International Journal of EXERCISE SCIENCE

Original Research

Progressive Arm Cycling Ergometry With 3- And 5-Minute Stage Durations Yields Similar Estimates of Substrate Oxidation in Healthy Adults

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ABSTRACT

International Journal of Exercise Science 17(2): 468-479, 2024. Arm cycling ergometry (ACE) leads to a lower maximal oxygen uptake (VO_2max) than cycling which is related to a smaller active muscle mass. This study compared estimates of fat and carbohydrate oxidation (FOx and CHOOx) between progressive exercise protocols varying in stage duration in an attempt to create a standard exercise protocol for determining substrate metabolism using ACE. Four men and seven women (age = 24 ± 9 yr) unfamiliar with ACE completed incremental exercise to determine peak power output and VO_2peak. During two subsequent sessions completed after an overnight fast, they completed progressive ACE using 3- or 5-min stages during which FOx, CHOOx, and blood lactate concentration (BLa) were measured. Results showed no difference ($p > 0.05$) in FOx, CHOOx, or BLa across stage duration, and there was no difference in maximal fat oxidation (0.16 ± 0.08 vs. 0.13 ± 0.07 g/min, $p = 0.07$). However, respiratory exchange ratio in response to the 3 min stage duration was significantly lower than the 5 min duration (0.83 ± 0.05 vs. 0.86 ± 0.03, $p = 0.04$, Cohen's d = 0.76). Results suggest that a 3 min stage duration is preferred to assess substrate metabolism during upper-body exercise in healthy adults.

KEY WORDS: Fat metabolism, blood lactate concentration, oxygen uptake, upper-body exercise, carbohydrate

INTRODUCTION

Fat and carbohydrate oxidation (FOx and CHOOx) functions to supply energy in the form of ATP at rest and during exercise. Data show that FOx peaks at low intensities after which carbohydrate becomes the primary fuel at moderate to high exercise intensities (7, 26). Venables et al. (28) reported that maximal fat oxidation (MFO) occurred at intensities equal to 48 % of maximal oxygen uptake (VO_2max) and 61 % maximal heart rate (HRmax) in adults performing incremental treadmill exercise to fatigue, further emphasizing the prioritization of FOx versus CHOOx at mild to moderate exercise intensities. It is evident that FOx is not just important for

exercise, but is also related to insulin sensitivity (25) and health status, as individuals who are prone to weight gain have lower FOx at rest and during exercise (30).
The majority of studies examining changes in FOx during exercise required cycling (1 ,3, 7, 10, 25, 26) which is frequently completed by athletes and available in fitness and health centers. Compared to treadmill exercise, FOx is lower during cycling due to the smaller working muscle mass and greater force required per unit of muscle (8). We (4) also showed lower FOx during cycling compared to rowing, further illustrating that changing the size of working muscle mass alters capacity for FOx.

A study by Achten et al. (1) in cyclists showed that an exercise protocol using 3 or 5 min stages of progressive cycling led to similar estimates of FOx. These results can be directly used by scientists when selecting an appropriate cycling protocol to determine CHOOx and FOx during exercise in trained adults. Nevertheless, in sedentary adults, Bordenave et al. (6) reported that 3 min stages underestimate CHOOx and overestimate FOx during cycling versus 6 min stages, suggesting that longer stages may be needed in nonathletic adults.

