Day to day variability in fat oxidation, and the effect after only one day of change in diet composition.

<table>
<thead>
<tr>
<th>Journal:</th>
<th><em>Applied Physiology, Nutrition, and Metabolism</em></th>
</tr>
</thead>
<tbody>
<tr>
<td>Manuscript ID</td>
<td>apnm-2015-0334.R2</td>
</tr>
<tr>
<td>Manuscript Type:</td>
<td>Article</td>
</tr>
<tr>
<td>Date Submitted by the Author:</td>
<td>04-Nov-2015</td>
</tr>
<tr>
<td>Complete List of Authors:</td>
<td>Støa, Eva; Telemark University College, Sport, Physical education and Outdoor Life Sciences</td>
</tr>
<tr>
<td></td>
<td>Nyhus, Lill-Katrin; Telemark University College, Department of Sport and Outdoor Life Studies</td>
</tr>
<tr>
<td></td>
<td>Claveau Børresen, Sandra; Telemark University College, Department of Sport and Outdoor Life Studies</td>
</tr>
<tr>
<td></td>
<td>Nygaard, Caroline; Telemark University College, Department of Sport and Outdoor Life Studies</td>
</tr>
<tr>
<td></td>
<td>Hovet, Åse Marie; Telemark University College, Department of Sport and Outdoor Life Studies</td>
</tr>
<tr>
<td></td>
<td>Bratland-Sanda, Solfrid; Telemark University College, Department of Sport and Outdoor Life Studies</td>
</tr>
<tr>
<td></td>
<td>Helgerud, Jan; Telemark University College, Department of Sport and Outdoor Life Studies; Norwegian University of Science and Technology, Faculty of Medicine, Department of Circulation and Medical Imaging; Hokksund Medical Rehabilitation Center, Støren, Øyvind; Telemark University College, Department of Sport and Outdoor Life Studies</td>
</tr>
<tr>
<td>Keyword:</td>
<td>exercise metabolism &lt; exercise, Keywords: Substrate oxidation, reliability, exercise, diet manipulation, exercise metabolism, physiological testing</td>
</tr>
</tbody>
</table>

https://mc06.manuscriptcentral.com/apnm-pubs
Day to day variability in fat oxidation, and the effect after only one day of change in diet composition.

Eva Maria Støa¹, Lill-Katrin Nyhus¹, Sandra Claveau Børresen¹, Caroline Nygaard¹, Åse Marie Hovet¹, Solfrid Bratland-Sanda¹, Jan Helgerud¹²³, Øyvind Støren¹.

¹Telemark University College, Norway, Department of Sport and Outdoor Life Studies
²Norwegian University of Science and Technology, Faculty of Medicine, Department of Circulation and Medical Imaging, Trondheim, Norway
³Hokksund Medical Rehabilitation Center, Hokksund, Norway

Running title: Reliability of substrate utilization

Corresponding author:
Eva Maria Støa,
Telemark University College, Norway, Department of Sport and Outdoor Life Studies
Eva.m.stoa@hit.no
Phone: +4741632015

Co-author email addresses: LK Nyhus; lill-katrin.nyhus@hit.no, SC Børresen, sandra_5454@hotmail.com, C Nygaard; 120847@student.hit.no, ÅM Hovet; amhovet@gmail.com, S Bratland-Sanda; solfrid.bratland-sanda@hit.no, J Helgerud; jan.helgerud@ntnu.no, Ø Støren; oyvind.storen@hit.no
ABSTRACT

Background: Indirect calorimetry is a common and non-invasive method to estimate rate of fat oxidation (FatOx) during exercise, and test – retest reliability should be considered when interpreting results. Diet also has an impact on FatOx. The aim of the present study was to investigate day to day variations in FatOx during moderate exercise given the same diet, and given two different isoenergetic diets.

Methods: 9 healthy moderately trained females participated in the study. They performed one VO\(_{2}\max\) test and four FatOx tests. Habitual diets were recorded and repeated to assess day to day variability in FatOx. FatOx was also measured after one day of fat-rich (26.8% CHO, 23.2% protein, 47.1% fat) and one day of CHO-rich diet (62.6% CHO, 20.1% protein, 12.4% fat).

Results: The reliability test revealed no differences in FatOx, RER, VO\(_{2}\), carbon dioxide production (VCO\(_{2}\)), heart rate (HR), blood lactate concentration [La\(_{b}\)] or blood glucose (BG) between the two habitual diet days. FatOx decreased after the CHO-rich diet compared to the habitual day 2 (from \(0.42\pm0.15\) to \(0.29\pm0.13\) g\(\cdot\)min\(^{-1}\), \(p<0.05\)). No difference was found in FatOx between fat-rich diet and the two habitual diet days. FatOx was 31% lower (from \(0.42\pm0.14\) to \(0.29\pm0.13\) g\(\cdot\)min\(^{-1}\), \(p<0.01\)) after the CHO-rich diet compared to the fat-rich diet. Using RER data to measure FatOx is a reliable method as long as the diet is strictly controlled. However, even a 1 day change in macronutrient composition will likely affect the FatOx results.

Keywords: Substrate oxidation, reliability, exercise, diet manipulation, exercise metabolism, physiological testing
Introduction

Estimates of fat oxidation (FatOx) are used to examine possible metabolic disturbances among individuals who are sedentary, among individuals with metabolic conditions like obesity and diabetes type 2, and also to investigate training effects on FatOx among both sedentary individuals and athletes (Brandou et al. 2003; Stisen et al. 2006; Venables and Jeukendrup 2008). An enhancement of FatOx during exercise may also have the potential to be a relevant strategy to prevent and treat overweight (Achten and Jeukendrup 2004).

