above, may shed further light on the potential role of apelin as an endogenous insulin sensitizer *in vivo* after exercise.

A broad time course for insulin-induced apelin induction and secretion signaling has been previously proposed, with *in vitro* insulin administration upregulating apelin mRNA significantly between three and twelve hours post-treatment in isolated adipocytes (Borghouts and Keizer, 2000; Daviaud et al., 2006). These findings, however, do not necessarily reflect *in vivo* insulin-apelin signaling or local apelin action on peripheral insulin-sensitive tissues. Apelin's short circulating $t_{1/2}$ could also have affected our findings. Despite the relatively short apelin $t_{1/2}$, substantive physiological effects may not be elicited during this time period, perhaps requiring longer duration to manifest changes in circulating apelin and influence insulin sensitivity. Apelin itself is also known to exert considerable influence on insulin secretion, inhibiting glucose-stimulated insulin release (Ringström et al., 2010; Sörhede-Winzell et al., 2005). Consequently, full consideration of this dynamic relationship should be further investigated, especially during the post- to one-hour post-treatment window and between the one to 24 hour post time.

Thirty minutes of treadmill running at 70-75% VO_2max in the present study elicited a significant improvement in insulin sensitivity at one hour post-exercise, per HOMA and QUICKI scores. However, VO_2max did not elicit insulin sensitivity improvements. Maximal aerobic exercise did not enhance insulin sensitivity likely due to its short exercise duration (7.21 ± 0.3 mins); moreover, subjects spent only several minutes of this time performing aerobic exercise near or at their maximal aerobic capacities. Submaximal exercise likely elicited significant improvements to HOMA and QUICKI scores through sufficient duration or threshold energy expenditure that exercise of shorter duration, independent of intensity, does not elicit (Magkos et al., 2008). Future studies also should focus on exercise chronicity, as repeated acute bouts or aerobic or interval training may elicit interesting findings, such as those noted in a recent study (Izadi et al., 2018). Interestingly, an improvement in insulin sensitivity was observed in our cohort following the submaximal bout, despite our subjects entering the study as healthy adults with normal glucose tolerance. Furthermore, significant relationships were noted between resting plasma apelin and HOMA-IR ($r = 0.626$, $p = 0.029$) and QUICKI ($r = 0.660$, $p = 0.019$) scores, supporting claims of elevated plasma apelin with insulin resistance (Alexiadou et al., 2012; Cavallo et al., 2012; Krist et al., 2013). Further studies of a similar acute nature should be undertaken in obese and/or T2D populations, so as to observe whether any differences in apelin alteration may be noted compared to our normal, apparently healthy cohort.

Conclusion

The current study demonstrated that circulating apelin does not respond significantly to either acute graded maximal exercise or steady state moderate intensity exercise and did not seem to contribute to hepatic exercise-induced improvements in insulin sensitivity one hour following steady state moderate exercise. Additionally, the transient enhancement in insulin sensitivity one hour following submaximal exercise suggests that this response was independent of circulating apelin changes. Other factors may have contributed to improved insulin sensitivity following submaximal exercise such as: nitric oxide (Roy et al., 1998), calcium and calmodulin/Ca^{2+}-dependent protein kinases (Stanford and Goodyear, 2014), AMPK or PI3K/Akt (O'Neill, 2013), hypoxia and ROS (Sandström et al., 2006) or GLUT4 upregulation. Research remains largely equivocal on the mechanism of exercise-mediated improvements in insulin-dependent insulin sensitivity. The improvement of insulin sensitivity following a single bout of exercise is undoubtedly a multifaceted phenomenon, involving a number of key proteins, blood factors and glucose receptor regulation in insulin-sensitive tissues.

Acknowledgements

The study comply with the current laws of the country in which were performed. The authors report no conflict of interest.

