Maximal fat oxidation during exercise is positively associated with 24-hour fat oxidation and insulin sensitivity in young, healthy men

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[1]*School of Sport, Exercise and Rehabilitation Sciences, University of Birmingham, Birmingham, United Kingdom;* [2]*University Hospitals Coventry and Warwickshire, United Kingdom;* [3]*University of Warwick, Coventry, United Kingdom; and* [4]*Nutrition and Dietetic Research Group, Imperial College, London, United Kingdom*

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* 118: 1415–1422, 2015. First published March 26, 2015; doi:10.1152/japplphysiol.00058.2015.—Disturbances in fat oxidation have been associated with an increased risk of obesity and metabolic disorders such as insulin resistance. There is large intersubject variability in the capacity to oxidize fat when a person is physically active, although the significance of this for metabolic health is unclear. We investigated whether the maximal capacity to oxidize fat during exercise is related to 24-h fat oxidation and insulin sensitivity. Maximal fat oxidation (MFO; indirect calorimetry during incremental exercise) and insulin sensitivity (Quantitative Insulin Sensitivity Check Index) were measured in 53 young, healthy men (age 24 ± 7 yr, $\dot{V}O_{2max}$ 52 ± 6 ml·kg^{-1}·min^{-1}). Fat oxidation over 24 h (24-h FO; indirect calorimetry) was assessed in 16 young, healthy men (age 26 ± 8 yr, $\dot{V}O_{2max}$ 52 ± 6 ml·kg^{-1}·min^{-1}) during a 36-h stay in a whole-room respiration chamber. MFO (g/min) was positively correlated with 24-h FO (g/day) ($R = 0.65$, $P = 0.003$; $R = 0.46$, $P = 0.041$ when controlled for $\dot{V}O_{2max}$ [l/min]), 24-h percent energy from FO ($R = 0.58$, $P = 0.009$), and insulin sensitivity ($R = 0.33$, $P = 0.007$). MFO (g/min) was negatively correlated with 24-h fat balance (g/day) ($R = -0.51$, $P = 0.021$) but not significantly correlated with 24-h respiratory quotient ($R = -0.29$, $P = 0.142$). Although additional investigations are needed, our data showing positive associations between MFO and 24-h FO, and between MFO and insulin sensitivity in healthy young men suggests that a high capacity to oxidize fat while one is physically active could be advantageous for the maintenance of metabolic health.

exercise; fat oxidation; insulin sensitivity; obesity

EXERCISE INTENSITY IS THE main determinant of fuel utilization during exercise. With increasing exercise intensity there is an obligatory rise in the contribution of carbohydrate and a decline in the contribution of fat to energy expenditure (6, 44, 55). The absolute rate of fat oxidation (FO) during exercise typically shows an inverted hyperbola with FO increasing to a maximum at moderate exercise intensities [45-65% maximal oxygen consumption ($\dot{V}O_{2max}$)] and decreasing to eventually become negligible at higher exercise intensities (1, 42). Interestingly, there is substantial intersubject variability in the relative contribution of fat to energy expenditure during exercise (20), and this large degree of variability is also apparent in the rates of maximal fat oxidation (MFO) that have been reported. For example, MFO determined during incremental treadmill exercise in 300 healthy men and women was 0.46 ± 0.17 g/min (mean ± SD) and varied by approximately fivefold between individuals (range, 0.18 to 1.01 g/min) (56). Part of the intersubject variability can be explained by aerobic capacity/training status (25), nutritional status (6, 13), physical activity level (56), fat-free mass (FFM) (56), and sex-related differences (51). Although appreciable efforts have been made to understand the determinants of FO during exercise (56), the significance of the observed variability in the capacity to oxidize fat during exercise for metabolic health requires clarification (27, 29, 45).

Reduced FO has previously been related to long-term changes in body weight or composition. For example, increased daily (i.e., 24 h) respiratory quotient (RQ; indicative of low relative FO) has been associated with a greater risk of body mass gain (58) and regain of body fat mass after diet-induced weight loss (16). As a high level of FO while one is physically active could make important contributions to daily FO, it is not unreasonable to consider that a high capacity to oxidize fat while being physically active is advantageous for the maintenance of body mass or body composition. Mechanistically, a reduced FO (and thus elevated carbohydrate oxidation) during exercise or on a daily basis may affect long-term body weight or composition through greater ad libitum food intake in free-living conditions (4, 41). Impairments in FO at the level of skeletal muscle have also been associated with reduced metabolic flexibility and insulin resistance (31), and enhancements in FO with exercise training are related to improvements in insulin sensitivity (IS) (22, 57). However, the evidence to date that links FO capacity during exercise with metabolic health is limited and inconsistent. For example, a low MFO was associated with a higher clustering of metabolic syndrome risk factors in overweight men (45), yet no association between variability in respiratory exchange ratio (RER) during exercise and IS was observed in endurance-trained men (20). Overall, our understanding of the links between the capacity to oxidize fat during exercise and metabolic health remain to be comprehensively studied.

Because exercise is a primary means to acutely increase FO and reduced FO has been linked to disorders such as obesity and insulin resistance, additional understanding of the significance of the observed intersubject variability in the capacity to oxidize fat during exercise could provide insights into the role of physical activity in the optimization of metabolic health. Thus the purpose of the present study was to test the hypothesis that the capacity for FO during exercise is related to 24-h FO and IS as markers for long-term metabolic health.