Despite the widespread availability of cycle ergometers, treadmills, and related modalities (Elliptical) which use the lower extremity, it is apparent that not all adults can perform these modalities. One potential alternative is arm cycling ergometry (ACE) which involves the upper body musculature including the arms, shoulders, and upper back. This form of exercise is applicable to adults who choose not to cycle, walk, or run and is widely used in adults with spinal cord injury, amputation, or heart disease. Long-term, ACE has been shown to increase cardiorespiratory fitness and other health-related indices (29). Arm cycling ergometry is characterized by a smaller exercising muscle mass, and in addition, the upper-body has a higher ratio of fast glycolytic muscle fibers than the lower-body, which should increase reliance on CHO as fuel. A recent study in active men (23) showed significantly lower FOx during ACE versus cycle ergometry, yet data can be questioned due to inaccurate reporting of the specific Frayn equation used to estimate FOx (fat oxidation = $6.21(VCO_2) - 4.21(VO_2)$, rather than $1.67(VO_2) - 1.67(VCO_2)$ (11). For example, at intensity equivalent to 70 percent of peak power output (%PPO) eliciting 89 % maximal heart rate (%HRmax) which should elicit significant blood lactate accumulation which is inversely associated with capacity for FOx (26), FOx was overestimated and equal to 0.31 – 0.33 g/min despite RER ranging from 0.95 – 0.96, which would result in a minimal 13 – 17 % contribution of fat towards energy expenditure. Their erroneous data describing metabolic responses to ACE merit a re-examination of FOx during ACE in healthy adults, as prevailing data concerning the metabolic response to ACE were acquired in those with obesity (21), spinal cord injury (SCI) (2), or heart disease (15). Effective exercise prescription targeting fat oxidation in healthy adults undergoing ACE, for example, cannot be created unless existing data are accurate.

This study compared estimates of FOx and CHOOx from two ACE exercise protocols varying in stage duration. Resultant data may be used by clinicians and scientists to 1) properly assign upper-body exercise to optimize fat and CHO oxidation for nutritional or exercise interventions and 2) to establish a standardized upper-body exercise protocol to be employed by scientists

who assess substrate metabolism, which is related to health status (18,21,24,30). It was hypothesized that no differences in any parameter would be exhibited between the protocols varying in stage duration, supporting prior work (1).

METHODS

Participants

A total of 11 healthy adults volunteered to take part in this randomized cross-over study. Potential participants were free of cardiovascular or metabolic disease, did not smoke, were non-obese, and were not taking medications or supplements altering metabolism. None had a history of participation in ACE or any upper-body exercise including boxing or swimming. The mean age, body mass, percent body fat, and physical activity were equal to 24 ± 9 yr, 69 ± 16 kg, 23 ± 5%, and 5 ± 1 h/wk, respectively. All habitually participated in physical activity including resistance training, non-competitive sport, and/or aerobic exercise and completed a minimum of 90 min/wk of physical activity. They initially filled out a Physical Activity Readiness Questionnaire and a health-history questionnaire, which were used to verify that they met all study requirements. Subsequently, they provided written informed consent, with the protocol approved by the University Institutional Review Board. Our study protocol aligns with the policies of the International Journal of Exercise Science (22). Our sample size is similar to that used in prior studies conducted to estimate MFO from progressive exercise (1, 9, 23).

Protocol

Participants were advised to refrain from exercise for 36 h prior, not eat for 3 h prior, and be well-rested and hydrated. During the initial session, height and body mass were initially measured using a balance scale and stadiometer. Percent body fat was determined by a trained technician using the sum of three skinfolds (15,16). Subsequently, VO_2peak was determined on an electrically braked arm ergometer attached to a wall (Angio, Lode, Groningen, the Netherlands). After a 3 min warm-up at 7 W, work rate was increased by 8, 15, or 20 W/min in a ramp-like manner to elicit volitional fatigue (cadence < 50 rev/min) in approximately 8 – 10 min. Participants were seated in a chair with the ergometer pedals at shoulder height, and they were required to keep their feet on the floor during exercise. Peak power output was identified as the work rate (in Watt) attained at volitional fatigue. Three minutes after this test, a fingertip was washed, dried, and a 0.7 µL blood sample was acquired to assess BLa using a portable device. Heart rate (HR, Polar, Woodbury, NY) and gas exchange data including VO_2, VCO_2, RER, and V_E (ParvoMedics TrueOne, Sandy, UT) were acquired every 15 s during exercise, and participants breathed into a three-way valve and wore headgear. VO_2peak was identified as the mean of the three values preceding volitional fatigue, and peak HR, RER, and V_E were identified as the highest value attained.

For 48 h prior to the subsequent trial, which was held at the same time of day as the VO_2peak test, participants recorded all food and drink ingested on a written log and refrained from voluntary exercise. This food intake was repeated for 48 h before the final session. They arrived at the lab after an overnight fast ($\geq$ 10 hr) and were well-rested and hydrated. These trials were

held > 72 hr after the baseline session and completed 4 – 7 d apart. The order of the 3- or 5-min stage duration was randomized across participants.

