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Original Research

No Effect of Body Fat on Fat Oxidation Rates in Lean and Overweight Females When Aerobic Fitness is Controlled and Muscle Mass is Similar

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Abstract

International Journal of Exercise Science 19(2): 2017, 2026. Fat oxidation (FATox) rates during exercise are largely determined by exercise intensity, aerobic fitness status, and body composition. FATox is proposed as a noninvasive means for assessing metabolic health in females. Overweight (OW) females rely less on fat than lean (LN) females during submaximal exercise although this remains unclear as previous studies have failed to control for aerobic fitness. This study compared FATox during submaximal exercise through indirect calorimetry between twenty female participants (N=20; mass: 63.1±9.0 kg; fat-free mass: 45.2±3.9 kg; fat mass: 17.8±6.0 kg; height: 165.0±5.7 cm; VO_{2peak}: 35.8±2.9 mL·kg^{-1}·min^{-1}) of a similar VO_{2peak} range (35±5 mL·kg^{-1}·min^{-1}), but stratified by bodyfat (BF%) into lean (LN) (N=10, ≤25 BF%) and overweight (OW) (N=10, ≥30 BF%). Participants exercised on a motorized treadmill for 3 min at 25%, 35%, 45%, and 55%, of their VO_{2peak}. Absolute (g·min^{-1}) and relative (mg·kgFFM^{-1}·min^{-1}) FATox rates were calculated from stoichiometric equations. There were no significant differences (p>0.05) at any stage for absolute, relative, or peak FATox rates between LN and OW females (absolute: LN: 0.30 ± 0.07 g·min^{-1} vs. OW: 0.32 ± 0.06 g·min^{-1}). In conclusion, FATox rates during exercise were not different between LN and OW females with similar aerobic fitness levels. Coaches and practitioners can choose similar exercises that promote adherence and enjoyment while subsequently improving aerobic capacity in female participants.

Keywords: Indirect calorimetry, body composition, fat metabolism, eumenorrheic females

Introduction

Substrate utilization during exercise is largely influenced by exercise intensity.[1] At low moderate exercise intensities (<65% VO_{2max}), endogenous lipids are the predominate fuel source, but as intensity increases, a shift in energy contribution occurs favoring carbohydrate oxidation.[2,3] However, exercise intensities[2,4] (55-65% of VO_{2max}) that predominately rely on fat oxidation (FATox) has garnered attention among coaches and athletes alike due to the suggestion that maximizing an individual's FATox may influence markers of metabolic health, aerobic performance, and improve body composition.[5]

Aside from exercise intensity, aerobic fitness and biological sex are the main contributors to FATox during exercise, with aerobically trained individuals exhibiting greater rates of FATox at higher relative intensities than untrained individuals.[6] This higher capacity for FATox in trained individuals reflects several mechanisms associated with chronic aerobic training such as an increase in intramuscular triacylglycerol concentrations,[7] mitochondrial density and

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function,[8] and increased fatty acid transport protein CD36 expression.[9] It is also well established that eumenorrheic females have an increased capacity for FATox compared to males because of physiological and hormonal differences.[10-12] Specifically, females have been reported to have greater concentrations of free fatty acids in circulation,[13,14] higher concentrations of lipid storage and lipid utilization.[15-17] and elevated concentrations of circulating estrogen which increases lipolytic activity.[18]

