

# Lipolytic markers, insulin and resting fat oxidation are associated with maximal fat oxidation

Robinson, Scott; Chambers, Edward; Fletcher, Gareth; Wallis, Gareth

DOI:  
[10.1055/s-0042-100291](https://doi.org/10.1055/s-0042-100291)

License:  
None: All rights reserved

Document Version  
Peer reviewed version

Citation for published version (Harvard):  
Robinson, S, Chambers, E, Fletcher, G & Wallis, G 2016, 'Lipolytic markers, insulin and resting fat oxidation are associated with maximal fat oxidation', *International Journal of Sports Medicine*, vol. 37, no. 08, pp. 607-613.  
<https://doi.org/10.1055/s-0042-100291>

[Link to publication on Research at Birmingham portal](#)

**Publisher Rights Statement:**  
Eligibility for repository: Checked on 9/3/2016

## General rights

Unless a licence is specified above, all rights (including copyright and moral rights) in this document are retained by the authors and/or the copyright holders. The express permission of the copyright holder must be obtained for any use of this material other than for purposes permitted by law.

- Users may freely distribute the URL that is used to identify this publication.
- Users may download and/or print one copy of the publication from the University of Birmingham research portal for the purpose of private study or non-commercial research.
- User may use extracts from the document in line with the concept of 'fair dealing' under the Copyright, Designs and Patents Act 1988 (?)
- Users may not further distribute the material nor use it for the purposes of commercial gain.

Where a licence is displayed above, please note the terms and conditions of the licence govern your use of this document.

When citing, please reference the published version.

## Take down policy

While the University of Birmingham exercises care and attention in making items available there are rare occasions when an item has been uploaded in error or has been deemed to be commercially or otherwise sensitive.

If you believe that this is the case for this document, please contact [UBIRA@lists.bham.ac.uk](mailto:UBIRA@lists.bham.ac.uk) providing details and we will remove access to the work immediately and investigate.

# **Lipolytic Markers, Insulin and Resting Fat Oxidation are Associated with Maximal Fat Oxidation**

## **ABSTRACT**

The maximal capacity to oxidise fat during exercise (MFO) is associated with 24-h fat balance and insulin sensitivity. Understanding factors that influence MFO could have implications for metabolic health. We investigated relationships between selected plasma metabolites, hormones and overnight-fasted resting fat oxidation rates (Resting), with MFO. Resting fat oxidation and MFO was measured in 57 men with blood collected at rest and during exercise. Plasma glycerol ( $R=0.39$ ,  $P=0.033$ ), non-esterified fatty acids (NEFA:  $R=0.27$ ,  $P=0.030$ ) and insulin ( $R=-0.36$ ,  $P=0.007$ ) measured at MFO correlated with MFO; only glycerol remained correlated when controlled for resting concentrations ( $R=0.36$ ,  $P=0.008$ ). The change in glycerol from rest to MFO correlated with exercise-induced fat oxidation ( $R=0.32$ ,  $P=0.012$ ).  $\dot{V}O_{2\max}$  correlated with resting fat oxidation ( $R=0.44$ ,  $P=0.001$ ) and MFO ( $R=0.52$ ,  $P<0.001$ ). Resting fat oxidation correlated with MFO ( $R=0.55$ ,  $P<0.001$ ); this remained when controlled for  $\dot{V}O_{2\max}$  ( $R=0.41$ ,  $P=0.001$ ). This study reports weak-to-moderate, albeit significant, relationships between plasma lipolytic markers, insulin and resting overnight-fasted fat oxidation with MFO and shows the plasma glycerol response to uniquely reflect exercise-induced fat oxidation.  $\dot{V}O_{2\max}$  correlates with fat oxidation but the relationship can be dissociated. Interventions to increase fat oxidation for optimal metabolic health would benefit from, but are not reliant on, increases in  $\dot{V}O_{2\max}$ .

## INTRODUCTION

Impairments in fat oxidation are associated with the development of cardio-metabolic risk factors. For example, an elevated daily (i.e. 24-h) respiratory quotient (RQ; indicative of a low relative fat oxidation) has been shown to be predictive of body mass gain [36] and regain of body fat mass after diet-induced weight loss [10]. Furthermore, a reduced fat oxidation under resting conditions at the level of skeletal muscle is associated with impaired insulin sensitivity and metabolic flexibility [25]. We have recently demonstrated that the maximal attainable rate of fat oxidation during exercise (maximal fat oxidation, MFO) is associated with 24-h fat oxidation, 24-h fat balance and insulin sensitivity as markers of long-term metabolic health [31]. A greater understanding of the factors that influence MFO could therefore provide insights into how to increase fat oxidation and optimise metabolic health.

The circulating metabolic and hormonal milieu has been shown to influence fat oxidation during fixed intensity exercise. For example, an increase in plasma glucose or insulin typically suppresses fat oxidation during exercise, whereas elevations in non-esterified fatty acid (NEFA) concentrations increase it [8, 15, 35]. However, the independent and relative influence of these plasma markers on MFO as reflective of the maximal capacity for fat oxidation has not been comprehensively studied. A significant and positive relationship between resting plasma NEFA concentration and MFO has been reported [32], although as plasma NEFA concentration was not measured during exercise the significance of this observation in the context of physical activity fat oxidation is unclear. Interestingly, recent work [26] demonstrates markedly higher circulating levels of plasma NEFA, glycerol and insulin across a range of exercise intensities in obese as compared to lean individuals yet reported no group differences in MFO. This indicates that the plasma lipolytic and insulin response to exercise may not be important in modulating MFO. However, the confounding presence of obesity could alter expected relationships between these plasma markers and

MFO. A better understanding of how plasma markers relate to MFO could inform strategies (e.g., dietary) to produce the most favorable metabolic and hormonal milieu to maximise fat oxidation whilst physically active, with potential benefits for metabolic health.