Prior to exercise after a 5-minute seated rest, a 0.7 µL blood sample was taken from a fingertip using a lancet (Owen Mumford Inc., Marietta, GA) and portable monitor (Lactate Plus, Sports Research Group, New Rochelle, NY) to assess BLa. The fingertip was cleaned with a damp towel, dried, and then the first drop of blood was wiped away. A second drop of blood was used to assess blood glucose concentration using a portable device (Bayer Contour, Ascencia, Parsippany, NJ), whose measurement was repeated 3 min post-exercise. Resting gas exchange data were acquired for 3 min to normalize values of RER. Progressive exercise began at a work rate = 15% PPO, with successive stages completed at intensities equal to 25, 35, 45, 55, 65, and 75% PPO for 3- or 5-min duration until mean RER was equal to 1.0 for the entire stage. All participants completed a minimum of four stages of ACE before RER exceeded 1.0. A workload = 15 %PPO was selected as the starting intensity to augment FOx, as recent work from our laboratory (5) shows that ACE performed at 20% PPO elicits 55% HRmax and RER of approximately 0.90, an RER prioritizing CHOOx. Cadence was maintained at 50 rev/min during exercise. Heart rate and gas exchange data were recorded every 15 s during the bout. Blood lactate concentration was acquired immediately at the end of each stage when the participant stopped cranking, and then 3 min after the final exercise stage. The last minute of data from each stage was averaged and used to estimate FOx and CHOOx in g/min using the Frayn (11) equation: FOx = 1.67(VO_2 in L/min) – 1.67(VCO_2 in L/min); CHOOx = 4.55(VCO_2 in L/min) – 3.21(VO_2 in L/min). Maximal fat oxidation (MFO) was recorded as the highest mean FOx value obtained from any stage during exercise and was expressed as g/min and as %HRpeak, %PPO, and %VO_2peak.

Statistical Analysis

Data are presented as mean ± standard deviation (SD) and were analyzed using SPSS Version 27 (Armonk, NY). The Shapiro-Wilks test was used to assess normality of all variables. Two-way ANOVA with repeated measures was used to assess differences in all outcomes across time (rest and 4 stages of exercise = 5 levels) and stage duration (2 levels). For blood glucose, a 2 X 2 repeated measures ANOVA was used. Aggregate gas exchange, metabolic, BLa, and HR data were only acquired from five stages (rest and four stages of exercise up to 45% PPO), as in various participants, RER surpassed 1.0 at 55% PPO, so exercise was terminated. In G-power, using N = 11, $\alpha = 0.05$, and effect size = 0.30 for a paired one-tailed t-test estimating differences in RER, power was equal to 0.25. Partial eta-squared (η^2p) was used to estimate effect size from the ANOVA, with values equal to 0.01, 0.06, and > 0.14 representing a small, medium, and large effect. The Greenhouse-Geisser correction was used if the sphericity assumption was violated. If a significant F ratio was obtained, Tukey's post hoc test was used to identify differences between means. Paired t-test was used to assess differences in outcomes between the 3- and 5-min stage duration. Pearson product moment correlation coefficient was used to identify associations between variables. Cohen's d was used as an estimate of effect size for pairwise comparisons. Statistical significance was set as $p < 0.05$.

RESULTS

Peak exercise data: Table 1 shows results from the VO_2peak test for 4 men and 7 women who completed all requirements of the study. The values of PPO, VO_2peak, and HRpeak are similar to those reported previously in untrained adults undergoing maximal ACE (5, 18).

Table 1. Data from peak exercise testing (N = 11).

Parameter	Mean ± SD	Range
VO_2peak (mL/kg/min)	23.5 ± 5.7	15.0 – 34.7
VO_2peak (L/min)	1.64 ± 0.67	0.90 – 3.05
PPO (W)	103 ± 40	66 - 187
$V_E$peak (L/min)	78 ± 27	43 - 134
HRpeak (b/min)	180 ± 16	136 - 192
RER	1.27 ± 0.17	1.02 – 1.60
BLa (mM)	8.8 ± 2.5	5.5 – 13.0
Duration (min)	9.1 ± 1.1	7 - 11

VO_2max = peak oxygen uptake; PPO = peak power output; V_E = ventilation; HR = heart rate; RER = respiratory exchange ratio; BLa = blood lactate concentration.