Interestingly, possessing excess adiposity does not appear to affect FATox in females as observed in males.[19,20] It has been speculated that elevated concentrations of estrogen in females, might protect lipolytic and oxidative proteins from reductions in FATox.[21-24] For example, Ruby et al[25] observed increased lipolytic activity, as reflected by decreases in glucose metabolism at rest and during moderate intensity exercise, as a result of elevated estrogen concentrations in amenorrhoeic females. In agreement with these findings, a recent review examining the influence of excess adiposity on rates of FATox during exercise found no differences between overweight/ obese and normal-weight females in six of the nine studies it examined.[26] Two of these studies reported an increase in FATox rates for females with excess adiposity compared to their leaner counterparts.[27-29] However, two important points should be taken into consideration when interpreting these findings. First, FATox rates are often reported in absolute measurements ($g \cdot min^{-1}$), with no adjustments for fat-free mass (FFM).[22,29-34] When ignoring the role of FFM in calculating FATox, decrements in FATox rates between overweight and lean participants may be masked as identified by Waldman et al,[35] thus creating a type II error. Second, to properly test the hypothesis that excess adiposity might affect FATox during submaximal exercise, lean and overweight individuals should display similar levels of aerobic fitness. Therefore, provided the role aerobic fitness and body composition might play on FATox rates in females, the purpose of this study was to compare FATox rates (absolute and relative to FFM) between lean and overweight eumenorrheic females who were similar in their aerobic fitness status (35 ± 5 $mL \cdot kg^{-1} \cdot min^{-1}$).

Methods

Participants

Participants were recruited using a convenience sampling approach, as they were volunteers who met the inclusion criteria and were readily accessible to the research team. Therefore, forty-five recreationally active, eumenorrheic females volunteered to participate in the study; however, twenty-five participants were excluded from the study following visit two due to BF% (N = 9) or VO_{2peak} (N = 16) that placed them outside the prior established criteria. Therefore, twenty (N = 20) recreationally active, eumenorrheic females completed the current study (Table 1). Participants provided verbal and written consent at the initial visit, prior to completing the Physical Activity Readiness Questionnaire (PAR-Q), a menstrual cycle questionnaire, and an exercise history questionnaire to ensure each participant met the inclusion criteria for the study. Additional inclusion criteria included: (a) no known cardiometablic diseases (e.g., diabetes, impaired fasting glucose), (b) currently between the age of 18-39 y, (c) currently not pregnant or attempting to become pregnant, (d) abstained from oral or hormonal contraceptives for the previous 6 months, and (e) currently defined as eumenorrheic which is assessed via menstrual cycle questionnaire.[36] All participants were nonsmokers (Table 1).

Table 1. Subject Characteristics.

	Lean	Overweight
Body mass index (kg/m^2)	20.9 ± 1.4	25.8 ± 2.0
Body mass (kg)*	56.9 ± 4.8	70.4 ± 8.1
Fat free mass (kg)	43.7 ± 3.6	46.8 ± 4.0
Fat mass (kg)*	13.2 ± 1.8	23.8 ± 4.9
Bodyfat (%)*	23.5 ± 1.9	33.7 ± 3.1
VO_{2peak}, (mL·kg^{-1}·min^{-1})	36.7 ± 2.8	34.3 ± 2.5

Values are means ± SD; n = 10 subjects in each group. VO_{2peak} = peak oxygen consumption *Significantly different from lean, $p < 0.05$.

Following the second visit, participants with a VO_{2peak} between 30-40 mL·kg^{-1}·min^{-1} were then stratified into two groups based on BF% (overweight (OW), ≥30% or lean (LN), ≤25%). No individual matching was performed, but fat free mass was recorded and considered in secondary analyses. Using the ACSM guidelines[37] and a previously published study examining BF% and FATox rates in females,[38] we chose a BF% range that placed our participants in either a OW (≥30%) or LN classification (≤25%). To examine the impact of adiposity on FATox rates in females the population was classified as Tier 1: recreationally active based on the framework established by Mckay et al.[39] A Tier 1 classification is described as an individual who is between the ages 18-64, who completes at least 150-300 min of moderate-intensity activity or 75-150 min of vigorous-intensity activity per week and a muscle-strengthening session ≥2 per week. This was assessed via an Exercise History Questionnaire that provided researchers with information regarding type of exercise, rating of perceived exercise for a typical exercise session, and min per day and days per week of physical exercise (aerobic or resistance training). Participants were excluded if they were collegiate athletes. Additionally, our chosen VO_{2peak} range placed our participants in an ACSM cardiorespiratory classification of "fair"[37] which we have found in past testing within our laboratory to encompass most of our healthy female participants who were not sedentary. Participants were excluded if their VO_{2peak} was <30 or >40 mL·kg^{-1}·min^{-1} and if their BF% fell between 25-30%. This study was performed in accordance with the Declaration of Helsinki and was approved by the University's Institutional Review Board (IRB #: 2023-026). This research was carried out fully in accordance with the ethical standards of the *International Journal of Exercise Science*.[40]