References

Alexiadou, K., Kokkinos, A., Liatis, S., Perrea, D., Katsilambros, N. and Tentolouris, N. (2012) Differences in plasma apelin and visfatin levels between patients with type 1 diabetes mellitus and healthy subjects and response after acute hyperglycemia and insulin administration. *Hormones* **11,** 444-450.

Attané, C., Daviaud, D., Dray, C., Dusaulcy, R., Masseboeuf, M., Prévot D., Carpene, C., Castan-Laurell, I. and Valet, P. (2011) Apelin stimulates glucose uptake but not lipolysis in human adipose tissue ex vivo. *Journal of Molecular Endocrinology* **46,** 21-28.

Avogaro, A., Bristow, J.D., Bier, D.M., Cobelli, C., and Toffolo, G. (1989) Stable-label intravenous glucose tolerance test minimal model. *Diabetes* **38,** 1048-1055.

Besse-Patin, A., Montasier, E., Vinel, C., Castan-Laurell, I,. Louche, K., Dray, C., Daviaud, D., Mir, L., Marques, M.A., Thalamas, C., Valet, P., Langin, D., Moro, C., and Viguerie, N. (2014) Effect of endurance training on skeletal muscle myokine expression in obese men: identification of apelin as a novel myokine. *International Journal of Obesity* **38,** 707-713.

Borghouts, L.B. and Keizer H.A. (2000) Exercise and insulin sensitivity: a review. *International Journal of Sports Medicine* **21,** 1-12.

Boucher, J., Masri, B., Daviaud, D., Gesta, S., Guigné, C. and Mazzucotelli, A. (2005) Apelin, a newly identified adipokine upregulated by insulin and obesity. *Endocrinology* **146,** 1764-1771.

Brestoff, J.R., Clippinger, B., Spinella, T., von Duvillard, S.P., Nindl, B.C. and Arciero, P.J. (2009) An acute bout of endurance exercise but not sprint interval exercise enhances insulin sensitivity.