Metabolic and HR data from progressive exercise: Table 2 shows the metabolic response to progressive ACE varying in stage duration. All participants completed a minimum of four stages until RER exceeded 1.0, leading to a minimum exercise duration equal to 12 or 20 min (total exercise duration = 19 ± 4 vs. 30 ± 8 min, $p < 0.001$, for the 3- and 5-min stage duration, respectively). VCO_2 and VO_2 increased more than threefold from rest to ACE at 45% PPO (η^2p =0.87 and 0.86, $p < 0.001$), and all values were different from each other ($p < 0.001$), yet there was no main effect of stage ($p = 0.66$ and 0.98, $\eta^2p = 0.02$ and 0.00) or timeXstage interaction ($p = 0.25$ and 0.76, η^2p =0.12 and 0.04). There was a main effect of intensity for RER ($p < 0.001$, η^2p =0.56), yet no effect of stage ($p = 0.18$, $\eta^2p = 0.17$) or interaction ($p = 0.61$, η^2p= 0.06). Post hoc analyses showed that all values were significantly different ($p < 0.03$) than RER recorded at 45 %PPO. For FOx, results showed no main effect of stage ($p = 0.18$, η^2p= 0.17), intensity ($p = 0.11$, η^2p= 0.17), or interaction ($p = 0.44$, η^2p= 0.09). There was a significant main effect of intensity on CHOOx ($p < 0.001$, $\eta^2p = 0.78$), yet no main effect of stage duration ($p = 0.07$, η^2p= 0.33) or interaction ($p = 0.07$, η^2p= 0.22) occurred. Heart rate and BLa data showed a significant effect of intensity ($p < 0.001$, η^2p= 0.78 and 0.71), but no main effect of stage ($p = 0.64$ and 0.61, η^2p =0.02 and 0.03) or interaction ($p = 0.34$ and 0.25, η^2p =0.08 and 0.12) occurred. All HR and BLa values were significantly different from each other ($p < 0.01$). Blood glucose significantly increased ($p < 0.001$, η^2p =0.66) from baseline to post-exercise in 3- and 5-min stages (88 ± 7 to 94 ± 10 mg/dL and 89 ± 10 to 92 ± 9 mg/dL), yet there was no effect of stage duration ($p = 0.64$, $\eta^2p = 0.03$,) or interaction ($p = 0.28$, $\eta^2p = 0.14$).

Table 3 depicts data comparing absolute MFO and related parameters between progressive exercise with 3- and 5-min stage duration. No differences ($p > 0.05$) in any outcome were revealed between stage durations except for RER at MFO, which was significantly lower in response to the 3-min stage duration.

Table 2. Metabolic and heart rate data from progressive ACE having 3 and 5 min stage duration (mean ± SD).

Outcome	Stage duration	Rest	15 %PPO	25 %PPO	35 %PPO	45 %PPO	Stage	%PPO	Int.
VCO_2 (L/min)	3 min	0.21±0.05	0.39±0.10	0.49±0.16	0.63±0.18	0.76±0.23	0.66	<0.001	0.25
	5 min	0.21±0.07	0.39±0.13	0.49±0.18	0.62±0.22	0.80±0.28			
VO_2 (L/min)	3 min	0.24±0.04	0.45±0.09	0.56±0.15	0.68±0.20	0.81±0.24	0.98	<0.001	0.76
	5 min	0.25±0.05	0.46±0.15	0.54±0.15	0.67±0.23	0.83±0.30			
RER	3 min	0.83±0.07	0.85±0.07	0.87±0.05	0.91±0.08	0.93±0.06	0.18	<0.001	0.61
	5 min	0.84±0.07	0.85±0.04	0.90±0.07	0.93±0.06	0.96±0.07			
FOx (g/min)	3 min	0.06±0.03	0.12±0.04	0.11±0.04	0.11±0.06	0.10±0.09	0.18	0.11	0.44
	5 min	0.07±0.03	0.12±0.04	0.09±0.06	0.08±0.07	0.07±0.08			
CHOOx (g/min)	3 min	0.14±0.07	0.32±0.20	0.45±0.24	0.57±0.28	0.82±0.31	0.07	<0.001	0.06
	5 min	0.14±0.09	0.30±0.14	0.47±0.23	0.67±0.21	0.95±0.26			
BLa (mM)	3 min	1.3±0.3	1.4±0.4	1.8±0.4	2.3±0.6	2.8±0.8	0.61	<0.001	0.25
	5 min	1.0±0.3	1.4±0.3	1.9±0.4	2.4±1.0	3.2±1.5			
HR (b/min)	3 min	76±8	92±15	102±14	110±16	125±17			
	5 min	77±9	95±14	102±13	114±14	127±14	0.32	<0.001	0.61