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METHODS

General Design and Study Participants

A total of 57 young, healthy, recreationally active Caucasian men participated in the study (see subject characteristics in Table 1). All subjects provided informed written consent and a local Research Ethics Committee in the United Kingdom approved the study. Each participant visited the laboratory on two occasions. During the first (familiarization) and second (exercise test) visits, which were separated by approximately 4 to 7 days, participants performed a graded treadmill exercise test to voluntary exhaustion with indirect calorimetry used to assess MFO and $\dot{V}O_{2max}$. Self-reported diet and physical activity were recorded via diaries in the 4 days immediately preceding the second visit. A resting, overnight-fasted blood sample was also collected for determination of IS on the second visit in 53 participants. Sixteen participants selected to represent a broad MFO range as determined during the exercise test also took part in a further trial. This trial consisted of a 36-h stay in a whole-room respiration chamber to measure 24-h energy expenditure (24-h EE) and substrate utilization by indirect calorimetry (24-h assessment). Chamber conditions were designed to simulate participant's habitual energy intake, percent macronutrient intake, and daily EE as estimated from the food and physical activity diaries.

Experimental Procedures

Familiarization. This visit was used for screening, to obtain participant consent, and familiarize participants with the exercise test. Height (Stadiometer, Seca, UK) and weight (OHaus, Champ II Scales) were measured to calculate body mass index [weight (kg)/height (m)^2]. A treadmill-based exercise test, identical to that described in more detail below, was then completed on a motorized treadmill (PPS 70sport-I; Woodway, Weil am Rhein, Germany) to familiarize participants with the exercise testing procedures. Participants were then given two sets of digital weighing scales (SF 400 Electronic Kitchen Scale; Zhejiang Province, China and Swees Digital Pocket Weighing Scales, Kent, UK), blank diaries, and detailed instructions to enable the completion of diet and physical activity diaries. Participants were instructed to follow their normal diet and activity between the first and second laboratory visits with the exception of the day immediately preceding the second visit (see below).

For the 4 days preceding the second laboratory visit, participants were asked to accurately weigh and record all consumed food and drinks and to document activity patterns in as much detail as possible. The activity diary was based on that described by Bouchard et al. (9) and required participants to record their level of activity every 15 min using a code from a 12-point scale provided for the entire 24 h of each of the 4 days. Participants were encouraged to make notes on any sport or exercise performed during the 4 days.

Exercise test. Participants reported to the laboratory between 7:00 and 8:00 A.M. following an overnight fast from 10:00 P.M. the evening before and having abstained from strenuous physical activity and alcohol consumption in the preceding 24 h. 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. Diet logs and physical activity diaries were checked, and any potential cases of underreporting or inaccuracies were clarified, after which body weight was recorded as described above, a resting blood sample was obtained, and the exercise test was performed.

The exercise test was based on the protocol described by Achten and colleagues (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 every 3 min until the participant's RER (as is conventionally used in studies of exercise metabolism to indicate relative fuel utilization) reached 1.00, after which the treadmill gradient was increased by 1% per minute until volitional exhaustion. Heart rate was measured continuously throughout exercise using a heart rate monitor (Polar FT-2; Finland) and recorded during the final 30 s of each exercise stage. Breath-by-breath measurements of oxygen consumption ($\dot{V}O_2$) and carbon dioxide production ($\dot{V}CO_2$) were obtained during exercise using an online automated gas analysis system (Oxycon Pro; Jaeger, Wuerzberg, 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). Environmental conditions were documented while the exercise test was conducted and remained constant with humidity at 51 ± 6%; and temperature at 22 ± 1°C.

Respiration chamber stay (24-h assessment). Participants (n = 16 out of the 57 initially tested) were provided scanned copies of their food and activity diaries recorded in the 4 days preceding the second laboratory visit and asked to repeat these during the four consecutive days leading up to the 24-h assessment visit. Participants arrived at the laboratory between 6:00 and 7:00 P.M. on the evening before the experimental period having abstained from alcohol and strenuous physical exercise that day. Upon arrival, height and weight were reconfirmed and body composition was assessed using air displacement plethysmography (BODPOD; Cosmed, Rome, Italy). Thereafter, participants entered and stayed in a dual-respiration whole-room metabolic chamber system as described previously (47) for a period of 36 h. Both chambers were occupied simultaneously to prevent feelings of isolation. Three airtight hatches were used for blood collection, and the exchange of food and any urine produced. The room was ventilated with fresh air at a rate of 7 m^3/h during the daytime period, and 5 m^3/h in the evening. Environmental conditions during the 24-h chamber assessment were kept stable with humidity at 57 ± 5% and temperature at 24 ± 1°C and 22 ± 1°C for day and night, respectively. The concentration of O_2 and CO_2 was measured using two separate sets of ABB AO2000 analyzers: O_2 was measured using a paramagnetic O_2 analyzer (Magnos 206; ABB, Germany), and CO_2 was measured using infrared analyzers (Uras 26; ABB). Flow was measured with dry bellows meters (G16; Meterfabriek Schlumberger, Germany). Ingoing air was analyzed every 15 min and outgoing air was analyzed once every 1 min. During the experiment the gas measurement system was calibrated every 5 min using nitrogen (N5.5) and a calibration gas (CO_2 8,000 parts per million, 18% O_2, balance N_2 ± 1%). Online analysis of $\dot{V}O_2$ and $\dot{V}CO_2$ was calculated using a microcomputer (Macintosh, Apple).