Applied Physiology Nutrient and Metabolism **34,** 25-32.
Cartee, G.D. (2015) Mechanisms for greater insulin-stimulated glucose uptake in normal and insulin-resistant skeletal muscle after acute exercise. *American Journal of Physiology Endocrinology and Metabolism* **309,** E949-E959.
Castan-Laurell, I., Dray, C., Knauf, C., Kunduzova, O. and Valet, P. (2012) Apelin, a promising target for type 2 diabetes treatment? *Trends in Endocrinology and Metabolism* **23,** 234-241.
Castan-Laurell, I., Vítkova, M., Daviaud, D., Dray C., Kováciková M., Kovacova Z. Hejnova, J., Stich, V. and Valet, P. (2008) Effect of hypocaloric diet-induced weight loss in obese women on plasma apelin and adipose tissue expression of apelin and APJ. *European Journal of Endocrinology* **158,** 905-910.
Cavallo, M.G., Sentinelli, F., Barchetta, I., Costantino C., Incani M., Perra L., Capoccia, D., Romeo, S., Cossu, E., Leonetti, F., Agati, L. and Baroni, M.G. (2012) Altered glucose homeostasis is associated with increased serum apelin levels in type 2 diabetes mellitus. *PLoS One* **7,** 1-6.
Christiansen, T., Paulsen, S.K., Bruun, J.M., Pedersen, S.B. and Richelsen, B. (2010) Exercise training versus diet-induced weight-loss on metabolic risk factors and inflammatory markers in obese subjects: a 12-week randomized intervention study. *American Journal of Physiology Endocrinology and Metabolism* **298,** E824-E831.
Daviaud, D., Boucher, J., Gesta, S., Dray C., Guigne C. and Quilliot, D. (2006) TNFalpha up-regulates apelin expression in human and mouse adipose tissue. *Federation of American Societies for Experimental Biology Journal* **20,** 1528-1530.
Dray, C., Debard, C., Jager, J., Disse, E., Daviaud, D. and Martin, P. (2010) Apelin and APJ regulation in adipose tissue and skeletal muscle of type 2 diabetic mice and humans. *American Journal of Physiology Endocrinology and Metabolism* **298,** E1161-1169.
Dray, C., Knauf, C., Daviaud, D., Waget, A., Boucher, J. and Buléon, M. (2008). Apelin stimulates glucose utilization in normal and obese insulin-resistant mice. *Cell Metabolism* **8,** 437-445.
Fontana, L., Klein, S. and Holloszy, J.O. (2010) Effects of long-term calorie restriction and endurance exercise on glucose tolerance, insulin action, and adipokine production. *Age* **32,** 97-108.
Goodyear, L.J. and Kahn, B.B. (1998) Exercise, glucose transport, and insulin sensitivity. *Annual Review of Medicine* **49,** 235-261.
Heath, G.W., Gavin, J.R., Hinderliter, J.M., Hagberg, J.M., Bloomfield, S.A. and Holloszy, J.O. (1983) Effects of exercise and lack of exercise on glucose tolerance and insulin sensitivity. *Journal of Applied Physiology* **55,** 512-517.
Henriksen, E.J. (2002) Effects of acute exercise and exercise training on insulin resistance. *Journal of Applied Physiology* **93,** 788-796.
Holloszy, J.O. (2005) Exercise-induced increase in muscle insulin sensitivity. *Journal of Applied Physiology* **99,** 338-343.
Izadi, M.R., Ghardashi Afousi, A., Asvadi Fard, M. and Babaee Bigi, M.A. (2018) High-intensity interval training lowers blood pressure and improves apelin and NOx plasma levels in older treated hypertensive individual.. *Journal of Physiology and Biochemistry* **74(1),** 47-55.
Jackson, A.S. and Pollock, M.L. (1978) *Generalized equations for predicting body density of men. British Journal of Nutrition* **40,** 497-504.
Japp, A.G., Cruden, N.L., Barnes, G., van Gemeren, N., Mathews, J. and Adamson, J. (2010) Acute cardiovascular effects of apelin in humans: potential role in patients with chronic heart failure. *Circulation* **21,** 1818-1827.
Kleinz, M.J. and Davenport, A.P. (2005) Emerging roles of apelin in biology and medicine. *Pharmacology Therapeutics* **107,** 198-211.
Krist, J., Wieder, K., Kloting, N., Oberbach, A., Kralisch, S., Wiesner, T., Schon, M.R., Gatner, D., Dietrich, A., Shang, E., Lohmann, T., DreBler M., Fasshauer, M., Stumvoll, M. and Bluher, M. (2013) Effects of weight loss and exercise on apelin serum concentrations and adipose tissue expression in human obesity. *Obesity Facts* **6,** 57-69.
LeBlanc, J., Nadeau, A., Richard, D. and Tremblay, A. (1981) Studies on the sparing effect of exercise on insulin requirements in human subjects. *Metabolism* **30,** 1119-1124.
Magkos, F., Mohammed, B.S. and Mittendorfer, B. (2010) Enhanced insulin sensitivity after acute exercise is not associated with changes in high-molecular weight adiponectin concentration in plasma. *European Journal of Endocrinology* **162,** 61-66.
Magkos, F., Tsekouras, Y., Kavouras, S.A., Mittendorfer, B. and Sidossis, L.S. (2008) Improved insulin sensitivity after a single bout of exercise is curvilinearly related to exercise energy expenditure. *Clinical Science* **114,** 59-64.
Marliss, E.B. and Vranic, M. (2002) Intense exercise has unique effects on both insulin release and its roles in glucoregulation: implications for diabetes. *Diabetes* **51,** S271-S283.
Midgley, A.W., McNaughton, L.R., Polman, R. and Marchant, D. (2007) Criteria for Determination of Maximal Oxygen Uptake. *Sports Medicine* **37,** 1019-1028.
Noble, B.J., Borg, G.A., Jacobs, I., Ceci, R. and Kaiser, P. (1983) A category-ratio perceived exertion scale: relationship to blood and muscle lactates and heart rate. *Medicine and Science in Sports and Exercise* **15,** 523-528.
O'Neill, H.M. (2013) AMPK and exercise: glucose uptake and insulin sensitivity. *Diabetes and Metabolism Journal* **37,** 1-21.
Pescatello, L.S. (2014) A*CSM's Guidelines for Exercise Testing and Prescription*. 9th Ed. Lippincott Williams and Wilkins. pp. 40-41, 73-145.
Ringström, C., Nitert, M.D., Bennet, H., Fex, M., Valet, P. and Rehfeld, J.F. (2010) Apelin is a novel islet peptide. *Regulatory Peptides* **162,** 44-51.
Roy, D., Perreault, M. and Marette, A. (1998) Insulin stimulation of glucose uptake in skeletal muscles and adipose tissues in vivo is NO dependent. *American Journal of Physiology* **274,** E692-E699.
Sandström ME, Zhang SJ, Bruton J, Silva JP, Reid MB, Westerblad H, Katz A.(2006) Role of reactive oxygen species in contraction-mediated glucose transport in mouse skeletal muscle. *Journal of Physiology* **575(Pt 1),** 251-262.
Sörhede-Winzell, M., Magnusson, C. and Ahrén, B. (2005) The apj receptor is expressed in pancreatic islets and its ligand, apelin, inhibits insulin secretion in mice. *Regulatory Peptides* **131,** 12-17.
Stanford, K.I. and Goodyear, L.J. (2014) Exercise and type 2 diabetes: molecular mechanisms regulating glucose uptake in skeletal muscle. *Advances in Physiology Education* **38,** 308-314.
Than, A., Zhang, X., Leow, M.K.-S., Poh, C.L., Chong, S.K. and Chen, P. (2014) Apelin attenuates oxidative stress in human adipocytes. *Journal of Biological Chemistry* **289,** 3763-3774.
Yue, P., Jin, H., Aillaud, M., Deng, A.C., Azuma, J. and Asagami, T. (2010) Apelin is necessary for the maintenance of insulin sensitivity. *American Journal of Physiology Endocrinology and Metabolism* **298,** E59-E67.