Previously, in a large cross-sectional study, indicators of physical fitness ( $\dot{V}O_{2\max}$ , physical activity level), body composition (fat free mass, fat mass) and sex-related metabolic differences have been identified as significant and independent determinants of MFO [34]. Whilst informative, that study did not consider the influence of resting overnight-fasted (Resting) substrate oxidation on MFO. Studies of metabolic flexibility have shown that dietary nutrient intake and energy balance can affect resting substrate oxidation and subsequent responses to metabolic challenges [16]. Thus, it is plausible that resting fat oxidation could influence the substrate oxidation response to a subsequent bout of exercise. Indeed, overnight-fasted RER measured at rest has been shown to predict RER during exercise in trained athletes and MFO in overweight men [17, 32]. However, whilst RER informs of the balance of carbohydrate and fat utilised, it does not provide information on rates of substrate oxidation. The relationship between resting rates of fat oxidation and MFO is not known. Understanding this relationship will clarify the necessity to account for resting fat oxidation rates in future studies using MFO as a marker of fat oxidation capacity during exercise.

In summary, the evidence to date linking circulating levels of plasma hormones and metabolites as potential correlates of MFO is limited and inconsistent and requires further clarification. Further, links between resting fat oxidation rate and MFO have not been established. Accordingly, the purpose of the present study was to investigate the relationships between selected plasma metabolites, hormones and resting fat oxidation with MFO.

## **METHODS**

### **Participants**

57 young, healthy, recreationally active Caucasian men were recruited for the study (age  $24 \pm 7$  y; height  $1.80 \pm 0.07$  m; weight  $78.7 \pm 11.3$  kg;  $\dot{V}O_{2\max}$   $52 \pm 6$  ml/kg/min; Body Mass Index [BMI]  $24.2 \pm 2.6$  kg/m<sup>2</sup>). Each participant provided written informed consent and a local Research Ethics Committee in the United Kingdom approved the study, which conformed to the ethical standards of the journal [21]. The data from 53 of the participants used in this study has been presented elsewhere [31]. The previous study focused on determinants of 24-h fat oxidation [31] whereas this study allows us to present new insights by focusing on what factors relate to fat oxidation specifically during an acute bout exercise as reflected by MFO.

### **General Design**

This cross-sectional study involved two laboratory visits each separated by ~4-7 days (Fig. 1). The first (Familiarization) and second (Main Experimental Trial) visit had participants perform a laboratory-based graded treadmill exercise test to volitional exhaustion to allow for the determination of MFO and  $\dot{V}O_{2\max}$  by indirect calorimetry. The second visit also involved an assessment of body composition (via dual-energy x-ray absorptiometry [DXA] or skinfolds), resting fat oxidation and blood sample collection at rest and during exercise. Self-reported diet and physical activity were documented using diaries in the 4-d immediately preceding the second visit.

INSERT FIGURE 1 HERE

### **Experimental Procedures**

#### **Familiarization**

The purpose of this visit was to obtain consent, screen, familiarize participants with the exercise test and provide instructions for diet and physical activity recording. Height (Stadiometer, Seca, UK) and weight (Ohaus, Champ II scales, USA) were measured to establish BMI (weight [kg]/height<sup>2</sup> [m]). Participants then performed an exercise test on a motorized treadmill (PPS 70sport-I, Woodway, Weil am Rhein, Germany), a detailed description of which is provided below. Upon completion of the exercise test participants were provided with two sets of digital weighing scales (Electronic Kitchen Scale, SF 400, Zhejiang Province, China and Swees Digital Pocket Weighing Scales, Kent, UK), blank diaries and thorough instructions to enable their completion. Participants were asked not to deviate from their normal diet and activity patterns in the period between the first and second visit, with the exception of the 24 hours prior to the second visit (see below).

For the 4 consecutive days preceding the secondary laboratory visit, clear instructions were provided to participants to weigh and record all consumed food and drinks items and document any physical activity precisely and in detail. The activity diary used required the participant to document their level of activity every 15 minutes, using a code from a 12-point scale provided, for 24 hours of each of the four days [5]. The scale ranged from sleeping to vigorous activity and offered examples of activities at each time point to help participants in determining their level of activity. To ensure the highest possible level of accuracy, participants were encouraged to document any sport or exercise performed during the four days.

### Main Experimental Trial

Participants arrived at the laboratory at 08:00 h following an overnight fast from 22:00 h the evening before and having abstained from alcohol consumption and strenuous physical activity in the preceding 24-hour period. Participants were asked not to perform any physical

activity on the morning of testing, such as brisk walking or cycling to the laboratory, and to consume 500 ml water upon waking to promote hydration. The researcher checked diet and physical activity diaries meticulously and any potential cases of misreporting were discussed and clarified, following which body weight was recorded and body composition assessed. For the first 29 participants, body fat percentage, lean-tissue mass and fat mass were calculated using DXA (QDR software, Hologic Inc., Bedford, MA). During the second half of the study the DXA became unavailable. Therefore, for the remaining 28 participants, body composition was determined using 4-site skinfolds and the updated sex and race/ethnicity specific equations [9]. All measurements were taken by an International Society for the Advancement of Kinanthropometry (ISAK) Level 1 Accredited Anthropometrist and followed International Standards for Anthropometric Assessment.

An indwelling cannula (20g IV catheter, BD Venflon, Helsingborg, Sweden) was inserted into an antecubital arm vein and connected to a 150 cm polyethylene extension line (V-Green I.V. Extension Line, Vygon, Swindon, UK). This was attached to a 3-way stopcock (BD Connecta, Vygon, Swindon, UK) to allow for repeated blood collection during treadmill running exercise without the need for changes in running speed or form. The cannula was kept patent for the duration of insertion by regular flushing of 0.9 % sodium chloride (B Braun, Melsungen, Germany). Participants then rested for 30 minutes in the supine position in a dimly lit, thermo-neutral, quiet room with indirect calorimetry used to assess resting energy expenditure and fat oxidation in the rested state. Participants were instructed to refrain from any movement during this period. Upon completion a resting blood sample (10 ml) was obtained and ~15 minutes later the exercise test was performed.