The first 12 h in the chamber was used to habituate participants with the room and the 24-h assessment commenced the following morning at 7:00 A.M. and finished at 7:00 A.M. the following day. A standard day in the room is outlined in Fig. 1. Meals and snacks were provided throughout the measurement period, which participants were asked to consume within 10–15 min of provision. Total energy and percent macronutrient intake was matched with participants' average daily intake (based on the 4-day weighed food intake analyzed previously). Alcohol was not provided during the 24 h assessment. To account for this, the calories consumed from alcohol during the 4-day measurement period were distributed evenly between carbohydrate, fat, and protein intake during the 24-h period. Participants performed

Table 1. *Participant characteristics*

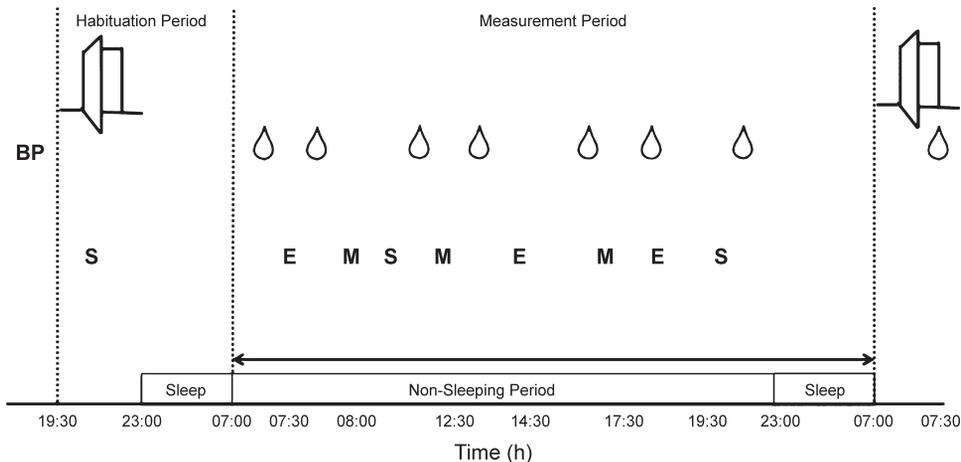|	24-h Assessment, *n* = 16	Insulin Sensitivity, *n* = 53
Age, yr	26 ± 8 (18–42)	24 ± 7 (18–44)
Height, m	1.81 ± 0.05 (1.72–1.89)	1.80 ± 0.07 (1.64–1.96)
Weight, kg	81.0 ± 11.2 (65.5–101.7)	78.7 ± 11.3 (54.7–113.0)
Body mass index, kg/m^2	24.5 ± 2.5 (21.3–29.7)	24.2 ± 2.6 (18.9–29.9)
Body fat, %	15 ± 5 (9–30)	
$\dot{V}O_{2max}$, ml·kg^{-1}·min^{-1}	52 ± 6 (39–60)	52 ± 6 (39–62)

Data are means ± SD (range).

Fig. 1. A schematic overview of proceedings during the 36-h stay in the respiration station. E, exercise; M, meal; S, snack; BP, body composition assessment. Drip symbols indicate blood draws; ↔ indicates urine collection.

a simple, nonexhaustive step activity at three separate time points during the 24-h assessment period. Each exercise period lasted between 10 and 20 min and was designed to ensure that 24-h EE reflected that calculated from the 4-day self-reported activity diary. Outside the designated exercise, eating, and sleeping periods, participants were not allowed to sleep or perform any additional physical activity other than light activities such as reading, watching television, or working on a computer. Blood was collected routinely (10 ml per sample) via an indwelling cannula inserted in an antecubital arm vein. Urine samples were collected for the entire 24-h measurement period. Participants were asked to empty their bladder prior to the measurement period (i.e., at 7:00 A.M.) to ensure that urine produced during the first night was not included in analyses. No alcohol, tea, coffee, or caffeinated beverages were allowed, but water and noncaffeinated herbal teas were available ad libitum.

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/day), absolute macronutrient intake (g/day), and percentage contribution of macronutrients to daily energy intake were established for each participant. Daily EE was estimated from the 4-day physical activity diary using the factorial approach (37). Each of the 12 codes, which had a corresponding metabolic equivalent value, was assigned a physical activity level (PAL; 50), 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 they had undertaken during the 4 days, the Compendium of Physical Activities (3) was used to calculate the specific PAL value. Daily EE was then estimated by multiplying the daily PAL value by the age- and sex-specific resting metabolic rate (kcal/day) using height, weight, and the following World Health Organization equations (17):

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

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

Plasma and Urine Analyses

Whole blood was collected into EDTA-containing vacutainers (BD Biosciences, Franklin Lakes, NJ). Samples were immediately centrifuged at 3,000 revolutions per minute for 15 min at 4°C. Aliquots containing plasma were stored at −80°C until analyzed. All collected samples were analyzed using enzymatic colorimetric assays for glucose (Glucose Oxidase kit; Instrumentation Laboratories, Cheshire, UK), nonesterified fatty acids (NEFA) (NEFA kit; Randox, London, UK), glycerol (GLY kit; Randox), and lactate (LAC kit; Randox) using an ILAB 650 Clinical Chemistry Analyzer (Instrumentation Laboratory, Warrington, UK). Insulin was analyzed by radioimmunoassay using a commercially available kit (HI-14K Human Insulin; Millipore, Billerica, MA). Collected urine volume was measured using digital weighing scales (model 323; Salter, Kent, UK) and analyzed enzymatically for urea (UREA kit; Roche, Mannheim, Germany) and creatinine (CREA kit; Roche) using an automated clinical chemistry analyzer (Cobas c702 Analyzer; Roche, Germany).