Indirect calorimetry is a non-invasive method commonly used to estimate energy expenditure and substrate utilization during different conditions (Battezzati and Viganò 2001). Respiratory exchange ratio (RER) is the ratio of carbon dioxide produced to oxygen consumed, and is often used to calculate FatOx during exercise (McArdle et al. 2010). The accuracy of estimating FatOx during exercise using RER is typically limited by the variations in work economy (WE) in addition to the accuracy of measuring oxygen consumption (VO$_2$) and carbon dioxide production (VCO$_2$). Variations in WE have been found to be relatively low, and in the area of 2% to 4% (Helgerud et al. 2009; Saunders et al. 2004), and test – retest variability in measuring VO$_2$ and VCO$_2$ is according to the producers of most analyzers 3% or less (Åstrand and Rodahl 1986). As variability in VO$_2$ or VCO$_2$ is an obvious part of the variability in WE, these numbers are not additive. The test – retest variability at the same relative work intensity should thus theoretically lie within 4-5%. Training or diet interventions may therefore be regarded as having affected FatOx if statistically significant results exceed 5% difference from baseline values. If FatOx results from RER measurements during exercise are to be used as expressions of health status or performance ability, they need to be reliable. Aerobic work capacity (Nordby et al. 2006; Van Loon et al. 1999), nutrient status (Gonzales and Stevenson 2012) and changes in diet (Burke and Hawley 2002; Carey et al. 2001; Helge et al. 2001) may affect FatOx during exercise at the same relative workload.
To evaluate the reliability of a FatOx test during exercise, it is thus crucial that diet is thoroughly controlled and standardized with equal macronutrient composition during the test days. Piers et al. (1992) found only small, non-significant variations in RER measured on different occasions during postabsorptive conditions (BMR) (CV 1.9%, p>0.05). Gonzales et al. (2012) showed similar results as Piers et al. (1992) when comparing RER during 2 postprandial conditions (CV 3.8%, p>0.05). However, Gonzales et al. (2012) also discovered a CV of 11.5% when comparing FatOx during postabsorptive conditions at two different occasions, and the CV increased to 20% during postprandial conditions.

A study investigating day to day variations in FatOx when diet is strictly controlled for is relevant to evaluate the tests’ reliability. The reliability test will thus make it easier to assess to what extent an eventual effect on FatOx during exercise is caused by an intervention or is a consequence of normal day to day FatOx variations.

Several studies have investigated the effects of a long-term (e.g several weeks) change in diet, and there is strong evidence that a major change in macronutrient composition will affect substrate utilization during aerobic exercise (Helge et al. 2001; Wycherley et al. 2014). However, also short term (1-3 days) changes in diet pattern before testing may affect FatOx during exercise (Guimaraes Couto et al. 2014; Patterson and Potteiger 2011; Stepto et al. 2002). These studies typically include many days between the diet manipulation days to “wash out” the effects of one specific diet before starting a new diet (Guimaraes Couto et al. 2014; Patterson and Potteiger 2011; Stepto et al. 2002). However, rate of FatOx during exercise is also affected by fitness level (Nordby et al. 2006; Van Loon et al. 1999). Therefore, too many days between diet manipulation days may increase the risk of a change in physical fitness. There is also a certain probability that changes in diet patterns among the participants in a study may unintendedly occur a few days before testing. Diet patterns typically differ between weekends and weekdays (Racette et al. 2008; Smith et al. 2015).
evaluation of possible changes in FatOx during exercise after only short-term changes in diet composition and after just a few days of “wash out” is thus relevant in a methodical perspective. In weight loss programs, where both exercise and diet are used as strategies to reduce weight, an evaluation like this may also illustrate which diet is optimal to increase FatOx during exercise. Only few studies have investigated the effects of a 1-2 days change in diet on FatOx during exercise (Guimaraes Couto et al. 2014; Patterson and Potteiger 2011; Stepto et al. 2002).

The aims of the present study were therefore to examine day to day variations in FatOx during moderate-intensity exercise (60% VO$_{2\text{max}}$) given the same test conditions, and to examine the effect of two different isoenergetic diets, either a high fat diet or high carbohydrate (CHO) diet one day prior to testing with a short “wash out” period (2 days) in between.

**Materials and methods**

*Subjects and general design*

Nine healthy and moderately trained (Heyward 2005) females (VO$_{2\text{max}}$: 43.8 ml·kg$^{-1}$·min$^{-1}$, with 2-3 training sessions per week) with a normal body mass index (BMI) (23.0±1.1 kg·m$^{-2}$) (Bouchard et al. 2007) participated in this study. Participant characteristics are presented in Table 1. The subjects performed one VO$_{2\text{max}}$ test and four FatOx tests during a nine days period. Inclusion criteria were age between 20 and 40 years and BMI between 18.5 and 30 (kg·m$^{-2}$). Participants were excluded from the study if they were or had been a) sick for more than 2 consecutive weeks before test start, b) using blood pressure medication, c) diagnosed with diabetes 1 or 2 or other metabolic disorders (e.g hyperthyreosis), d) dieting during the last 6 weeks before test start, e) diagnosed with eating disorders or revealed a disturbed
relationship to food (SCOFF questionnaire, Morgan et al. 1999). Participants with an energy intake below 1500 Kcal per day were excluded from the study. This initial 1500 kcal threshold criterion was set due to the recognition that an energy intake below 30 Kcal per kg fat free mass per day is critical low (Mountjoy et al. 2014). In addition, each participant’s individual energy intake was evaluated up against their physical activity level to ensure energy balance.