The exercise test was a modified version of that described previously [2] with the starting speed and treadmill inclination set to 3.5 km/h and 1%, respectively. The treadmill speed was

increased by 1 km/h until an RER of 1.00 was reached for a minimum of 30 seconds, following which the treadmill speed remained constant and the gradient increased by 1% every minute until volitional exhaustion. Heart rate was measured continuously during exercise using a heart rate monitor (Polar FT-2, Finland) and recorded during the final 30 seconds of each exercise stage. A venous blood sample (5 ml) was obtained prior to the start of exercise (with the participant standing still on the treadmill) and during the final 30 seconds of each exercise stage until RER reached 1.00. A final blood sample (10 ml) was drawn at  $\dot{V}O_{2\max}$ .

Breath-by-breath measurements of oxygen consumption ( $\dot{V}O_2$ ) and carbon dioxide production ( $\dot{V}CO_2$ ) were collected during rest and exercise using an online automated gas analysis system (Oxycon Pro, Jaeger, Wuerzburg, Germany). The gas analyzers were calibrated with a 5.07 %  $CO_2$ , 14.79 %  $O_2$ , 80.14 %  $N_2$  gas mixture (BOC Gases, Surrey, UK), and the volume transducer was calibrated with a 3-liter calibration syringe (Jaeger, Wuerzburg, Germany). Environmental conditions during testing were: humidity  $50 \pm 5$  %; temperature  $22 \pm 2$  °C.

### **Self-Report Diet and Activity Analysis**

Food diaries were analyzed using dietary analysis software (Dietplan Version 6.7, Forestfield Software, Horsham, UK). Daily energy intake (EI; kcal/d), macronutrient intake (g/kg body mass) and percentage contribution of macronutrients to daily energy intake were established for each participant. Daily energy expenditure was estimated using the factorial approach [27]. Here, each of the 12 codes, which had a corresponding metabolic equivalent (MET) value, was assigned a Physical Activity Level (PAL) [33] and a daily PAL was determined by multiplying each of the 12 codes by the total amount of time spent at the activity level. Where participants had made notes on a specific sport and exercise activity undertaken during the 4 days, the Compendium of Physical Activities [4] was used to calculate the specific PAL value. Daily energy expenditure was then estimated by multiplying the daily PAL value by the age



and sex specific resting metabolic rate (RMR; kcal/d) using height, weight and the World Health Organization (WHO) equation [12]:

Energy expenditure (kcal/d; men < 30 years):  $\text{Daily PAL value} * \text{RMR} (15.4 * \text{body mass [kg]} - (27.0 * \text{height [m]}) + 717)$

Energy expenditure (kcal/d; men > 30 years):  $\text{Daily PAL value} * \text{RMR} (11.3 * \text{body mass [kg]} - (16.0 * \text{height [m]}) + 901)$

### **Plasma Analyses**

Whole blood was collected into EDTA-containing Vacutainers (BD, New Jersey, USA). Samples were immediately centrifuged at 1361g for 15 minutes at 4°C. Aliquots containing plasma were stored at -80°C until analyzed. Plasma glucose (mmol/L), glycerol (μmol/L), NEFA (mmol/L) and insulin (μIU/mL) concentrations were analysed at rest, at the exercise stage that corresponded to MFO, and at  $\dot{V}O_{2\text{max}}$ . Plasma lactate concentration (mmol/L) was analyzed pre-exercise, at each stage during the exercise test and at  $\dot{V}O_{2\text{max}}$ . Sample analysis was performed using enzymatic colorimetric assays for plasma glucose (Glucose Oxidase, Instrumentation Laboratories, Cheshire, UK), glycerol (GLY, Randox, London, UK), NEFA (NEFA, Randox, London, UK), lactate (L-Lac, Randox, London, UK) using an ILAB 650 Clinical Chemistry Analyser (Instrumentation Laboratory, Warrington, UK). Plasma insulin and leptin (rest only) were analysed by radioimmunoassay using commercially available kits (HI-14K Human Insulin and HL-81K Human Leptin; both Millipore, Billerica, MA).

### **Calculations**

Resting energy expenditure and fat oxidation rates were calculated during a stable measurement period (i.e., a deviation in  $\dot{V}O_2$  of <10% of the average  $\dot{V}O_2$  between minutes 20-

30; mean $\pm$ SD recording period was 5 $\pm$ 2 minutes) using appropriate equations (14) and with a protein correction factor of 0.11 mg/kg/min, as used previously [11, 20]. During the exercise test  $\dot{V}O_2$  and  $\dot{V}CO_2$  were averaged over the last minute of each sub-maximal exercise stage and fat and carbohydrate oxidation were calculated [14], with the assumption that the urinary nitrogen excretion rate was negligible.  $\dot{V}O_2$  was considered as maximal when two of the following three criteria were met; an RER >1.1, heart rate within 10 beats of predicted maximum (calculated as 220-age) [13], or an increase of < 2ml/kg/min in  $\dot{V}O_2$  with a further increase in workload.  $\dot{V}O_{2max}$  was calculated as the highest rolling 60 second average  $\dot{V}O_2$ .  $\dot{V}O_2$  peak (highest rolling 30 seconds average) was calculated for participants who failed to demonstrate at least two of the above criteria.

For each participant, two previously characterized [1] features were established: 1) the Maximal rate of Fat Oxidation during exercise (MFO), obtained by visual inspection as originally described [1]; 2) Fat<sub>max</sub>, defined as the exercise intensity that elicits MFO. Lactate threshold (LT) was determined manually for each participant by plotting plasma lactate concentration against exercise intensity and was recorded as the intensity at which the first increase in lactate concentration above baseline was observed (LIAB; lactate increase above baseline [baseline is resting in the present study]), as used previously [19].