Protocol

This study incorporated an experimental, cross-sectional design to examine whether body composition impacted FATox rates in females with similar aerobic fitness classifications. All participants reported to the laboratory on three separate occasions: 1) participants completed informed consent and inclusion questionnaires, 2) completion of a VO_{2peak} test and body composition assessment via bioelectrical impedance analysis (BIA) to determine eligibility for trial three, and 3) an experimental trial to assess FATox rates. During visit one, participants provided written informed consent and completed a series of screening questionnaires (detailed below). Participants were then provided with two 500-mL water bottles and were informed to drink one bottle the night before visit 2 and one the morning of, prior to arrival, to ensure euhydration during the body composition measurement via BIA for each participant. For visits two and three, participants refrained from alcohol consumption and strenuous exercise for 48 h and caffeine consumption for at least 12 h prior to testing. For visit three, participants alerted an investigator at the onset of menses and were tested in their early follicular phase (identified as the first five days following onset of menses[36]) and asked to arrive following at least a 10 h overnight

fast[41]. Participants were instructed to maintain their habitual diet throughout the duration of the study and were excluded if they were currently following any form of carbohydrate-restricted or fasting protocols.

Body composition

Upon arrival to the laboratory, participants' urine specific gravity was assessed via refractometer (Master Refractometer, ATAGO, Tokyo, Japan). Upon hydration status confirmation (1.013 ± 0.005), participants height (Invicta Plastics Limited, Leicester, England) and body mass (BWB-800, Tanita Inc., Japan) measurements were obtained. Participants were then assessed for body composition via bioelectrical impedance analysis[42] (BIA; mBCA 514; SecaGmbH & Co., Hamburg, Germany). If participants were <25% or >30% BF, they then completed a VO_{2peak} test. Body composition analysis measurements included: body fat percentage (BF%), fat mass (FM), and fat free mass (FFM).

VO_{2peak}

A VO_{2peak} test was completed on a motorized treadmill (Woodway, Waukesha, WI, USA). Participants were fitted with a face mask (Hans Rudolph, Kansas City, MO, USA) for the collection of cardiorespiratory measures via indirect calorimetry[43] (Parvo Medics TrueOne 2400, Sandy, UT, USA) and provided a chest strap heart rate monitor (Polar Electro Ltd, Kempele, Finland). The protocol began with a period of 3 min, at a walking speed of 1.6 km·h^{-1} and at a clamped 3% grade. The treadmill speed then increased by 1.6 km·h^{-1} every 3 min until the participant reached volitional exhaustion, or if the participant reached a respiratory exchange ratio >1.05 and a maximal heart rate value within ±10 beats per min of age-predicted values.[44]

Experimental Trial

Following an overnight fast (~10 h), participants reported to the laboratory to complete a submaximal, graded exercise test. Participants were fitted with a heart rate monitor and a metabolic face mask for the collection of expired air via indirect calorimetry during the graded exercise test. The graded exercise test consisted of 4×3 min stages, with each subsequent stage increasing in intensity (workload km·h^{-1} increased; grade remained 1%). Using data from each participant's initial VO_{2peak} test, treadmill speeds corresponding to 25%, 35%, 45%, and 55% of their VO_{2peak} were identified based on their individual speed-VO_2 relationship and then used for each respective 3 min stage bout. We chose these intensities based on pilot data from our laboratory which found that recreationally trained females elicit peak FATox rates at ~40% of their VO_{2peak}. Duration for each stage was determined based on previous research which found that 3 min stages produced similar FATox values as 5 min stages.[2]

Indirect Calorimetry and Calculations

FATox rates were calculated based on average cardiorespiratory measures from expired gases. These values were then calculated by averaging the last two, 30 s increments during the last (i.e. third) min of each stage. Absolute (g·min^{-1}) and relative (mg·kg FFM^{-1}·min^{-1}) FATox rates were calculated from stoichiometric equations with an assumption that protein contributions were negligible.[45] Further, peak FATox (g·min^{-1}) was determined using a third-degree polynomial regression analysis constructed from the measured FATox data on each individual participant.[2,4] The highest value detected (g·min^{-1}) by the regression analyses and the relative intensity (VO_{2peak}%) at which it occurred were collected and the mean value was used for statistical analysis.