Calculations

Insulin sensitivity was calculated using the Quantitative Insulin Sensitivity Check Index (QUICKI), which has been validated against and shown to have a strong positive correlation ($R = 0.78$) with IS measured using the gold standard hyperinsulinemic euglycemic clamp technique (30). During the exercise test $\dot{V}O_2$ and $\dot{V}CO_2$ were averaged over the last minute of each submaximal exercise stage, and fat and carbohydrate oxidation were calculated according to the equations presented by Frayn (19), with the assumption that the urinary nitrogen excretion rate was negligible. $\dot{V}O_{2max}$ was calculated as the highest rolling 60-s average $\dot{V}O_2$ for participants displaying two of the three following criteria: an RER >1.1; heart rate within 10 beats of predicted maximum (calculated as $220 - \text{age}$) (18), or an increase of <2 ml·kg^{-1}·min^{-1} in $\dot{V}O_2$ with a further increase in workload. $\dot{V}O_{2\text{ peak}}$ (the highest rolling 30-s average) was calculated for participants who failed to demonstrate at least two of the above criteria. For each participant, two previously characterized features were established (1): *1*) MFO, obtained by visual inspection as originally described (1); and *2*) Fat$_{max}$, defined as the exercise intensity ($\%\dot{V}O_{2max}$) that elicits MFO.

For the 24-h assessment, $\dot{V}O_2$ and $\dot{V}CO_2$ were initially averaged in 30-min intervals and extrapolated to 24-h values and used to calculate the 24-h RQ ($RQ = \dot{V}CO_2/\dot{V}O_2$, as the expression of relative fuel utilization conventionally used in metabolic chamber studies), 24-h EE (54), 24-h carbohydrate, and 24-h FO (19) with urinary nitrogen correction where appropriate. Urinary nitrogen content was estimated by correcting urinary urea and creatinine by 1.11 to account for nonmeasured nitrogen sources (e.g., ammonia, urate) (8). Twenty-four-hour protein oxidation (g/day) was calculated by multiplying total N (g/day) by 6.25. Twenty-four-hour fat balance was calculated as dietary fat intake over 24 h (g/day) − 24-h FO (g/day). The food quotient (FQ) for the chamber diet was calculated as previously described (52). RQ and substrate utilization were also estimated for periods of nonsleep (encompassing 7:00 A.M. to 11:00 P.M.) and sleep [encompassing 1:45 A.M. to 4:45 A.M. (46)] periods. As with the 24-h determinations, $\dot{V}O_2$ and $\dot{V}CO_2$ were averaged in 30-min intervals and extrapolated to the corresponding period duration to

calculate RQ and substrate utilization (19). We corrected substrate oxidation rates using 24-h urinary nitrogen excretion values rather than using excretion values specific to the nonsleep and sleep periods, and we acknowledge this as a methodological limitation.

Statistical Analyses

Data were analyzed using SPSS software (version 21; Chicago, IL). All data were normally distributed according to the Shapiro-Wilk test of normality and are presented as means ± SD (and range where appropriate). Statistical significance was accepted at $P < 0.05$. Paired-sample *t*-tests were used to compare differences in habitual and chamber 24-h energy intake (kcal/day), 24-h EE (kcal/day), 24-h energy balance (kcal/day), and plasma hormone and substrate responses over time. Bivariate correlations were used to explore relationships between MFO and *1*) 24-h FO; *2*) other potential correlates of 24-h FO [e.g., $\dot{V}o_{2max}$, blood metabolite/hormone responses assessed as 24-h area under the curve (trapezoid method)]; and *3*) IS. Partial correlations were used where applicable to adjust for potential relevant confounders.

RESULTS

Results for the 24-h assessment are presented for 16 participants with the exception of the blood data, which due to difficulty in blood collection, is presented for 15 participants. Associations between MFO and IS are based on 53 participants.

Diet and Energy Expenditure

Table 2 shows that as planned, there were no significant differences between participants' energy and macronutrient intake, 24-h EE, or 24-h energy balance estimated from 4-day food and physical activity diaries and that measured during the 24-h assessment in the respiration chamber.