Key points

- Acute steady state aerobic exercise enhances insulin sensitization without an increase in plasma apelin in apparently healthy young subjects.
- Graded exercise to VO_2max increases plasma glucose and insulin without a concomitant change in plasma apelin.
- An acute glucose load can increase plasma glucose and insulin but does not increase plasma apelin in apparently healthy young subjects.

AUTHOR BIOGRAPHY

Justin D. WALLER
Employment
University of North Carolina at Greensboro
Degree
MSc
Research interests
The endocrine effects of physical activity and exercise, sport nutrition and the metabolic effects of acute and chronic dietary intervention.
E-mail:
justin.waller@granvillecounty.org

Emily H. MCNEILL
Employment
University of North Carolina at Greensboro
Degree
MSc
Research interests
Body composition and the distribution of adipose tissue throughout the body.
E-mail: Emily@bos.digital

Frank ZHONG
Employment
University of North Carolina at Greensboro
Degree
MSc
Research interests
Aerobic exercise and its effect on inflammatory and anti-inflammatory markers in the plasma.
E-mail: Zhofrank@gmail.com

Lauren S. VERVAECKE
Employment
University of North Carolina at Greensboro
Degree
PhD
Research interests
Understanding changes in brain physiology and endocrine responses with aerobic and resistance training exercise and diet.
E-mail: ervaeck@uscupstate.edu

Allan H. GOLDFARB
Employment
University of North Carolina at Greensboro
Degree
PhD
Research interests
Metabolism, endocrine effects with exercise and training, antioxidants and cell signaling molecules.
E-mail: ahgoldfa@uncg.edu

✉ **Allan H. Goldfarb, Professor**
Department of Kinesiology, University of North Carolina at Greensboro, 260 Coleman Building, 1408 Walker Avenue, USA