17 individuals volunteered for the study. Eight persons were excluded due to high age (n=2), too low daily energy intake (n=3), metabolic disturbance (n=1) and risk of disturbed eating patterns (n=2). All of the participants gave their written consent to participate after receiving both oral and written information about procedures, possible risks, and benefits. The study was approved by the Regional Committee for Medical and Health Research Ethics in Southern Norway, as well as the institutional review board at Telemark University College, and was conducted in accordance with ethical principles of the Helsinki Declaration. The participants’ preparation procedures included no strenuous exercise the day before testing and they could only ingest water the last two hours before testing. All food and fluid intake during test day was thoroughly monitored. The fat oxidation tests were always performed at the same time during the day (± 1 hour). The participants agreed to maintain their habitual physical activities during the study period to prevent changes in total energy expenditure.

(Table 1)

Test equipment

The physical tests were carried out using a Lode Excalibur Sport (Lode, Groningen, Netherlands) cycle ergometer. Between 100- and 1500 watt, the workload accuracy of the test bike is ± 2% according to the manufacturer’s ergometer specifications. The cycling sitting
position was accurately fitted to each subject and registered for the next test to ensure the same position at each test and thereby lowering the risk of influencing WE. Ergo spirometry was performed using the metabolic test system Sensor Medics Vmax Spectra (Sensor Medics 229, Yorba Linda, California, USA). The volume and gas analyzers were calibrated with a 3 L calibration syringe (Hans Rudolph, Kansas City, MO, USA) and calibration gas (26% O2 and 16% O2). Measurements of VO$_2$ and VCO$_2$ with the Sensor Medics Vmax Spectra are accurate within a range of ± 3%, in accordance with manufacturer recommendations. It is possible that the use of oxygen expenditure measurements could be slightly different with the Douglas bag equipment due to the changes in the composition of the ambient air (Betts and Thompson 2012). However, we have no reason to suspect greater difference in measurements than the up to ± 3% range of accuracy already accounted for. Also, test-to-test variations with the Vmax Spectra in our laboratory are shown to be less than ±1%, with a SEM of 0.1–0.2 in different tests, as reported in Helgerud et al. (2010). It may also be argued that newer equipment such as the Vmax Spectra, continuously measures the ambient air and thus the problem raised in Bell and Thompson (2012) should not apply to the same extent as with the Douglas bag method. Heart rate (HR) was recorded continuously during all stages of the testing procedure using Polar rs400 (Polar Kempele, Finland). Blood lactate concentration ([La$^-$]$_b$) was registered using an Arcray Lactate Pro LT-1710 analyzer, venous whole blood (Arcray Inc. Kyoto, Japan). Before each fat oxidation test, the participants’ blood glucose (BG) was measured using a Accu-Chek Compact Plus blood sugar meter (Roche Diagnostics, Germany). Body weight was measured with the body scale Tefal Sensitive Computer Pp 6010, France.
Diet manipulation and registration (table 2)

Diet was thoroughly registered all 9 days in the testing period, using food registration forms and a 1 g accurate kitchen scale (Wilfa, KW-4, Hagan, Norway). Two days before test start the participants’ daily eating habits and activity level were obtained using a 1-day food diary and interview about physical activity habits. The information about physical activity level and their habitual diet was used to discover potential over- or under eating. Their habitual diets were carefully registered, and thus repeated during all normal days (i.e days 1,2,3,6,7). At day 4, half of the participants were randomly chosen to change to a fat-rich diet aimed to consist of approximately 50% fat and 10% carbohydrates. The other half of the participants changed to CHO-rich diet consisting of approximately 10% fat and 50% carbohydrates. The participants were given an individual menu based on these contributions, and they maintained this diet until testing was completed on day 5. On day 6 and 7 the participants ate as normal, to ensure a normalization of their diet before the next days with diet manipulation. A longer wash-out period was specifically not chosen in order to avoid a long study time line. A long study time line would increase the risk of changes in aerobic capacity in the participants. Additionally, we wished to explore to what extent diet changes during shorter periods of time and with less time of normalization in diet influence FatOx during exercise. There was no physical testing during day 6 and 7. On day 8 the participants’ diets were changed again, to either a fat-rich or a CHO-rich diet (depending on the previous diet on days 4-5). This diet was maintained until end of testing at day 9.
Testing procedures

All subjects were thoroughly familiarized with testing procedures and equipment before test start. On the first day, the participants underwent anthropometric measurements (weight and height) before completing three submaximal 5 min bouts at gradually increasing workload and a VO$_{2\text{max}}$ test. When performing the three 5 min submaximal workloads, the subjects started at a brake power corresponding to approximately 50% VO$_{2\text{max}}$. At the next two 5 min bouts, the brake power was increased to obtain an intensity equivalent to approximately 75- and 85% VO$_{2\text{max}}$, respectively. As the three submaximal workloads were performed, the intensities were subjectively calculated from HR, RER and VO$_2$. The percentages were then evaluated up against VO$_{2\text{max}}$ to ensure that the subjective calculations were valid. The VO$_{2\text{max}}$ test was performed using an incremental protocol. The participants started at a brake power assumed to be approximately 75% VO$_{2\text{max}}$, and the brake power was increased every 30 sec by 10 or 20 watt depending on the individual VO$_2$ curve and the subjective evaluation of the test leader. The VO$_2$ values were registered every 20 sec. The test ended at voluntary exhaustion and the following criteria, as previously used in Støren et al. (2008), Sunde et al. (2010) and Helgerud et al. (2010), were used to evaluate if VO$_{2\text{max}}$ was accomplished; a flattening of the VO$_2$ curve, RER $\geq$ 1.05, HR$_{\text{peak}}$ $\geq$ 95% of expected HR$_{\text{max}}$ and concentration of blood lactate above 8 mmol·L$^{-1}$. The average of the two highest subsequent VO$_2$ measurements was registered as VO$_{2\text{max}}$. The highest heart rate during the VO$_{2\text{max}}$ test was registered as peak heart rate (HR$_{\text{peak}}$).