MFO and 24-h Substrate Oxidation

MFO for the 16 participants who undertook the 24-h assessment was 0.59 ± 0.21 g/min (range, 0.30–1.02 g/min). In these participants, Fat$_{max}$ was 56 ± 17% $\dot{V}o_{2max}$ and ranged from 30 to 78% $\dot{V}o_{2max}$. During the 24-h assessment, 24-h RQ was 0.86 ± 0.03 (range, 0.81–0.90). Twenty-four-hour fat, carbohydrate, and protein oxidation rates were 81 ± 30 g/day (range, 27–135 g/day), 346 ± 75 g/day (range, 153–470 g/day), and 120 ± 54 g/day (range, 25–204 g/day), respectively. The relative contributions of fat, carbohydrate, and protein oxidation to total EE throughout the 24-h assessment are shown in Fig. 2, and this corresponded to 28 ± 10, 53 ± 10, and 19 ± 9%, respectively. Twenty-four-hour fat balance was 31 ± 46 g/day. Sleep RQ (1:45 A.M. to 4:45 A.M.) was significantly lower than non-sleep RQ (7:00 A.M. to 11:00 P.M.) (0.82 ± 0.02 and 0.88 ±
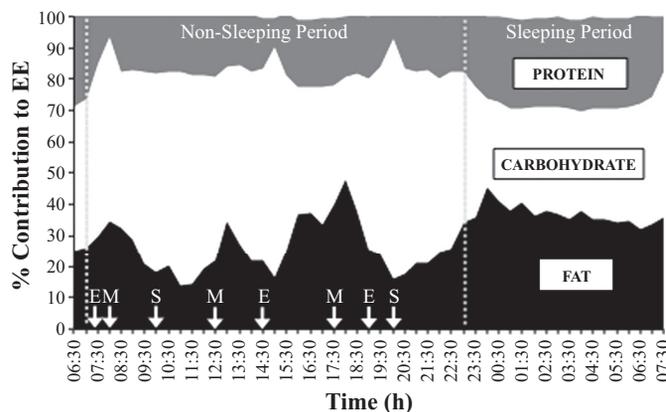

Fig. 2. Percentage contribution of carbohydrates, fat, and protein to energy expenditure; $n = 16$.

0.03, respectively, $P < 0.001$). As can be seen in Fig. 2, the relative contributions of each macronutrient to 24-h EE differed between sleep and nonsleep (fat 36 ± 10% and 25 ± 10%; carbohydrate 28 ± 6% and 59 ± 12%; protein 29 ± 11% and 16 ± 8%; all $P < 0.001$).

MFO (g/min) was significantly and positively correlated with 24-h FO (g/day), 24-h percent of energy from fat oxidation (%EnFO), and negatively correlated with 24-h fat balance (Fig. 3) but was not significantly associated with 24-h RQ ($R = -0.29$, $P = 0.142$). $\dot{V}o_{2max}$ (l/min) was positively correlated with MFO (g/min) and 24-h FO (g/day) ($R = 0.72$, $P = 0.001$ and $R = 0.51$, $P = 0.022$, respectively). MFO (g/min) remained positively correlated with 24-h FO (g/day) when controlled for $\dot{V}o_{2max}$ as a potential confounder (l/min) ($R = 0.46$, $P = 0.041$). The findings were similar when MFO expressed relative to FFM (mg·kg FFM^{-1}·min^{-1}) was correlated with 24-h FO (g·kg FFM^{-1}·day^{-1}, $R = 0.70$, $P = 0.001$), 24-h %EnFO ($R = 0.60$, $P = 0.007$), and 24-h RQ ($R = 0.31$, $P = 0.12$). $\dot{V}o_{2max}$ (l/min) was positively correlated with MFO (mg·kg FFM^{-1}·min^{-1}; $R = 0.58$, $P = 0.009$) but not 24-h FO (g·kg FFM^{-1}·day^{-1}, $R = 0.35$, $P = 0.093$); therefore, partial correlations between MFO and 24-h FO (with both expressed relative to FFM) controlled for $\dot{V}o_{2max}$ were deemed unnecessary.

When the data were separated into sleep (1:45 A.M. to 4:45 A.M.) and nonsleep (7:00 A.M. to 11:00 P.M.) periods, MFO (g/min) was significantly negatively correlated with sleep RQ ($R = -0.51$, $P = 0.022$) but was not significantly correlated with nonsleep RQ ($R = -0.24$, $P = 0.187$). MFO (g/min) was positively correlated with FO during both the sleep and non-sleep periods ($R = 0.71$, $P = 0.001$ and $R = 0.58$, $P = 0.01$, respectively; %EnFO $R = 0.59$, $P = 0.008$, and $R = 0.51$, $P = 0.021$, respectively). Similar findings prevailed when MFO expressed relative to FFM (g·kg FFM^{-1}·min^{-1}) was correlated with sleep and nonsleep FO (g/kg FFM $R = 0.73$, $P = 0.001$ and $R = 0.63$, $P = 0.004$, respectively).

Twenty-four-hour carbohydrate intake (g/day) and 24-h energy balance (kcal/day) were significantly negatively correlated with 24-h FO (g/day, $R = -0.59$, $P = 0.008$ and $R = -0.53$, $P = 0.017$, respectively), 24-h %EnFO ($R = -0.62$, $P = 0.003$ and $R = -0.56$, $P = 0.013$, respectively) and 24-h FO relative to FFM (g·kg FFM^{-1}·day^{-1}, $R = -0.62$, $P = 0.005$ and $R = -0.58$, $P = 0.009$, respectively). Twenty-four-hour

Table 2. *Comparison between 4-day self-report and chamber food intake, energy expenditure and energy balance*