VCO_2 = carbon dioxide production; VO_2 = oxygen uptake; RER = respiratory exchange ratio; FOx = fat oxidation; CHOOx = carbohydrate oxidation; BLa = blood lactate concentration; HR = heart rate; PPO = peak power output; Stage = p value for the main effect of stage duration; PPO = p value for the main effect of intensity; Int. = p value for the stage X intensity interaction.

Table 3. Differences in maximal fat oxidation, RER, and blood lactate concentration between the 3 min and 5 min stage duration (mean ± SD).

Parameter	3 min stage	5 min stage	p value	Cohen's d
MFO (g/min)	0.16 ± 0.08	0.13 ± 0.07	0.07	0.42
MFO (%HRpeak)	58.4 ± 8.4	57.4 ± 8.1	0.61	0.13
MFO (%VO_2peak)	37.3 ± 8.1	37.5 ± 9.3	0.95	0.03
RER at MFO	0.83 ± 0.05	0.86 ± 0.03*	0.04	0.76
Workload at MFO (%PPO)	25.9 ± 12.0	24.1 ± 10.4	0.17	0.17
BLa at MFO (mM)	1.94 ± 0.96	1.96 ± 0.81	0.93	0.02

MFO = maximal fat oxidation; HR = heart rate; VO_2 = oxygen uptake; RER = respiratory exchange ratio; PPO = peak power output; BLa = blood lactate concentration; * = $p < 0.05$ versus 3 min duration.

Differences in MFO and RER at MFO in our participants: Figure 1 compares MFO (g/min) and RER at MFO across stage duration in all 11 participants. With exception of one participant, a 24 yr old female with MFO equal to 0.08 versus 0.17 g/min, 10 of 11 participants had higher values of MFO in response to 3 compared to 5 min stage duration. Unsurprisingly, RER at MFO was lower in 82% of participants with 3 min versus 5 min stage durations. Across participants, MFO and RER at MFO varied widely, from 0.08 – 0.32 g/min to 0.78 – 0.91, respectively.

Correlation analyses: Between the 3-min and 5-min stage duration, results revealed strong, significant relationships for MFO ($r = 0.82$, $r^2 = 0.67$, $p = 0.02$), RER at MFO ($r = 0.74$, $r^2 = 0.55$, $p = 0.01$), BLa at MFO ($r = 0.70$, $r^2 = 0.49$, $p = 0.02$), workload at MFO ($r = 0.95$, $r^2 = 0.90$, $p < 0.001$),

and MFO expressed as %HRpeak ($r = 0.75$, $r^2 = 0.56$, $p = 0.008$), yet not for MFO expressed as %VO_2peak ($r = 0.50$, $r^2 = 0.25$, $p = 0.12$).