The FatOx test was performed at the relative workload of 60% VO$_{2\text{max}}$. To calculate each person’s individual workload in watts, three 5 min submaximal workloads were used to establish the linear regression for VO$_{2\text{max}}$ and watts (As in Helgerud et al. 2010). With a VO$_{2\text{max}}$-test to set 100% VO$_{2\text{max}}$, this linear regression was used to determine the wattage at 60% VO$_{2\text{max}}$. This calculation is based on the physiological principle that oxygen
consumption is linearly related to the workload, and is previously used in Støren et al. (2008), Sunde et al. (2010) and Helgerud et al. (2010). The linearity from this regression in Sunde et al. (2010) averaged an $R^2 = 0.992 \pm 0.005$, $p < 0.0001$. The fat oxidation tests were completed on days 2, 3, 5, and 9 (table 2). FatOx results from days 2 and 3 with normal diets are the baseline data for evaluating day to day variability, while results from days 5 and 9 show the acute effects of diet manipulation on FatOx. 10 minutes before each FatOx test the participants’ BG were measured, since a difference before testing can reveal a change in eating pattern and thereby affect the FatOx results. The FatOx test used a 10 minutes protocol were $V_O^2$, $V_CO^2$ and thus RER values were measured every 20 sec and averaged between 4 and 10 minutes. At this stage of the work period, a stable plateau of gas exchange is reached giving a reliable basis for estimating substrate utilization. The length of the test is in accordance with Bordenave et al’s (2007) recommendation using longer than 3 min steps to allow accurate calculations of substrate utilization using indirect calorimetry. FatOx was calculated using the following formula from Frayn (1983): FatOx (g·min$^{-1}$) = ($V_O^2 \cdot 1.67$) – ($V_CO^2 \cdot 1.67$) – 0.307 \cdot (POX). Where POX (g·min$^{-1}$) is the protein oxidation rate, assumed to be (KJ·min$^{-1}$) \cdot (0.12g ·J) / 17.74 KJ

Statistics

Experimental data are presented as mean ± standard deviation, as well as delta values (Δ) and coefficient of variance (CV) in percent. A general linear model with Tukey post hoc test was used to assess potential differences in $V_O^2$, $V_CO^2$, RER, FatOx, HR, [La]$_b$, BG, BW and BMI between different test days. The general linear model (ANOVA), with Tukey post hoc tests (ANCOVA) were used to account for the four different steps (test days 1-4) in which FatOx was measured. CV was used to assess variability between subject at the different test days and variability in difference between test days. Helgerud et al. (2009) have previously
used these statistical methods in assessing potential differences in running economy at different velocities. In addition, we wanted to assess whether or not the size of FatOx would have an impact on the test-retest variability between the two test days with identical diet (test days 1-2). A Bland-Altman plot, as previously used in e.g. Gonzales et al. (2012), was used for this purpose. To further assess the reliability, intraclass correlation coefficient tests were performed. A Pearson bivariate correlational test was used to determine a possible relationship between VO\textsubscript{2}\text{max} and FatOx at baseline. In all tests, significance was accepted at p<0.05. Analyses were performed using Statistical Package for the Social Sciences software version 22 (SPSS, Chicago, Illinois, USA).

Results

Energy intake and nutrient composition (table 3)

There were no significant changes in total energy intake (TEI) or in distribution from the different nutrients (CHO, fat and proteins) to TEI between the two habitual diet days. No significant changes were found in TEI and %TEI protein between the high fat diet and the high CHO diet. The high CHO diet had a significant higher distribution (134%; 62.6±8.5 vs 26.8±5.4 %TEI, p<0.01) of CHO and a significant lower distribution from fat (-74%; 12.4±4.8 vs 47.1±6.9 %TEI, p<0.01) than that of the high fat diet. The high CHO diet also had a significant higher distribution from CHO and lower distribution from fat compared to both habitual diet day 1 (p<0.01) and habitual diet day 2 (p<0.01). The high fat diet had a significant lower %TEI CHO than that of habitual day 1 and 2 (p<0.01), but no significant change was found in %TEI fat between high fat diet and the habitual diet days. There was no significant change in %TEI proteins between high fat and the habitual days.
The participants body weights were unchanged between day 1 and day 9 (62.1±7.2 kg and 62.5±7.3 kg respectively, ∆ = 0.4, CV% = 0.5).

(Table 3)

Day to day variability in metabolic responses and changes from diet manipulation (table 4).

No significant differences in VO₂, VCO₂, RER, FatOx, HR, [La⁻]b and BG were found between the two habitual days. The non-significant difference in FatOx was 7.6% (0.39±0.08 vs 0.42±0.15 g·min⁻¹, p>0.05) between the two test days. The intraclass correlation coefficient (ICC) was 0.823. The CV of this difference was 1.3%.

(Table 4)

No change was found in FatOx between high fat diet day and habitual diet days. The size of the participants FatOx did not affect the day to day variability (Figure 1).