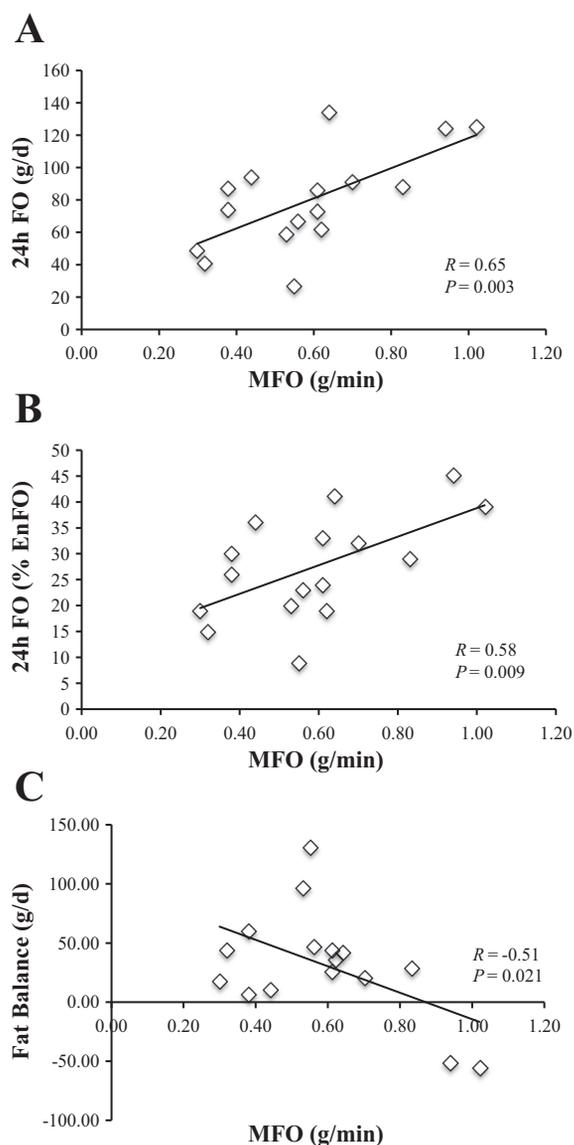|	Four-day Self-Report	24-h Assessment
Energy intake, kcal/day	2,779 ± 652	2,773 ± 657
Energy expenditure, kcal/day	2,705 ± 219	2,621 ± 203
Energy balance, kcal/day	74 ± 638	153 ± 601
Carbohydrate, %	42 ± 10	43 ± 11
Fat, %	34 ± 7	36 ± 6
Protein, %	20 ± 7	20 ± 7
Alcohol, %	4 ± 5	
Food quotient	0.84 ± 0.03	0.84 ± 0.03

Fig. 3. Correlations between maximal fat oxidation (MFO, g/min) and 24-h fat oxidation (FO, g/day) (*A*), 24-h FO (percent of energy from fat oxidation, %EnFO) (*B*), and fat balance (g/day) (*C*).

measured plasma metabolite and hormone responses did not correlate with any expression of 24-h FO.

Plasma Metabolites and Hormones

Plasma metabolite and hormone responses to the different activities performed in the respiratory chamber are shown in Table 3. Plasma glycerol concentration increased significantly from before to after exercise in the fasted state, whereas plasma glucose, insulin, and NEFA showed no change. In response to feeding, plasma insulin and glucose concentrations increased, whereas plasma glycerol and NEFA concentrations remained similar. Overnight, plasma insulin concentration decreased and plasma glycerol and NEFA concentrations both increased, whereas plasma glucose showed no change.

Insulin Sensitivity

MFO assessed in the larger cohort of participants was 0.60 ± 0.18 g/min (range, 0.30–1.02 g/min), and this corresponded to an exercise intensity of $58 \pm 17\%$ $\dot{V}_{O_{2max}}$ (range, 21–83% $\dot{V}_{O_{2max}}$). Resting plasma glucose and insulin concentration was 5.0 ± 0.4 mmol/l and 11 ± 4 μIU/ml, respectively. IS estimated using the QUICKI was 0.34 ± 0.02 (range, 0.31–0.40 arbitrary units). MFO (g/min) was significantly positively correlated with IS ($R = 0.33$, $P = 0.007$). $\dot{V}_{O_{2max}}$ (l/min) was significantly positively correlated with MFO (g/min) but not with IS ($R = 0.72$, $P = 0.001$ and $R = 0.11$, $P = 0.223$, respectively); therefore, partial correlations between MFO and IS controlled for $\dot{V}_{O_{2max}}$ were deemed unnecessary.

DISCUSSION

The purpose of the present study was to investigate the hypothesis that the capacity for FO during exercise is related to daily FO and IS as markers for long-term metabolic health. Accordingly, for the first time we report significant and positive relationships between MFO during exercise and 24-h FO, and between MFO and IS in healthy young men.

In seeking to explain the observed association between MFO and 24-h FO we explored the influence of a variety of factors on these two variables. We found that $\dot{V}_{O_{2max}}$ was a strong correlate of 24-h FO, and because $\dot{V}_{O_{2max}}$ was also correlated with MFO it suggests that aerobic capacity could be a driver of the association between MFO and 24-h FO. The findings from the present study support those of Venables and colleagues (56) who showed $\dot{V}_{O_{2max}}$ to be a significant predictor of MFO assessed in 300 healthy men and women and are consistent with the well-established effect of endurance exercise training to increase $\dot{V}_{O_{2max}}$ and the capacity to oxidize fat during exercise (32, 38). A parallel with our finding can be drawn

glycerol concentration area under the curve (AUC) (38.6 ± 10.6 mmol·l^{-1}·day^{-1}) was positively correlated with 24-h FO (g·kg FFM^{-1}·day^{-1}, $R = 0.44$, $P = 0.048$) and showed a tendency toward a positive correlation with 24-h FO when expressed otherwise (g/day, $R = 0.40$, $P = 0.071$ or %EnFO, $R = 0.40$, $P = 0.069$). Twenty-four-hour AUC for the other