Statistical Analysis

Data are reported as mean ± SD. The α level was set at $p \leq .05$ to be considered statistically significant. Data were first assessed for normality and homogeneity using a Shapiro-Wilk and Levene's test, respectively. Following confirmation of normality and homogeneity tests, primary analyses compared absolute FATox (g·min^{-1}) between groups at each respective stage (25%, 35%, 45%, and 55% VO_{2peak}) using independent *t*-tests. During data inspection, we observed that despite no intentional matching, the two groups exhibited similar FFM ($p = 0.16$). To address potential confounding by FFM—a known determinant of metabolic capacity—we conducted a planned secondary analysis normalizing FATox to FFM (mg·kg FFM^{-1}·min^{-1}). This approach aligns with recent recommendations for interpreting substrate oxidation relative to metabolically active tissue.[35] Where significance occurred, effect sizes were calculated and reported as Cohen's *d*[46] to compare the magnitude of effect between independent groups and are interpreted as [trivial (<0.20), small (0.20-0.59), moderate (0.60-1.19), large (1.20-1.99), very large (2.00-3.99), and extremely large (≥4.00). All statistical analysis were performed in SPSS (version 28; IRBM, Chicago, IL).

Results

Anthropometric characteristics for the 20 participants are presented in Table 1. Although FFM was not statistically different between groups ($p = 0.16$), the LN group had a significantly lower BF% ($p < 0.001$, $d = 4.21$), fat mass ($p < 0.001$, $d = 2.86$), and total body mass ($p < 0.001$, $d = 2.04$) than the OW group. Regarding FATox, no significant group differences were found for absolute (g·min^{-1}; Table 2) or relative (mg·kg FFM^{-1}·min^{-1}; Table 3) FATox rates, or peak values (absolute: LN: 0.30 ± 0.07 g·min^{-1} vs. OW: 0.32 ± 0.06 g·min^{-1}) (all $p > 0.05$). Lastly, while treadmill speed from participant's VO_{2peak} trials were used to predict and to elicit VO_{2peak} intensities from 25-55% for each participant, *post hoc* analysis revealed a ±5% fluctuation from the intended target at some stages. The actual achieved VO_2 ranges are as follows: 25% (LN: 9.2 ± 1.2 mL·kg^{-1}·min^{-1}; OW: 8.8 ± 0.8 mL·kg^{-1}·min^{-1}), 35% (LN: 12.4 ± 1.6 mL·kg^{-1}·min^{-1}; OW: 11.7 ± 1.5 mL·kg^{-1}·min^{-1}), 45% (LN: 15.8 ± 1.3 mL·kg^{-1}·min^{-1}; OW: 14.7 ± 1.0 mL·kg^{-1}·min^{-1}), and 55% (LN: 19.9 ± 2.0 mL·kg^{-1}·min^{-1}; OW: 17.6 ± 1.7 mL·kg^{-1}·min^{-1}). *Post-hoc* comparisons indicated that VO_2 differed significantly between groups at 45% ($p = 0.04$) and 55% ($p = 0.02$), and no differences at 25-35% ($p \geq 0.40$).

Table 2. Absolute Fat Oxidation Rates.