(Figure 1)

FatOx was significantly lower in CHO diet than habitual day 2 (p<0.05), but no significant change was found in FatOx between the CHO diet day and habitual day 1. RER was significantly higher (4.8%; 0.87±0.04 vs 0.83±0.04, p<0.05), and FatOx significant lower (-31%; 0.29±0.13 vs 0.42±0.14 g·min⁻¹, p<0.05) in the high CHO diet than in high fat diet. VO₂ was significantly lower during the high CHO diet than the high fat diet (-4.1%; 1.65±0.17 vs 1.72±0.20, p<0.05). There were no significant changes in VCO₂, HR, [La⁻]b and BG between high fat days and high CHO days.

No correlation was found between VO₂max and FatOx.

Day to day variability in metabolic responses and changes from diet manipulation (table 4).

RER was significantly higher (4.8%, p<0.05), and FatOx significant lower (-31%, p<0.05) in the high CHO diet than in the high fat diet. VO₂ was significantly lower during the high CHO diet than the high fat diet (-4.1%, p<0.05). FatOx was significantly lower in CHO diet than...
habitual day 2 (p<0.05), but no significant change was found in FatOx between the two different diet manipulation days and habitual day 1. No significant differences in VO$_2$, VCO$_2$, RER, FatOx, HR, [La]$_b$ and BG were found between the two habitual days. There were no significant changes in VCO$_2$, HR, [La]$_b$ and BG between high fat days and high CHO days. (Table 4)

**Discussion**

The day to day test – retest showed a non-significant difference in FatOx between the two habitual days of 7.6%, with a CV in difference of 1.3%, and a ICC of 0.823. Although not directly comparable due to differences in methods, the difference and the CV between the two habitual test days is smaller than reported in Pierce et al. (1992) and Gonzales et al. (2012). The acute effect of diet manipulation was significantly lower FatOx (-31%, p<0.05) during the low fat - high CHO diet compared to the high fat – low CHO diet. This effect may partly be explained by a day to day variability (7.6%, p>0.05) in FatOx as shown between habitual day 1 and habitual day 2. This difference will include the 4-5 % expected variations in WE and test – retest variability in measuring VO$_2$ and VCO$_2$. This indicates that 2-3 % of the day to day FatOx variability could be due to small non-significant diet differences. Even when accounting for the 4-5 % expected variations in WE and VO$_2$, 26-27% of the difference in FatOx between the diet manipulation days is probably caused by the different contents of fat and CHO.

We found no correlation between VO$_{2\max}$ and FatOx. This is in opposition to the results in Nordby et al. (2006). The lack of correlation in the present study is probably due to homogenous VO$_{2\max}$ values (CV 9.8%) compared to the more heterogenous VO$_{2\max}$ values from Nordby et al. (2006) (CV> 15%). The VO$_{2\max}$ values in Nordby et al. (2006) represents training status. Nordby et al. (2006) actually divides the participants into trained and
untrained based on VO$_{2\text{max}}$ values. The participants in the present study were all moderately trained and this is also expressed by the low diversity in VO$_{2\text{max}}$.

The lower FatOx during high CHO diet compared to high-fat diet in our study, may be caused by higher levels of CHO in glycogen stores in muscle after a high CHO diet. Increased glycolytic flux from hyperglycemia and hyperinsulinemia has also been found to reduce fatty acid oxidation during exercise (Coyle et al. 1997). The lower FatOx during the high-CHO diet in our study is in accordance with Patterson and Potteiger (2011). In Patterson and Potteiger (2011), a higher FatOx (P<0.05) was found during exercise after a low CHO (20% CHO, 40% protein, 40% fat) diet compared to a moderate CHO diet (55% CHO, 15% protein, 30% fat). Our study was very similar to the study of Patterson and Potteiger (2011) in several areas; female participants, the number of participants, short term diet manipulation, the participants had very similar anthropometrics, and the FatOx tests were performed at similar intensity. However, the present study had only 2 wash-out days between the different diets, while Patterson and Potteiger (2011) had at least 7 days. There is a possibility that a longer wash-out period in the present study could have increased the differences in FatOx between the low- and high CHO diets. The habitual diet did not differ substantially from the high fat diet in the present study. A longer wash-out period with a habitual diet could possibly decrease circulating insulin levels, leading to a higher utilization of fatty acids. However, insulin levels were not measured in the present study.

Guimaraes Couto et al. (2014) also investigated the effect of a short-term (2 days), isoenergetic fat-rich (24.2% CHO, 15.5% protein, 60.4% fat), CHO rich diet (69.3% CHO, 15.1% protein, 15.9% fat) or habitual (56.1% CHO, 16.5% protein , 27.5% fat) on substrate oxidation rates during submaximal exercise (65% VO$_{2\text{peak}}$) in trained, mid- to late-pubertal boys. The three different diet periods were performed with 7 to 14 washout days in between. Although they did not measure FatOx per se, the results revealed a significant lower (P<0.05)
RER and CHO contribution (P=0.01) to energy expenditure, and higher contribution from fat in the fat rich diet than in the CHO rich diet. Similar to the present study, the RER in the fat rich diet was not significantly different from the habitual diet. Additionally, and contrary to the findings in our study, RER was not different after the CHO rich diet than after habitual diet. This might be due to a higher proportion of CHO in the participants’ habitual diets in Guimares Couto et al. (2014) compared to our study (56% TEI versus ~36% TEI).

A randomized cross-over study by Stepto et al. (2002) also conducted a short-term (3 days) diet manipulation to examine the effect of either a high fat (~60% TEI) or a high CHO diet (~67% TEI) on high-intensity aerobic interval training in endurance athletes. An 18 day washout period separated the two different diets. They found a significant decrease in RER (P<0.05) after the high fat diet, leading to an increased FatOx of 96%. Contrary to the participants in the present study who had a high distribution of fat in their habitual diet, the endurance athletes in Stepto et al. (2002) habitually consumed a high proportion of CHO in their habitual diet (60% of TEI). Similar to the findings in our study, the results from Stepto et al. (2002) indicate that the effect on FatOx after diet manipulation is dependent on the degree of similarity to habitual diet.