## **Statistical Analyses**

Data were analyzed using SPSS (SPSS Version 21, Chicago, IL). All data were normally distributed according to the Shapiro-Wilk test of normality and are presented as Mean $\pm$ SD (and range where appropriate). A One-Way Repeated Measures ANOVA was used to investigate the temporal differences in plasma hormone and metabolite concentrations with post hoc paired samples t-tests (using the Bonferroni correction) applied where necessary for multiple comparisons. Bivariate correlations were used to investigate relationships between

MFO and: 1) metabolite/hormone concentrations and their response to exercise; and 2) other potential correlates of MFO (e.g., resting fat oxidation,  $\dot{V}O_{2\max}$ , FFM). Partial correlations were used where applicable to adjust for potential relevant confounders. A paired samples t-test was employed to compare the mean exercise intensities at MFO and LIAB. Statistical significance was accepted at  $P < 0.05$ .

## RESULTS

The data described below represents 57 participants, with the exception of resting fat oxidation whereby due to scheduling issues we report data for 51 participants and blood data where due to difficulty withdrawing blood from some participants at certain time-points we report data for between 45-57 participants.

### Diet and Activity

Self-reported energy intake, energy expenditure and energy balance were  $2854 \pm 604$  kcal/d,  $2808 \pm 384$  kcal/d and  $45 \pm 613$  kcal/d, respectively. The contribution of carbohydrate, fat, protein, fiber and alcohol to daily energy intake was  $42 \pm 9$  % ( $4.00 \pm 1.71$  g/kg),  $34 \pm 8$  % ( $1.30 \pm 0.52$  g/kg),  $18 \pm 6$  % ( $1.58 \pm 0.64$  g/kg),  $2 \pm 3$ %, and  $4 \pm 5$ %, respectively.

### Resting and Exercise Substrate Oxidation

Resting energy expenditure was  $1.37 \pm 0.28$  kcal/min (range 0.81-2.03 kcal/min). Resting RER, fat oxidation (g/min) and (% energy from FO [%EnFO]) was  $0.80 \pm 0.04$  (range 0.73-0.87),  $0.08 \pm 0.03$  g/min (0.02-0.15 g/min) and  $53 \pm 16$ % (range 14-80%), respectively. The MFO arising from the exercise test was  $0.60 \pm 0.18$  g/min (range 0.30-1.02 g/min) or  $9.18 \pm 2.65$  mg/kg FFM/min (range 4.13-15.98 mg/kg FFM/min) and this corresponded to an exercise intensity of  $58 \pm 17$  %  $\dot{V}O_{2\max}$  (range 21-83 %  $\dot{V}O_{2\max}$ ).

## Plasma Metabolite and Hormonal Responses

Plasma metabolite responses are presented in Table 1.

INSERT TABLE 1 HERE

In response to exercise, plasma glycerol ( $P=0.001$ ), NEFA ( $P=0.002$ ) and lactate ( $P=0.011$ ) concentration increased significantly from rest to MFO, whereas glucose did not change. The change from MFO to  $\dot{V}O_{2\max}$  resulted in a significant rise in plasma glucose ( $P<0.001$ ), glycerol ( $P<0.001$ ) and lactate ( $P<0.001$ ), however plasma concentration of NEFA was unaltered. Plasma glucose ( $P<0.001$ ), glycerol ( $P<0.001$ ) and lactate ( $P<0.001$ ) were all significantly higher at  $\dot{V}O_{2\max}$  than under resting conditions. Plasma lactate increase above baseline (LIAB) was positioned at  $61\pm12\%$   $\dot{V}O_{2\max}$  and did not differ significantly from  $\text{Fat}_{\max}$  ( $P=0.624$ ). Plasma insulin did not change from rest to MFO ( $10.9\pm3.5$   $\mu\text{IU/mL}$  and  $8.6\pm3.2$   $\mu\text{IU/mL}$ , respectively;  $P>0.05$ ) or from MFO to  $\dot{V}O_{2\max}$  ( $8.6\pm3.2$   $\mu\text{IU/mL}$  and  $8.3\pm6.9$   $\mu\text{IU/mL}$ , respectively;  $P>0.05$ ) but was significantly lower at  $\dot{V}O_{2\max}$  than at rest ( $10.9\pm3.5$   $\mu\text{IU/mL}$  and  $8.3\pm6.9$   $\mu\text{IU/mL}$ , respectively;  $P=0.026$ ). Resting plasma leptin concentration was  $3.5\pm3.6$   $\text{ng/mL}$ .

## Correlational analysis

For clarity, correlations of specific variables with fat oxidation rates are presented with oxidation rates expressed in absolute terms (i.e., g/min). Unless explicitly stated, expressing fat oxidation relative to lean body mass does not change the outcomes.

*Resting fat oxidation:*  $\dot{V}O_{2\max}$  (L/min,  $R=0.44$ ,  $P=0.001$ ), FFM (kg;  $R=0.39$ ,  $P=0.002$ ) and energy expenditure (kcal/d;  $R=0.34$ ,  $P=0.009$ ) were significantly and positively associated with resting fat oxidation. No measured plasma hormones or metabolites were significantly

associated with resting fat oxidation, with the exception of resting plasma NEFA concentration which was significantly and positively correlated with resting fat oxidation expressed relative to lean body mass ( $R=0.29$ ,  $P=0.019$ ).

*Maximal Fat Oxidation during exercise:* Resting RER ( $R=-0.47$ ,  $P<0.001$ ), resting fat oxidation ( $R=0.55$ ,  $P<0.001$ ; Figure 2),  $\dot{V}O_{2\max}$  (L/min;  $R=0.52$ ,  $P<0.001$ ), body mass (kg;  $R=0.24$ ,  $P=0.037$ ), FFM (kg;  $R=0.28$ ,  $P=0.018$ ) and BMI ( $\text{kg/m}^2$ ;  $R=0.25$ ,  $P=0.031$ ) were all significantly correlated with MFO. Resting fat oxidation (g/min) remained positively correlated with MFO when controlled for  $\dot{V}O_{2\max}$  as a potential confounder (L/min) ( $R=0.41$ ,  $P=0.001$ ). Fat mass, energy intake, energy expenditure, energy balance and macronutrient intakes were not significantly correlated with MFO ( $P>0.05$ ).