Table 3. *Plasma hormone and metabolite responses to different activities performed within the respiratory chamber*

	Fasted Exercise, 7:25–07:55 A.M.		Lunch, 12:30–1:15 P.M.		Dinner, 5:00–6:30 P.M.		Overnight 9:55 P.M.–7:25 A.M.	
	Before	After	Before	After	Before	After	Before	After
Insulin, μIU/ml	15 ± 6	13 ± 6	15 ± 7	59 ± 47*	11 ± 4	77 ± 47†	23 ± 12	13 ± 5†
Glucose, mmol/l	5.2 ± 0.5	5.4 ± 0.7	5.1 ± 0.9	6.1 ± 1.9*	5.9 ± 1.8	9.0 ± 3.7†	5.3 ± 0.7	5.0 ± 1.5
Glycerol, μmol/l	30 ± 16	51 ± 24†	16 ± 4	18 ± 7	30 ± 11	29 ± 14	17 ± 10	35 ± 24†
NEFA, mmol/l	0.32 ± 0.19	0.38 ± 0.17	0.26 ± 0.17	0.34 ± 0.22†	0.47 ± 0.24	0.51 ± 0.24	0.21 ± 0.14	0.36 ± 0.14†

NEFA, nonesterified fatty acids. *Significance change in concentration Before to After sample at $P < 0.05$. †Significance change in concentration Before to After sample at $P < 0.01$.

from a prior report showing $\dot{V}_{O_{2max}}$ to explain the major (76%) proportion of variance in adaptation of 24-h fat balance to increased dietary fat intake (49), suggesting that aerobic capacity can exert an important influence over 24-h FO under these conditions. However, and in contrast to what might be expected, cross-sectional studies do not report higher 24-h FO in endurance-trained individuals compared with sedentary counterparts (36), suggesting a high aerobic capacity may not be a necessary prerequisite for high daily FO. In support of this, the present study showed that MFO was still significantly and positively related to 24-h FO when controlled for $\dot{V}_{O_{2max}}$, which suggests an independent relationship. Overall, although we reveal a correlation between MFO and 24-h FO, the present study highlights the need for longitudinal training studies to delineate the relative role of changes in maximal aerobic capacity and the capacity to oxidize fat during exercise on daily patterns of substrate oxidation.

It could be argued that variables other than $\dot{V}_{O_{2max}}$ may also be contributing to our observations relating MFO to 24-h FO. Previous research shows that carbohydrate intake and the state of energy balance (28) can influence fuel utilization. In the present study, carbohydrate intake and energy balance were significantly and negatively associated with 24-h FO, although they were not associated with MFO (data not shown). The lack of association of carbohydrate intake with MFO is consistent with the findings of Goedecke et al. (20) who found no association between dietary carbohydrate intake and intersubject variability in exercising RER over a range of predetermined workloads. Moreover, Rosenkilde (45) found no differences in habitual carbohydrate intake or energy balance between two groups of moderately overweight men who differed markedly in their MFO during exercise. Thus whereas it is well established from intervention studies that nutritional status can exert large effects on the balance of fuels oxidized during exercise (6, 13), we do not believe this to be underlying the associations between MFO and 24-h FO in the present study.

Regular blood draws were taken throughout the 24-h assessment in an attempt to explore relationships between plasma metabolic responses and 24-h FO. Although the majority of these were uninformative, including NEFA concentration AUC (correlation with 24-h FO: g/day, $R = 0.22$, $P = 0.220$; g·kg $FFM^{-1}·day^{-1}$, $R = 0.25$, $P = 0.185$), glycerol concentration AUC was significantly and positively associated with 24-h FO (correlation with 24-h FO: g·kg $FFM^{-1}·day^{-1}$, $R = 0.44$, $P = 0.048$). Our glycerol data reflect static concentration measurements, and we cannot infer whether these glycerol concentrations are driven by alterations in lipolytic rate or glycerol clearance. Furthermore, because the relationship between MFO and 24-h FO did not alter substantially ($R = 0.61$, $P = 0.011$) when glycerol AUC was included as a partial correlate, the observation should be treated with caution. Nonetheless, previous work suggests a link between lipolytic rate and FO. For example, Horowitz et al. (26) found that a reduced lipolytic rate was associated with a suppression of FO, whereas Coggan et al. (12) observed a higher lipolytic rate in trained compared with untrained subjects, and this was associated with higher FO during 30 min of exercise at 75% to 80% $\dot{V}_{O_{2max}}$. More recently, Isacco et al. (27) found that women with a preferential body fat distribution (a lower abdominal-to-lower-body-fat mass ratio) oxidized more fat during a 45-min cycling exercise compared with women who had a less preferential body fat

distribution (higher abdominal-to-lower-body-fat mass ratio), and this was associated with higher glycerol concentrations. The use of isotopic tracers of lipid metabolism would be needed to fully investigate the potential involvement of lipolytic rate in the observed association between MFO and 24-h FO.