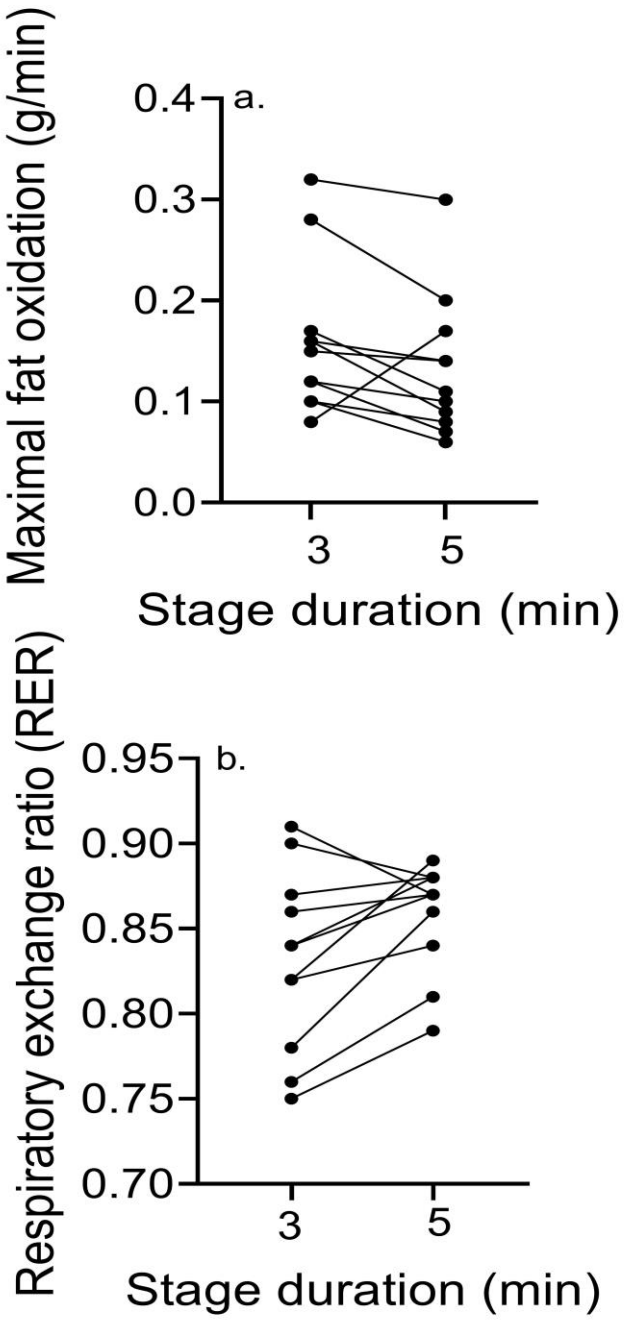

Figure 1. Individual differences in a) MFO (g/min) and b) RER at MFO in 11 participants performing progressive ACE with 3- and 5-min stage duration.

DISCUSSION

This study compared estimates of FOx and CHOOx during progressive ACE using 3- and 5-min stage durations, as prior work was performed in clinical populations (15, 18, 21) or reported erroneous data (23), which merits a re-investigation of this topic in healthy adults. Results show similar estimates of MFO, FOx and CHOOx, as well as BLa between stage durations of 3 and 5 minutes, Nevertheless, RER at MFO was significantly lower in response to 3-min stage duration, reflecting higher FOx. These results may be used by scientists to implement a time-efficient 3 min per stage ACE protocol, which is less burdensome for participants, when estimating substrate metabolism, which is relevant to exercise and nutritional programming in many adults.

Data show that RER at MFO was significantly lower (large effect) in response to the 3 versus 5 min stage duration, representing a 10% higher rate of FOx. However, previous data (3) reveal a standard error of the mean of 0.03 for RER and coefficient of variation (CV) equal to 30% for MFO when progressive cycling was performed by sedentary women on two separate days preceded by a 6 hr fast and abstention from physical activity for 48 hr. In moderately-trained men, Croci et al. (9) demonstrated a CV for MFO and RER at MFO determined from progressive cycling ranging from 23 – 26% and 1.6 – 1.7%. In addition, the CV for estimates of FOx and CHOOx during progressive exercise was substantial (9 – 49%). Overall, the dramatic day-to-day variation in outcomes associated with capacity for FOx suggests that the attenuated RER in response to the 3-min stage duration is not clinically meaningful.

Our MFO values ranging from 0.13 - 0.16 g/min are substantially lower than those reported from other exercise modalities including cycling (1) and treadmill running (27), which is attributed to the small muscle mass inherent with ACE. They are also lower than values (~0.20 g/min) shown in trained men with SCI completing progressive ACE (19). Nevertheless, they are markedly higher than values (0.06 g/min) reported for able-bodied adults (18), despite these individuals having higher VO_2peak (27 mL/kg/min) than our sample. Yet in this study, exercise was completed after a 3 – 4 h fast, which should elicit lower estimates of FOx compared to an overnight fast (22). Moreover, different stoichiometric equations were used (19), which may elicit different estimates of FOx and CHOOx across studies.