INSERT FIGURE 2 HERE

While resting plasma glycerol concentration was not significantly correlated with MFO, plasma glycerol concentration at MFO was significantly and positively associated with MFO (Fig. 3), and this correlation remained when controlled for potential confounding variables (i.e., resting plasma glycerol concentration,  $R=0.36$ ,  $P=0.008$ ;  $\dot{V}O_{2\max}$ ,  $R=0.51$ ,  $P<0.001$ ). Furthermore, the change in plasma glycerol concentration from rest to MFO correlated with the change in fat oxidation from rest to MFO ( $R=0.32$ ,  $P=0.012$ ).

INSERT FIGURE 3 HERE

The plasma concentration of NEFA in the resting state and at MFO was significantly and positively associated with MFO ( $R=0.36$ ,  $P=0.003$  and  $R=0.27$ ,  $P=0.030$ , respectively), however the significant relationship between plasma NEFA concentration at MFO and MFO was lost

when resting plasma NEFA concentration was considered as a potential confounding variable ( $R=0.14$ ,  $P=0.162$ ). Furthermore, the change in plasma NEFA concentration rest to MFO did not correlate with the change in resting fat oxidation to fat oxidation at MFO ( $R=0.10$ ,  $P=0.236$ ). Plasma glucose and lactate concentration measured in the resting state and at MFO were not significantly associated with MFO.

Plasma insulin concentration at rest and at MFO was significantly and negatively associated with MFO ( $R=-0.29$ ,  $P=0.016$  and  $R=-0.36$ ,  $P=0.007$ , respectively), and the relationship between plasma insulin concentration at MFO and MFO showed a trend for significance when resting plasma insulin concentration was included as a partial correlate ( $R=-0.23$ ,  $P=0.060$ ). The change in plasma insulin concentration from rest to MFO did not correlate with the change in resting fat oxidation to MFO ( $R=-0.115$ ,  $P=0.224$ ). Resting plasma leptin concentration was not associated with overnight-fasted fat oxidation or MFO but was significantly and positively associated with the following parameters of body composition: body weight (kg;  $R=0.24$ ,  $P=0.048$ ), BMI ( $R=0.33$ ,  $P=0.011$ ), waist circumference ( $R=0.40$ ,  $P=0.002$ ) and % body fat ( $R=0.59$ ,  $P<0.001$ ).

## DISCUSSION

The purpose of the present study was to investigate the relationship between selected plasma metabolites, hormones and resting fat oxidation with MFO during exercise. Accordingly, we report weak to moderate, albeit significant relationships between circulating concentrations of plasma NEFA, glycerol and insulin measured during exercise and MFO. We also show that resting fat oxidation is significantly and positively associated with MFO and provide new insights into the role of  $\dot{V}O_{2\max}$  as a determinant of fat oxidation across a range of physiological contexts.

The first main set of observations in the current study were that plasma NEFA or glycerol concentrations and plasma insulin concentrations measured during exercise at the point of MFO, were positively and negatively associated with MFO, respectively. To the best of our knowledge, such associations specific to MFO have not previously been reported. The findings are however consistent with what might be predicted based on the observations of others. For example, previous work [17] showed that plasma NEFA concentration measured during exercise was a significant determinant of the inter-individual variability in RER during low ( $\sim 41\% \dot{V}O_{2\text{peak}}$ ) and moderate ( $\sim 63\% \dot{V}O_{2\text{peak}}$ ) intensity exercise in trained cyclists. This is consistent with the important role of adipose tissue-derived NEFA for muscle fuel supply during exercise performed in the overnight-fasted state [for Review see 15]. Furthermore, as compared to untrained individuals, endurance-trained men exhibited elevated plasma glycerol concentrations and rates of fat oxidation during 30 minutes of exercise at 75-80%  $\dot{V}O_{2\text{max}}$  [7]. Finally, others have also shown that an elevation in plasma insulin concentration suppresses fat oxidation during low (25-45%  $\dot{V}O_{2\text{max}}$ ) and moderate (68%  $\dot{V}O_{2\text{max}}$ ) intensity exercise [22, 23]. Collectively, the results of the present study extend support for the integrated influence of lipolysis (as reflected by plasma glycerol concentration), the anti-lipolytic action of plasma insulin, and plasma NEFA availability as determinants of fat oxidation during exercise. This information could inform strategies (e.g., dietary) to manipulate the hormonal and metabolic milieu in an attempt to optimize fat oxidation whilst physically active, which ultimately could confer improvements for metabolic health [31, 32].

Interestingly, MFO has been shown to be similar in lean and obese individuals, yet obese individuals appeared to have markedly higher circulating levels of plasma NEFA, glycerol and insulin across a range of exercise intensities [26]. While it should be noted that the lean group were considerably fitter than the obese group ( $\dot{V}O_{2\text{peak}}$ :  $44.9 \pm 1.3$  versus  $53.8 \pm 1.7$  ml/kg

FFM/min), the findings do contrast our current observations and would indicate that these plasma responses are not important modulators of MFO. In that study, fat oxidation rates were higher in the obese group at lower exercise intensities (20-30%  $\dot{V}O_{2\text{peak}}$ ) and higher in the lean group at higher exercise intensities (65-85%  $\dot{V}O_{2\text{peak}}$ ). Furthermore, studies comparing fitness-matched lean and obese subjects have shown higher rates of fat oxidation in obesity despite the presence of hyperinsulinemia and similar or elevated plasma NEFA concentration during exercise [17, 20]. Therefore, the role of circulating levels of plasma NEFA, glycerol, and insulin in determining fat oxidation during exercise may be altered as a function of obesity.