The participants in the present study were all females and represented a moderately trained group (VO\textsubscript{2max}; 43.8 ml·kg\textsuperscript{-1}·min\textsuperscript{-1}) (Heyward 2005) with a normal BMI (23.0±1.1) (Bouchard et al. 2007). A reduced TEI, and/or a sudden increase in exercise intensity or volume during the diet manipulation days could lead to a negative energy balance and a possible increase in FatOx during exercise (Kempen et al. 1998; van Loon et al. 2001). However, there was no change in TEI or body weight, and none of the participants in this study reported changes in physical activity during the study period.

The participants had somewhat lower energy intake than recommended by nutrition guidelines. Basal- and resting metabolic rate, thermic effect of food and physical activity are
the most important components determining a person’s total daily energy expenditure (Bouchard et al. 2007). According to Harvard Medical School (www.health.harvard.edu), a moderately active person would require approximately 16 calories per pound, which in this group of participants would mean an average caloric need of 2180. Another often used daily calorie need formula is the Mifflin St Jeor equation. If using Mifflin St Jeor equation in this participant group the estimation would average 2208 Kcal. The participants average TEI during the nine days was 1967 Kcal per day. Although this energy intake is somewhat lower than recommended, we did not find it critically low. The participants did not lose weight during the nine days of study participation which indicates energy balance.

The participants in Patterson and Potteiger (2011) were also all females in the same age class, and with very similar anthropometrics. The participants in Patterson and Potteiger (2011) had a higher average TEI compared to the participants in our study (2366 vs 1967 Kcal per day, respectively). But the participants in Patterson and Potteiger (2011) were also more physically active which is also shown by higher VO$_{2\text{max}}$ values (47.9 vs 43.8 ml·kg$^{-1}$·min$^{-1}$ and 3.0 vs 2.77 L·min$^{-1}$). The higher TEI in Patterson and Potteiger (2011) may at least partly be explained by higher fitness levels and higher physical activity levels.

Recommended intake of CHO, fat and proteins are 45%-65%, 20%-35% and 10%-35% respectively (Manore 2005). In the present study, the distribution of fat in the participants’ habitual diets were above 40% which is higher than recommended. Other studies have used a higher distribution of fat (60-65%) in typical high fat diets compared to the present study (Guimaraes Couto et al. 2014; Helge et al. 2001; Wycherley et al. 2014). When preparing the menus and calculating the fat distribution in the present study, the fat distribution was estimated to lie within 40 % above the daily recommended fat distribution. This consideration was done to ensure that the two manipulated diets were very different from each other in nutrient composition, but still realistic in a test situation.
The use of ergospirometry measurements is the most time saving way to assess FatOx during exercise, and it also put a minimum of stress on the participating subjects. Due to practical and economic reasons, most of exercise laboratories do not have the possibility to measure substrate utilization in vivo for example by the isotopic tracer techniques. The use of RER measurements when RER is below 0.90, and when metabolic mechanisms like gluconeogenesis and lipogenesis are at a minimum are therefore the most common and widely used method to measure rate of substrate utilization during exercise (Battezzati and Vigano 2001; Brandou et al 2003). It was thus a clear choice in this study to evaluate this method of measuring FatOx. However, as the protein oxidation was set as a constant and was not measured by urinary nitrogen excretion, the RER method may not be quite as accurate as for example isotopic tracer techniques. Isotopic tracer techniques together with indirect calorimetry have been recommended to give more accurate and complementary data about substrate metabolism (Schutz 1997). However, Romjin et al. (1992) found that FatOx and CHO oxidation measured with a stable isotope method were not significantly different from those measured with indirect calorimetry during exercise at 80-85% VO_{2max} (CV approximately 12% for both).

It has been proposed that acute effects from different CHO glycemic indexes (GI) in meals prior to testing may influence FatOx during moderate exercise. However, studies investigating this are somewhat inconsistent. For example, a low GI (LGI) compared to high GI (HGI) breakfast preceding substrate utilization measurement during moderate exercise has been shown to result in both increased FatOx (Wee et al. 2005), lower FatOx (Moore et al. 2010) and no change (Zakrzewski et al. 2012). Therefore, in the present study we did not take this into consideration when preparing meal plans for the different diet manipulations.

None of the participants reported to be in their menstrual period. However, menstrual cycle status was not reported. During moderate intensity exercise, some studies have indicated a
higher FatOx and a lower CHO oxidation in the luteal phase compared to the follicular phase (Campbell et al. 2001; Zderic et al. 2001), while other studies found no differences in substrate utilization between the two phases (Devries et al. 2006; Vaiksaar 2011; Suh et al. 2002). Since these studies are not conclusive, a possible influence of menstrual cycle phase on FatOx cannot be excluded.

The habitual diets, high-fat diets and high CHO diets in our study and the three studies mentioned, all differ somewhat in macronutrient composition. It is likely that this will affect the adaptations to the different diets and thereby the results of the studies. Our study had only 2 days of “wash-out” between the two different diet days, which is very short compared to Patterson and Potteiger (2011), Guimaraes Couto et al. (2014) and Stepto et al. (2002) who had at least 7 days. We cannot be certain that 2 days is sufficient to normalize the metabolism after a diet manipulation.