MFO occurred at intensities equal to approximately 25% PPO and 58% HRpeak, respectively, which corroborate prior work. Maximal fat oxidation in response to progressive treadmill exercise performed by 300 adults occurred at 48% VO_2max and 61% HRmax, respectively (28). In 15 active young men performing progressive cycling on two separate occasions, MFO occurred at intensities equal to 32 – 36% PPO, 44 – 50% VO_2max, and 59 – 63% HRmax, respectively (9). The fact that MFO occurs at similar fractions of peak HR is surprising due to differences in participant characteristics, exercise protocols, and methods used to determine FOx across studies. Nevertheless, the differences in MFO expressed as %VO_2max/VO_2peak across studies are due to the blunted VO_2 response of arm cycling compared to whole-body exercise including running and cycling. Overall, our results demonstrate that MFO during ACE occurs

at relatively low power outputs and a workload attendant with light intensity according to the ACSM (12).

Our results (Figure 1) reveal highly variable estimates of MFO and RER at MFO across participants, which are likely due to discrepancies in VO_2peak, body composition, and sex within our sample. Moreover, dietary fat intake and training volume are related to capacity for fat oxidation (13), and it is likely that our participants ingest different amounts of fat and accumulate dissimilar amounts of physical activity which would alter resultant estimates of substrate metabolism. Significant correlations were shown between absolute VO_2peak and MFO from the 3 ($r = 0.62$, $r^2 = 0.38$, $p = 0.04$) and 5 min protocol ($r = 0.79$, $r^2 = 0.63$, $p = 0.004$), which corroborate prior data (10). Data from Goedecke et al. (13) in trained cyclists demonstrated marked variability in resting and exercise RER, which they attributed to different muscle glycogen and free fatty acid concentration and BLa across participants. In the present study, neither muscle glycogen nor free fatty acid concentration was estimated, so we can only speculate as to what explains this variation across participants. Nevertheless, our data show that BLa at MFO explained 49 - 66% of the variance in MFO, which suggests that this metabolite is strongly associated with capacity for fat oxidation during upper-body exercise, as previously demonstrated for cycling in a diverse group of participants (26).

This study has a few limitations. First, only four stages of progressive ACE were completed by all participants due to the relatively low exercise tolerance of three participants whose RER surpassed 1.0 at 55% PPO in the 5 min stage protocol. In future studies, a lower initial work rate and smaller increments in power output may be required to enable completion of an additional number of stages. However, this approach may lead to a substantially longer exercise bout which may be unpleasant for nonathletic adults. Second, we used the Frayn equations (11) to estimate substrate metabolism, so our data do not apply to studies in which alternate equations are used (19). Third, due to known differences in substrate metabolism between trained and untrained muscle (14), our results acquired in individuals unfamiliar with ACE do not apply to individuals who regularly complete ACE and/or endurance training of the upper-body, as performed by swimmers, boxers, etc. Fourth, the sample was small and heterogeneous in sex, body mass, and VO_2peak, which may alter interpretation of our results. Nevertheless, the within subjects design attendant with this study may minimize potential effects of our diverse sample. In addition, our study is strengthened by precise allocation of intensities during progressive ACE as well as standardization of pedal cadence and body position. We also employed an overnight fast, and our blood glucose results suggest that participants arrived in the post-absorptive state. Lastly, we required participants to abstain from voluntary activity and standardize food intake for 48 h pre-trial, which elicit stable muscle glycogen and in turn, more precise estimates of substrate metabolism.

Overall, progressive ACE using 3- or 5-min stage duration yields similar estimates of substrate oxidation and blood lactate concentration, although RER at MFO was significantly lower in response to the 3-min duration. Maximal fat oxidation occurred at a workload equal to ~25% PPO and 58% HRpeak, so if optimizing fat oxidation is the goal of exercise programming,

individuals should perform low intensity ACE. Amongst participants, there was dramatic variability in substrate metabolism during exercise.

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