We also explored associations between the resting concentrations of plasma glycerol, NEFA, insulin and leptin with MFO. In line with others [32] we observed a significant and positive relationship between resting plasma NEFA concentration and MFO. We also identified previously undocumented significant positive and inverse correlations between resting concentrations of plasma glycerol and insulin with MFO, respectively. Interestingly, the relationships between the concentrations of plasma NEFA and insulin during exercise with MFO were lost when controlled for their resting concentrations using partial correlations. Moreover, the exercise-induced changes in plasma NEFA and insulin were not correlated with the exercise-induced change in fat oxidation (i.e., resting to MFO). This suggests that while plasma NEFA and insulin concentrations at rest and during exercise are generally correlated with MFO, they may not be the prominent drivers of the exercise-induced increase in fat oxidation. While leptin has been shown to promote fat oxidation in muscle [28], we did not observe a significant relationship between resting plasma leptin concentration and whole-body fat oxidation. Nevertheless, our data is consistent with previous studies that show leptin concentration is positively associated with body mass and body fat [28].



By contrast, the significant and positive relationship between plasma glycerol concentration during exercise and MFO remained when controlled for resting plasma glycerol concentration and the exercise-induced change in plasma glycerol was significantly and positively correlated with the exercise-induced change in fat oxidation. Others have recently demonstrated that 33% of the variation in fat oxidation observed during 90 minutes exercise at 70%  $\dot{V}O_{2\max}$  could be explained by the subcutaneous abdominal adipose tissue dialysate glycerol concentration (as a marker of lipolysis) area under the curve during the exercise period [29]. Additionally, previous studies have reported that changes in lipolytic rate (i.e., rate of appearance of whole-body glycerol) coincide temporally with changes in fat oxidation during exercise [7, 22]. In this light, we interpret our observations to indicate that plasma glycerol concentrations uniquely reflect the metabolic processes underlying the regulation of fat oxidation during exercise. Nonetheless, we acknowledge that further work is necessary to fully delineate the relationship between lipolytic rate and plasma NEFA kinetics as determinants of the inter-individual variability in MFO.

Consistent with the findings of others [32] we report a large inter-subject variability in MFO, which could be partly explained by the significant and positive influences of  $\dot{V}O_{2\max}$  [2, 34] and FFM [34]. However, these previous reports did not consider the role of resting fat oxidation as a potential correlate of MFO. Indeed, overnight-fasted RER determined in the resting state has been shown to predict both RER during low-moderate exercise intensities in trained athletes and MFO in overweight men [17, 32]. Accordingly, we confirm previously reported observations [32] by showing resting RER to be negatively correlated to MFO and extend this to demonstrate that the actual rate of resting fat oxidation is significantly and positively correlated with MFO. In line with previous suggestions, our results indicate that resting fat oxidation should be considered in the design and interpretation of studies investigating exercise-induced fat oxidation [17]. For instance, accounting for resting fat oxidation within

an experimental design will enable researchers to isolate exercise specific effects of interventions or between-group comparison versus more generalized effects on fat oxidation.

Our further analysis shows that  $\dot{V}O_{2\max}$  is positively correlated with both resting fat oxidation and MFO. We previously reported significant and positive correlations between  $\dot{V}O_{2\max}$  and 24-hour fat oxidation [31]. Collectively this implicates  $\dot{V}O_{2\max}$  as an important determinant of fat oxidation under a range of physiological conditions. Interestingly, the significant relationships between resting fat oxidation and MFO reported herein, and MFO and 24-hour fat oxidation reported previously [31], remain when controlled for the influence of  $\dot{V}O_{2\max}$ . This suggests that metabolic factors either directly or indirectly associated with  $\dot{V}O_{2\max}$  such as skeletal muscle fibre type, capillary density and/or muscle oxidative capacity are also important determinants of an individual's overall capacity to oxidize fat. Our finding that  $\dot{V}O_{2\max}$  and fat oxidation could be dissociated implies that improvements in cardio-respiratory fitness (i.e.,  $\dot{V}O_{2\max}$ ) per se would increase fat oxidation but is not essential for an increase in fat oxidation. This is consistent with recent work showing exercise training-induced improvements in insulin sensitivity to be unrelated to changes  $\dot{V}O_{2\max}$  [6].

In conclusion, we report new associations between plasma glycerol, NEFA and insulin concentration and overnight-fasted resting fat oxidation with MFO and identify the plasma glycerol response to exercise to uniquely reflect exercise-induced fat oxidation. These results should be considered when developing strategies to modulate the metabolic and hormonal milieu to maximize fat oxidation and when designing future studies of exercise induced fat oxidation. Finally,  $\dot{V}O_{2\max}$  was shown to correlate with fat oxidation across a range of physiological contexts but the relationship can be dissociated. Thus, interventions to increase fat oxidation for optimal metabolic health would benefit from, but are not reliant on, increases in  $\dot{V}O_{2\max}$ .

## CONFLICTS OF INTEREST

There are no conflicts of interest to report.

## REFERENCES

1. *Achten J, Gleeson M, Jeukendrup, AE.* Determination of the exercise intensity that elicits maximal fat oxidation. *Med Sci Sports Exerc* 2002; 34: 92-97
2. *Achten J, Jeukendrup AE* Maximal fat oxidation during exercise in trained men. *Int J Sports Med* 2003; 24: 603-608
3. *Achten J, Jeukendrup AE.* Relation between plasma lactate concentration and fat oxidation rates over a wide range of exercise intensities. *Int J Sports Med* 2004; 25: 32-37
4. *Ainsworth BE, Haskell WL, Whitt, MC, Irwin ML, Swartz AM, Strath SJ, O'Brien WL, Bassett DR, Schmitz KH, Emplaincourt PO, Jacobs DR, Leon AS.* Compendium of physical activities: A second update of codes and MET values. *Med Sci Sports Exerc* 2011; 43: 1575-1581
5. *Bouchard C, Tremblay A, Leblanc, C, Lortie G, Savard R, Theriault, G.* A method to assess energy expenditure in children and adults. *Am J of Clin Nutr* 1983; 37: 461-467