We chose to assess relationships between MFO with 24-h FO because the latter may be more reflective of long-term susceptibility to obesity. For example, previous work links high daily RQ, which is indicative of a low relative FO, with an increased risk of body mass gain (58) and regain of body fat mass after diet-induced weight loss (16). Although we did not observe a significant association between MFO and 24-h RQ, the correlation was directionally consistent ($R = -0.29$) with what would be predicted. Nonetheless, our data did reveal a significant and negative correlation between MFO and sleeping RQ, which could suggest that the relationship becomes stronger under conditions where the suppressive effects of insulin on lipid metabolism may be less apparent. Indeed, previous studies have demonstrated that compared with nonexercise control conditions FO in response to acute aerobic exercise was higher during the sleep period but not the nonsleep period (7, 23). Collectively, this suggests the sleep period may be particularly sensitive to alterations in lipid metabolism in response to physiological perturbations. Although we did not observe a significant correlation between MFO and 24-h RQ, we reiterate that we report positive associations between MFO and 24-h absolute FO, and between MFO and 24-h FO as a proportion of daily energy expenditure. These relationships, which incorporate energy expenditure, are likely more relevant than RQ because they will ultimately contribute as determinants of absolute and relative substrate balances, respectively. The negative association between MFO and fat balance observed in the present study reinforces this concept. In additional analysis, we explored relationships between MFO and FO measured during the various activities performed in the respiratory chamber (data not shown). This analysis indicated that the relationship between MFO and 24-h FO appears to reflect an overall elevated oxidation of fat rather than being restricted to a particular time period or to certain activities. Thus MFO could reflect an overall capacity for daily whole-body FO and our interpretation of the present data is that MFO could be a marker for long-term regulation of body mass or composition, although we accept that further work is necessary to test this concept.

We also explored relationships between MFO and IS using a surrogate marker of IS (QUICKI). In contrast to the findings by Goedecke et al. (21) who observed no association with exercising RER, we found a small to moderate correlation between MFO and IS. The precise reason for this discrepancy is unclear, but it could be explained by methodological differences. For instance, in the present study, rates of FO were determined over a wide range of exercise intensities providing the resolution to identify the maximal capacity for FO during exercise, whereas the study by Goedecke and colleagues assessed RER during three predetermined exercise workloads. Also, exercise RER considers only relative fuel utilization, whereas MFO, which incorporates metabolic rate, could better reflect metabolic capacity and thus be more likely to correlate with IS. However, this suggestion is not consistent with the observations of Chu and colleagues (11) who recently showed

MFO to be positively correlated with insulin resistance (estimated from the homeostatic model assessment of insulin resistance) and unrelated to whole body IS index (determined from oral glucose tolerance testing). However, this was observed in a group of normal and overweight girls, which is quite different from the study population tested herein.

The data from the present study, which are suggestive of a link between MFO and IS in young, healthy men, is consistent with observational studies showing that impairment in fat metabolism at the level of skeletal muscle is associated with reduced metabolic flexibility and insulin resistance (31) and longitudinal studies showing enhancement in FO with exercise training is associated with improvements in IS (22, 57). Furthermore, greater exercise training-induced improvements in IS have been observed using exercise training strategies that enhance FO during acute exercise [e.g., targeting training intensity Fat_{max} or exercising in the overnight-fasted state (57)]. From a mechanistic standpoint, this association between MFO and IS seems plausible. It has been suggested that incomplete metabolism of fatty acids in the β-oxidation pathway induces an accumulation of acyl carnitines in the mitochondria, and this is associated with insulin resistance (34). Furthermore, the accumulation of lipid metabolites such as ceramide, diacylglycerol, and long-chain fatty acyl-CoA has also been linked with insulin resistance in skeletal muscle (48). We acknowledge that we have not studied IS using direct methods (e.g., hyperinsulinemic euglycemic clamp technique), but our observations could imply that a higher capacity for FO during physical activity could alleviate lipotoxic stress in skeletal muscle and confer improvements in IS.

Finally, we believe there are two further aspects of our study that warrant consideration in discussion. First, we purposely chose to feed subjects their habitual diet and to replicate activity levels in the metabolic chamber trial to reflect variation between individuals under conditions that mimicked their daily lives, and we were successful in achieving this. It would be important in future work to confirm these observations under conditions of standardized dietary and physical activity levels. Second, our findings linking MFO with 24-h FO advance a potential role for MFO as a determinant of long-term regulation of body mass or composition. However, it is notable that obese individuals do not always have a lower MFO compared with their lean counterparts (5, 36), even when matched for cardiorespiratory fitness (14). Nonetheless, Rosenkilde et al. (45) found that even within a relatively homogenous group of overweight men, those with a lower MFO exhibited a higher clustering of metabolic syndrome risk factors than those with a higher MFO. Therefore, we believe that the intersubject variability in MFO should be considered as a potential marker of risk for metabolic disorders within defined population groups (e.g., normal weight, overweight).

In conclusion, we observed a significant and positive association between MFO during exercise and 24-h FO, and MFO during exercise and IS in healthy young men. Future work should seek to establish whether this is a causal relationship and address in a variety of subject populations the long-term implications of the apparent intersubject variation in MFO during exercise and risk for disorders associated with disturbances in fat metabolism, such as obesity, insulin resistance, and type 2 diabetes.

AUTHOR CONTRIBUTIONS

S.L.R. and G.A.W. conception and design of research; S.L.R., J.H., G.S.F., E.S.C., and G.A.W. performed experiments; S.L.R., J.H., and G.A.W. analyzed data; S.L.R. and G.A.W. interpreted results of experiments; S.L.R. prepared figures; S.L.R. and G.A.W. drafted manuscript; S.L.R., J.H., G.S.F., E.S.C., and G.A.W. edited and revised manuscript; S.L.R., J.H., G.S.F., E.S.C., and G.A.W. approved final version of manuscript.

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