We found no difference in FatOx between high-fat diet days and habitual diet days. The most obvious reason is that the participants’ habitual diets already consisted of a high relative proportion of fat (≈42 %TEI compared to ≈47 %TEI in high-fat diet). Another reason may be the short duration of only one day of diet manipulation, since adaptations to a high-fat diet takes longer than the almost immediate response to a high-CHO diet (Jeukendrup 2003). Similar to our study, Guimaraes Couto et al. (2014) found no changes in RER during exercise between high-fat diet and habitual diet. Interestingly, this occurred although there was a higher difference in fat content between fat-rich diet days and habitual diets than in the present study, and despite that their habitual diets contained a higher proportion of CHO than in the present study.

This study has demonstrated the importance of dietary macronutrient composition on FatOx during exercise, and that shifts in substrate utilization during aerobic exercise may occur after only 1 day of change in diet composition.
**Conclusion**

There were no significant day to day variations in FatOx between two habitual diet days with equal macronutrient composition.

A low fat – high CHO diet led to a 31% reduction in FatOx during moderate exercise compared to high fat – low CHO diet and habitual diet.

**Conflict of interest statement**

Authors have no conflict of interest to report.

**Acknowledgement**

We wish to thank our statistician Per Christian Hagen (Telemark University College) for help with the statistical analyzes.

**Abbreviations**

- BG: Blood glucose
- BMI: Body mass index
- BW: Body weight
- CHO: Carbohydrate
- CV: Coefficient of variance
- FatOx: Fat oxidation
- GI: Glycemic index
- HR: Heart rate
- HR$_{\text{max}}$: Maximal heart rate
- HR$_{\text{peak}}$: Peak heart rate
KCAL  Kilo calories
KJ    Kilo joules
\([La^-]_b\)  Blood lactate concentration
POX   Protein oxidation
RER   Respiratory exchange ratio
TEI   Total energy intake
VCO_2 Volume of carbon dioxide
VO_2  Oxygen uptake
VO_{2\text{max}} Maximal oxygen uptake
WE    Work economy

References


www.health.harvard.edu
# Tables

## Table 1. Participant characteristics (N=9)

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>22.4±1.1</td>
</tr>
<tr>
<td>BW (kg)</td>
<td>62.1±7.2</td>
</tr>
<tr>
<td>BMI (kg⋅m⁻²)</td>
<td>23.0±1.1</td>
</tr>
<tr>
<td>Height (cm)</td>
<td>165.1±6.3</td>
</tr>
<tr>
<td>VO₂max (ml⋅kg⁻¹⋅min⁻¹)</td>
<td>43.8±4.3</td>
</tr>
<tr>
<td>VO₂max (L⋅min⁻¹)</td>
<td>2.77±0.33</td>
</tr>
</tbody>
</table>

Values are mean ± standard deviation.

BW, body weight. BMI, body mass index. VO₂max, maximal oxygen consumption.
Table 2. Testing procedures from day 0 to day 9

<table>
<thead>
<tr>
<th>Day</th>
<th>Test</th>
<th>Diet</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>Information day(^1)</td>
<td>Habitual</td>
</tr>
<tr>
<td>1</td>
<td>Anthropometrics &amp; calculation of 60% $\dot{VO}_{2\text{max}}$</td>
<td>Habitual</td>
</tr>
<tr>
<td>2</td>
<td>FatOx test</td>
<td>Habitual</td>
</tr>
<tr>
<td>3</td>
<td>FatOx test</td>
<td>Habitual</td>
</tr>
<tr>
<td>4</td>
<td>No physical tests</td>
<td>High fat or high CHO</td>
</tr>
<tr>
<td>5</td>
<td>FatOx test</td>
<td>High fat or high CHO</td>
</tr>
<tr>
<td></td>
<td>(habitual diet after testing)</td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>No physical tests</td>
<td>Habitual</td>
</tr>
<tr>
<td>7</td>
<td>No physical tests</td>
<td>Habitual</td>
</tr>
<tr>
<td>8</td>
<td>No physical tests</td>
<td>High fat or high CHO</td>
</tr>
<tr>
<td>9</td>
<td>FatOx test</td>
<td>High fat or high CHO</td>
</tr>
</tbody>
</table>

FatOx, fat oxidation test at 60% $VO_{2\text{max}}$. $VO_{2\text{max}}$, maximal oxygen consumption. High fat diet, 50% fat and 10% CHO. CHO, carbohydrates.

\(^1\) Information about the study. Assessment of eating habits and physical activity level.

\(^2\) Calculation of 60% $VO_{2\text{max}}$ was based on three 5 min submaximal work periods at increasing workload and a $VO_{2\text{max}}$ test.
Table 3. Dietary variability (N=9)