6. *Brennan AM, Lam M, Stotz P, Hudson R, Ross R.* Exercise-induced improvement in insulin sensitivity is not mediated by change in cardiorespiratory fitness. *Diabetes Care* 2014; 37: 95-97
7. *Coggan AR, Raguso CA, Gastaldelli A, Sidossis LS, Yeckel CW.* Fat metabolism during high-intensity exercise in endurance-trained and untrained men. *Metabolism* 2007; 49: 122-128
8. *Costill DL, Coyle E, Dalsky G, Evans W, Fink W, Hoopes D.* Effects of elevated plasma FFA and insulin on muscle glycogen usage during exercise. *J Appl Physiol* 1977; 43: 695-699
9. *Davidson LE, Wang J, Thornton JC, Kaleem Z, Silva-Palacios F, Pierson RN, Heymsfield SB, Gallagher D.* Predicting fat percent by skinfolds in racial groups: Durnin and Womersley revisited. *Med Sci Sports Exerc* 2011; 43: 542-549
10. *Ellis AC, Hyatt TC, Gower BA, Hunter GR.* Respiratory quotient predicts fat mass gain in premenopausal women. *Obesity* 2010; 18: 2255-2259
11. *Flatt JP, Ravussin E, Acheson KJ, Jequier E.* Effects of dietary fat on postprandial substrate oxidation and on carbohydrate and fat balances. *J Clin Invest* 1985; 76: 1019-1024
12. *Food and Agricultural Organization/World Health Organization/United Nations University.* Energy and Protein Requirements: Report of a Joint FAO/WHO/UNU Expert

Consultation World Health Organization Technical Report Series 724. Geneva, WHO, 1985

13. *Fox SMI, Naughton JP, Haskell WL.* Physical activity and the prevention of coronary heart disease. *Ann Clin Res* 1971; 3: 404-432
14. *Frayn KN.* Calculation of substrate oxidation rates in vivo from gaseous exchange. *J Appl Physiol* 1983; 55: 628-634
15. *Frayn KN.* Fat as a fuel: emerging understanding of the adipose tissue-skeletal muscle axis. *Acta Physiologica* 2010; 199: 509-518
16. *Galgani JE, Moro C, Ravussin E.* Metabolic flexibility and insulin resistance. *Am J Physiol Endocrinol Metab* 2008; 295: 1009-1017
17. *Goedecke JH, Gibson AS, Grobler L, Collins M, Noakes TD, Lambert EV.* Determinants of the variability in respiratory exchange ratio at rest and during exercise in trained athletes. *Am J Physiol Endocrinol Metab* 2000; 279: 1325-1334
18. *Goodpaster BH, Wolfe RR, Kelley DE.* Effects of obesity on substrate utilisation during exercise. *Obesity* 2002; 10: 575-584
19. *Hagberg JM, Coyle EF.* Physiological determinants of endurance performance as studied in competitive race-walkers. *Med Sci Sports Exerc* 1983; 15:287-289

20. *Hall LML, Moran CN, Milne GR, Wilson J, MacFarlane NG, Forouhi NG, Hariharan N, Salt IP, Sattar N, Gill JM.* Fat oxidation, fitness and skeletal muscle expression of oxidative/lipid metabolism genes in South Asians: implications for insulin resistance? PLOS ONE 2010; 5: e14197
21. *Harriss DJ, Atkinson G.* Ethical standards in sports and exercise science research: 2014 update. Int J Sports Med 2013; 34: 1025-1028
22. *Horowitz JF, Klein S.* Oxidation of non-plasma fatty acids during exercise is increased in women with abdominal obesity. J Appl Physiol 2000; 89: 2276–2282
23. *Horowitz JF, Mora-Rodriguez R, Byerley LO, Coyle EF.* Lipolytic suppression following carbohydrate ingestion limits fat oxidation during exercise. Am J Physiol Endocrinol Metab 1997; 273: 768–775
24. *Horowitz JF, Mora-Rodriguez R, Byerley LO, Coyle EF.* Substrate metabolism when subjects are fed carbohydrate during exercise. Am J Physiol 1999; 276: 828-835
25. *Kelley DE, Simoneau, JA.* Impaired free fatty acid utilization by skeletal muscle in non-insulin-dependent diabetes mellitus. J Clin Invest 1994; 94: 2349-2356
26. *Lanzi S, Codecasa F, Cornacchia M, Maestrini S, Salvadori A, Brunani A, Malatesta D.* Fat oxidation, hormonal and plasma metabolite kinetics during a submaximal incremental test in lean and obese adults. PLoS ONE 2014; 9: e88707

27. *Manore M, Meyer NL, Thompson J.* Sport Nutrition for Health and Performance. Champaign, IL: Human Kinetics, 2009
28. *Minokoshi Y, Toda C, Okamoto S.* Regulatory role of leptin in glucose and lipid metabolism in skeletal muscle. *Indian J Endocrinol Metab* 2012; 16: 562-568
29. *Moro C, Harant I, Badin PM, Patarca FX, Guillard JC, Bourlier V, Langin D, Glisezinski I.* Influence of lipolysis and fatty acid availability on fuel selection during exercise. *J Physiol Biochem* 2013; 70: 583-591
30. *Ostlund RE, Wang JW, Klein S, Gingerich R.* Relation between plasma leptin concentration and body fat, gender, diet, age, and metabolic covariates. *J Clin Endocrinol Metab* 2013; 81: 3909-3913
31. *Robinson SL, Hattersley J, Frost GS, Chambers ES, Wallis GA.* Maximal fat oxidation during exercise is positively associated with 24-hour fat oxidation and insulin sensitivity in young, healthy men. *J Appl Physiol* 2015; 118: 1415-1422
32. *Rosenkilde M, Nordby P, Nielsen LB, Stallknecht BM, Helge JW.* Fat oxidation at rest predicts peak fat oxidation during exercise and metabolic phenotype in overweight men. *Int J Obesity* 2010; 34: 871-877
33. *Subcommittee on the Tenth Edition of the Recommended Dietary Allowances, Food and Nutrition Board, Commission on Life Sciences, National Research Council.* Recommended Dietary Allowances: 10th Edition. Washington D.C.: The National Academies Press, 1989.