VO_{2PEAK} %	Lean	Overweight	*P*-value	Cohen's *d*
25	0.23 ± 0.05	0.27 ± 0.04	0.09	.88
35	0.26 ± 0.06	0.30 ± 0.05	0.09	.72
45	0.27 ± 0.07	0.30 ± 0.07	0.35	.42
55	0.21 ± 0.10	0.24 ± 0.10	0.56	.30

Values are mean ± standard deviation. *P*-values represent between-group comparisons at each stage. Cohen's *d* reflects effect size for lean relative to overweight. Statistical significance was set at $p<0.05$.

Table 3. Relative Fat Oxidation Rates.

VO_{2PEAK} %	Lean	Overweight	*P*-value	Cohen's *d*
25	5.30 ± 1.03	5.77 ± 1.00	0.27	.46
35	6.06 ± 1.49	6.58 ± 1.06	0.33	.40
45	6.32 ± 1.73	6.50 ± 1.46	0.79	.11
55	5.02 ± 2.34	5.18 ± 2.09	0.86	.07

Values are mean ± standard deviation. *P*-values represent between-group comparisons at each stage. Cohen's *d* reflects effect size for lean relative to overweight. Statistical significance was set at $p<0.05$.

Discussion

The aim of this study was to assess if varying levels of adiposity impacted FATox rates in eumenorrheic females, when controlling for aerobic fitness status. The main findings from this study demonstrated there were no significant differences at any stage for absolute and relative FATox rates between LN and OW females during submaximal exercise.

It has been suggested that to properly assess if adiposity affect FATox rates in females, participants should first be matched for aerobic fitness status[7,26] to prevent the occurrence of a type II error. With this consideration in mind, our findings align with a recent review[26] and previous studies[12,21-23] which concluded that increased adiposity does not appear to impair FATox in females. For example, Blaize et al[39] observed no differences in absolute and relative peak FATox rates between LN (0.39 ± 0.10 g·min^{-1}, 8.5 ± 2.7 mg·kg FFM^{-1}·min^{-1}) and OW (0.49 ± 0.13 g·min^{-1}, 10.81 ± 2.80 mg·kg FFM^{-1}·min^{-1}) females with no significant differences in FFM (LN: 46.0 ± 4.9 kg; OW: 45.8 ± 4.6 kg) and aerobic fitness status (LN: 61.3 mg·kg FFM^{-1}·min^{-1}; 65.1 mg·kg FFM^{-1}·min^{-1}). Likewise, Kerherve´ et al[23] reported similar FATox values during submaximal exercise (30-60% VO_{2max}) across LN, OW, and obese females. These findings were reported although significant differences were present among VO_{2max} values (LN: 38.2 ± 7.2 mL·kg^{-1}·min^{-1}; OW: 33.3 ± 5.9 mL·kg^{-1}·min^{-1}; obese: 23.8 ± 3.7 mL·kg^{-1}·min^{-1}) which contrast a recent study reporting that FATox values were highest for the more aerobically fit females and lowest for the OW, sedentary group across all submaximal cycling intensities.[35] Clearly discrepancies exist regarding whether a FATox decrement exists in females characterized by excess adiposity. While our data add to the growing body of evidence suggesting this is not the case when participants reflect a similar aerobic capacity, additional variables such as FFM are often overlooked in previously reported studies which warrant additional consideration.

For example, Horowitz et al[27] evaluated plasma fatty acid availability and FATox between obese and LN females and found obese females to have a greater FATox during moderate-intensity exercise compared to their LN counterparts. This elevated FATox rate in the obese group occurred in the absence of any significant differences between fatty acid appearance, plasma fatty acid uptake, or plasma fatty acid oxidation. Therefore, the differences in FATox between the groups likely occurred inside the skeletal muscle, presumably via a greater reliance on intramuscular triglycerides for oxidation in the obese group. This is an important consideration as Horowitz's obese group had significantly higher FFM (~9 kg) compared to the LN group.[27] It is plausible that had the present investigation recruited obese females as opposed to OW, a greater discrepancy in FFM might have elicited higher FATox values than the ones reported (see Tables 2 and 3). In the present study, no significant differences existed in FFM between LN (44.1 ± 3.6 kg) and OW (46.5 ± 4.1 kg) females (Table 1), although it should be mentioned this was not intentionally controlled. A unique challenge in such a study design would be the controlling of aerobic fitness between participants considered *obese* and LN. While only speculation, participants possessing an aerobic fitness status deemed "fair" would likely self-limit adiposity from reaching a status considered obese. Regardless, since FFM is the primary site of FATox, our findings collectively suggest that the total contracting FFM during exercise is arguably a stronger regulator of FATox rates than the level of adiposity a female possesses.