<table>
<thead>
<tr>
<th></th>
<th>Habitual 1</th>
<th>Habitual 2</th>
<th>Δ</th>
<th>CV(%)</th>
<th>High Fat</th>
<th>High CHO</th>
<th>Δ</th>
<th>CV(%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>TEI (Kcal)</td>
<td>1983±363</td>
<td>2096±222</td>
<td>113</td>
<td>3.8</td>
<td>1893±254</td>
<td>1894±386</td>
<td>1</td>
<td>4.1</td>
</tr>
<tr>
<td>CHO (g)</td>
<td>180.5±35.8</td>
<td>181.6±51.4</td>
<td>1.1</td>
<td>12.4</td>
<td>296.3±67.4§</td>
<td>127.0±23.8*§</td>
<td>169.3*</td>
<td>20.8</td>
</tr>
<tr>
<td>Fat (g)</td>
<td>92.9±29.2</td>
<td>100.2±20.6</td>
<td>7.3</td>
<td>22.8</td>
<td>25.7±7.5§</td>
<td>99.8±24.7*</td>
<td>73.9*</td>
<td>30.1</td>
</tr>
<tr>
<td>Prot (g)</td>
<td>97.5±25.7</td>
<td>107.8±27.5</td>
<td>10.3</td>
<td>22.7</td>
<td>93.6±29.8</td>
<td>105.3±23.8</td>
<td>11.7</td>
<td>27.4</td>
</tr>
<tr>
<td>%TEI CHO</td>
<td>37.2±6.7</td>
<td>35.0±9.7</td>
<td>-2.2</td>
<td>3.6</td>
<td>26.8±5.4§</td>
<td>62.6±8.5*§</td>
<td>35.8*</td>
<td>3.7</td>
</tr>
<tr>
<td>%TEI Fat</td>
<td>41.2±7.6</td>
<td>42.6±7.3</td>
<td>1.4</td>
<td>2.7</td>
<td>47.1±6.9</td>
<td>12.4±4.8*§</td>
<td>-34.7*</td>
<td>2.7</td>
</tr>
<tr>
<td>%TEI Prot</td>
<td>20.0±3.8</td>
<td>20.8±4.8</td>
<td>0.8</td>
<td>1.3</td>
<td>23.2±7.1</td>
<td>20.1±6.4</td>
<td>-3.1</td>
<td>4.9</td>
</tr>
</tbody>
</table>

Values are mean ± standard deviation, delta values (Δ), and coefficient of variance (CV) in per cent. Kcal, kilocalories. TEI, total energy intake. CHO, carbohydrates. Prot, proteins. Habitual 1, based on habitual diet day 1. Habitual 2, based on habitual diet day 2. High Fat, fat-rich diet day 4-5 or day 8-9. High CHO, carbohydrate-rich diet day 4-5 or day 8-9.

* p<0.01 different from High Fat
§ p<0.01 different from Habitual 1
# p<0.01 different from Habitual 2
*§ p<0.01 different from Δ Habitual
Table 4. Day to day variability in FatOx during normal diet and changes in FatOx during a fat rich diet and a carbohydrate rich diet respectively (N=9)

<table>
<thead>
<tr>
<th></th>
<th>Habitual 1</th>
<th>Habitual 2</th>
<th>Δ, CV(%)</th>
<th>ICC</th>
<th>High Fat</th>
<th>High CHO</th>
<th>Δ, CV(%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>VO₂ (L·min⁻¹)</td>
<td>1.70±0.17</td>
<td>1.72±0.20</td>
<td>0.02, 3.8</td>
<td>0.958</td>
<td>1.72±0.20</td>
<td>1.65±0.17*§</td>
<td>-0.07*, 4.1</td>
</tr>
<tr>
<td>VCO₂ (L·min⁻¹)</td>
<td>1.41±0.14</td>
<td>1.43±0.13</td>
<td>0.02, 3.6</td>
<td>0.928</td>
<td>1.42±0.16</td>
<td>1.43±0.14</td>
<td>0.01, 3.7</td>
</tr>
<tr>
<td>RER (VCO₂ / VO₂)</td>
<td>0.83±0.02</td>
<td>0.83±0.04</td>
<td>0.0, 2.7</td>
<td>0.710</td>
<td>0.83±0.04</td>
<td>0.87±0.04*</td>
<td>0.04*, 2.7</td>
</tr>
<tr>
<td>FatOx (g·min⁻¹)</td>
<td>0.39±0.08</td>
<td>0.42±0.15</td>
<td>0.03, 1.3</td>
<td>0.823</td>
<td>0.42±0.14</td>
<td>0.29±0.13*§</td>
<td>-0.13*, 4.9</td>
</tr>
<tr>
<td>HR (b·min⁻¹)</td>
<td>143±12</td>
<td>144±12</td>
<td>1, 0.3</td>
<td>0.952</td>
<td>142±11</td>
<td>139±11</td>
<td>-3, 1.6</td>
</tr>
<tr>
<td>[La]₀ (mmol·L⁻¹)</td>
<td>2.7±0.8</td>
<td>2.6±0.7</td>
<td>-0.1, 2.7</td>
<td>0.838</td>
<td>2.8±0.6</td>
<td>3.2±0.8</td>
<td>0.4, 11.8</td>
</tr>
<tr>
<td>BG (mmol·L⁻¹)</td>
<td>5.4±0.9</td>
<td>4.9±0.4</td>
<td>-0.5, 7.8</td>
<td>0.304</td>
<td>5.3±1.0</td>
<td>5.3±0.4</td>
<td>0.0, 0.5</td>
</tr>
</tbody>
</table>

Values are mean ± standard deviation, delta values (Δ), coefficient of variance (CV) in per cent and intraclass correlation coefficient (ICC). VO₂, oxygen consumption in liters per minute. VCO₂, carbon dioxide production in liters per minute. RER, respiratory exchange ratio. FatOx, fat oxidation in grams per minute. HR, heart rate in beats per minute. [La]₀, blood lactate concentration in millimoles per liter. BG, Blood glucose in millimoles per liter.

* p<0.05 different from High Fat
§ p<0.05 different from Habitual 2
*§ p<0.05 different from Δ Habitual
## p<0.01 different from Δ Habitual
Figure 1: Bland-Altman Plot on mean FatOx (x-axis) versus difference in FatOx (y-axis) between habitual days 1 and 2 (N=9). FatOx, fat oxidation. Upper (ULA) and bottom (LLA) lines show the upper and lower 95% confidence interval in differences. Medial line shows mean difference between the two test days. No plots appeared outside ULA and LLA.
Difference in FatOx (g·min⁻¹) between habitual days

Mean FatOx (g·min⁻¹)

ULA = 0.15
Mean = -0.03
LLA = -0.21