34. *Venables MC, Achten J, Jeukendrup AE*. Determinants of fat oxidation during exercise in healthy men and women: a cross-sectional study. *J Appl Physiol* 2005; 98: 160-167
35. *Vukovich MD, Costill DL, Hickey MS, Trappe SW, Cole KJ, Fink WJ*. Effect of fat emulsion infusion and fat feeding on muscle glycogen utilisation during cycle exercise. *J Appl Physiol* 1993; 75: 1513-1518
36. *Zurlo F, Lillioja S, Puente EA, Nyomba BL, Raz I, Saad MF, Swinburn BA, Knowler WC, Bogardus C, Ravussin E*. Low ratio of fat to carbohydrate metabolism as predictor of weight gain: study of 24-h RQ. *Am J Physiol Endocrinol Metab* 1990; 259: 650-65



**Table 1. Plasma metabolite responses from rest to MFO to  $\dot{V}O_{2\max}$**

	<b>Rest</b>	<b>MFO</b>	<b><math>\dot{V}O_{2\max}</math></b>
<b>Glucose (mmol/L)</b>	5.0±0.4	5.1±0.5	5.8±0.1** ^
<b>Glycerol (μmol/L)</b>	40.0±19.8	80.2±53.5*	151.8±85.2** ^
<b>NEFA (mmol/L)</b>	0.57±0.19	0.81±0.45*	0.66±0.37
<b>Lactate (mmol/L)</b>	0.8±0.2	2.1±1.4*	10.2±3.3** ^

Values are mean ± SD. \* denotes a significant change from rest to MFO, \*\* from MFO to  $\dot{V}O_{2\max}$ , and ^ from rest to  $\dot{V}O_{2\max}$

**Fig. 1 Schematic representation of the study design**

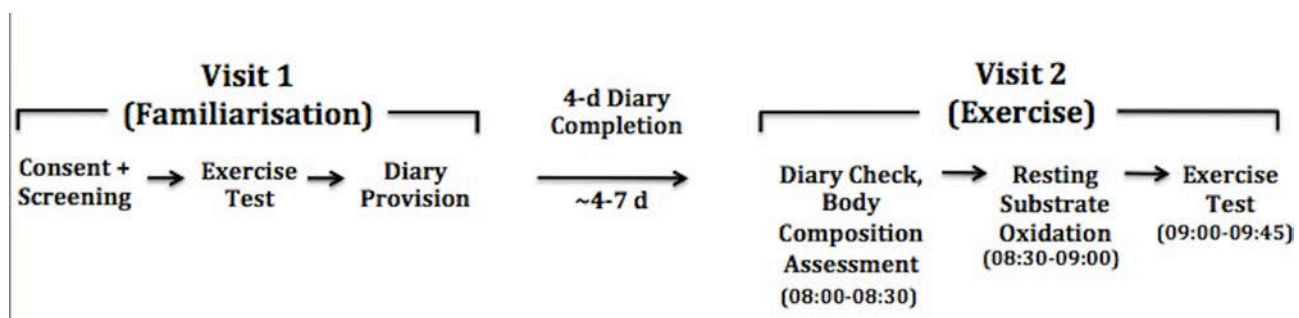


Fig. 2 Correlation between resting fat oxidation (gmin) and MFO during exercise

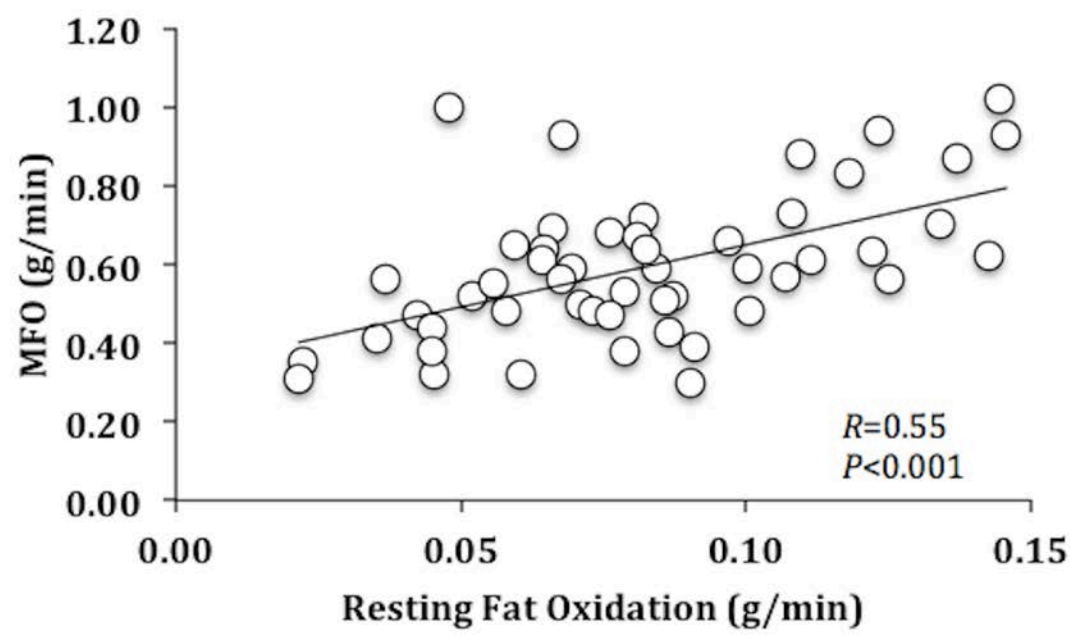


Fig. 3 Correlations between (A) Plasma glycerol concentration at rest and MFO; and (B) plasma glycerol concentration at MFO and MFO