It is often overlooked that OW females tend to carry more FFM compared to LN females,[7,20,26-28] although not always.[38] This has also been confirmed by a recent review reporting that OW and obese females had higher FFM in 14 of their 16 included studies which examined the effect of adiposity on FATox.[26] This is an important consideration for future research since increased

FFM is known to impact energy expenditure and the type of energy (e.g., lipid vs. CHO) during submaximal exercise,[20] and when comparing FATox rates.[28,30] These findings would also strongly suggest that while FATox is often calculated and reported in absolute measurements (g·min^{-1}), the value should be also expressed relative to FFM (mg·kg FFM^{-1}·min^{-1}) since it is influenced by the amount of metabolically active tissue the female possesses. These considerations can be observed in study's such Keim et al.[20] who reported their LN group having both significantly lower FFM and energy expenditure than that of their obese group. However, any significant differences were eliminated once energy expenditure was expressed relative to FFM. Other female focused studies where FATox is reported as a function of FFM show an elevated rate in FATox capacity among those possessing greater FFM[27,28] or no differences at all.[7,14,21,45] However, this is not always the case as Waldman et al[35] demonstrated that even among OW females possessing greater amounts of FFM (~4.5 kg) than the LN participants, the females characterized by higher aerobic fitness levels had significantly higher absolute and relative FATox rates during submaximal exercise compared to the OW group. Collectively these findings suggest that while the amount of FFM serves an important role in FATox, the type of skeletal muscle (type 1 vs 2) and metabolic machinery within (e.g., mitochondrial count and density, lipolytic enzyme count, etc.) determines the rate and duration of FATox and serves as an important consideration in future research for investigators interested in the confounding effects of variables which influence FATox.

It is important to note that our study has some limitations, with the first limitation being the assumption of low circulating hormone concentrations, such as estradiol, provided our participants were all tested in the early follicular phase. This is also an important consideration with regard to our population tested. Our study included college-age (~20 y), eumenorrheic females and so our findings may not necessarily extrapolate to females of peri- or post-menopausal age. Our second limitation was using submaximal workloads based off of VO_{2peak} to examine FATox rates. Our stage intensity prediction equations generated workloads that differed from our intended VO_2 range by approximately ±5%. However, we would point out that both groups were working at similar workloads of their aerobic capacity at each stage, with the greatest difference occurring at the final stage (55%), where a 2% discrepancy occurred between LN and OW participants. While this is the most common approach in past studies, it could be argued that the mechanisms which limit the rate of O_2 consumption differ from those which limit FATox. Thus, using other proxy measures such as lactate thresholds where changes in FATox can still be observed between participants without a disruption of other byproducts (e.g., reactive oxygen species) may serve as a better parameter than VO_{2peak}. Lastly, while FFM similarity between groups strengthened internal validity, future studies should intentionally control or stratify for FFM to confirm our findings.

Our study demonstrated no differences in FATox rates between OW and LN females when controlling first for aerobic fitness status. Females with excess adiposity are often considered as having an impairment in their ability to oxidize fat, with an additional underlying assumption that exercise prescription should be altered to reflect this metabolic state. Indeed, if female participants were unable to oxidize endogenous lipid sources, prescribing an exercise intensity that targeted mitochondrial function (e.g., high-intensity interval training) may be optimal by ensuring an adequate rate of weight loss. However, our findings suggest that from an exercise prescription standpoint, metabolically healthy, young LN and OW females are able to oxidize fat at similar rates and therefore, choosing exercises that promote adherence and enjoyment while subsequently improving aerobic capacity should be prioritized for optimizing metabolic health in female participants.

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