Balance of Substrates at Exercise in Athletes: Lipodependent vs Glucodependent Sports [Version 1, Awaiting Peer Review]

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Abstract

Recent development of exercise-tests assessing the balance of substrates used for oxidation at exercise evidences various profiles among various populations, and training appears to influence these parameters. This study aimed at comparing selected groups of subjects submitted to different well-defined training protocols. We investigated the balance of substrates oxidized at exercise in 90 athletes submitted to different training protocols (28 cyclists, 32 soccer players, 19 male rugby players, 11 rugby women, compared to 41 controls) during an exercise-test consisting of five six-minute steady-state workloads designed for measuring carbohydrate and fat oxidation with indirect calorimetry. The power at which lipid oxidation reached a maximum expressed as a percentage of VO2max capability ranked as follows: cyclists (59.5 ± 2.8) > female rugby players (45.1 ± 4.0) > male rugby players (39.5 ± 6.5) > female controls (39.6 ± 3.7) > male controls (32.5 ± 4.5) > soccer players (17.9 ± 2.0). Thus, beside the expected picture of athletes oxidizing higher quantities of lipids than controls (cyclists and male rugby players had a high ability to oxidize lipids), we evidence here, in a sample of soccer players, an opposite pattern of “early glucodependence”.

Keywords

Substrate Crossover; Lipid Oxidation; Power of Maximal Lipid Oxidation; Soccer; Rugby; Cycling; Exercise; Lipid Oxidation; LIPOXmax
Introduction

While the balance of substrates utilized for oxidation at exercise has long been subject of interest [1-2], exercise-tests designed to assess this balance in athletes or patients referred for metabolic diseases have only recently been developed [3-4]. Since low intensity training increases lipid oxidation [5-6] while high intensity training improves the ability to oxidize carbohydrates (CHO) [7-8], this latter effect being reversed by overtraining [7], the individual assessment of this balance of substrates used at exercise may provide an evaluation of the metabolic effects of training protocols designed for improving lipid oxidation [4,6,9].

However, the reason for the inter-individual variability of these parameters remains poorly understood [10-13]. Clearly, energetic pathways favored by specific training programs may be markedly different among sports, some of them involving endurance activity and thus lipid oxidation and other ones involving mostly CHO oxidation. Thus, this study aimed at clarifying this issue by comparing selected groups of subjects submitted to different training protocols, in order to define which, if any, is the ‘specific metabolic profile’, in terms of balance of substrates, of each of these groups.

Research Design and Methods

Subjects

Subjects used in this study were 90 trained athletes: 28 cyclists, 32 male soccer players, 19 male rugby players, 11 rugby by female players (national level in soccer and male rugby and regional level in cyclism and female rugby) and 41 healthy sedentary volunteers. The subjects were checked to be on good health and were free of medication. Written informed consent if any, is the ‘specific metabolic profile’, in terms of balance of substrates, of each of these groups.

**Values are expressed as mean ± SEM; BMI = Body Mass Index; a Units: VO₂max, ml/min/kg body weight. b Units: %VO₂max**

<table>
<thead>
<tr>
<th>Source</th>
<th>Cyclists</th>
<th>Soccer players</th>
<th>Rugby players</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number</td>
<td>19</td>
<td>22</td>
<td>22</td>
</tr>
<tr>
<td>Age (yr)</td>
<td>30.87 ± 1.86</td>
<td>24.34 ± 0.63</td>
<td>25.91 ± 1.25</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>79.91 ± 1.26</td>
<td>73.59 ± 1.04</td>
<td>104.45 ± 2.43</td>
</tr>
<tr>
<td>Height (cm)</td>
<td>177.98 ± 0.28</td>
<td>177.56 ± 0.59</td>
<td>188.09 ± 2.05</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>22.73 ± 0.33</td>
<td>22.86 ± 0.50</td>
<td>24.56 ± 0.52</td>
</tr>
<tr>
<td>Waist circumference</td>
<td>79.06 ± 1.46</td>
<td>79.4 ± 1.30</td>
<td>82.05 ± 1.45</td>
</tr>
<tr>
<td>Hip circumference</td>
<td>94.70 ± 0.99</td>
<td>94.50 ± 1.19</td>
<td>108.30 ± 1.47</td>
</tr>
<tr>
<td>Overtraining score</td>
<td>10.07 ± 0.71</td>
<td>11.48 ± 0.48</td>
<td>15.50 ± 0.84</td>
</tr>
<tr>
<td>VO₂max</td>
<td>47.20 ± 3.54</td>
<td>47.00 ± 3.76</td>
<td>48.70 ± 1.42</td>
</tr>
<tr>
<td>MPO mg/kg</td>
<td>3.56 ± 0.81</td>
<td>4.65 ± 0.04</td>
<td>4.02 ± 0.26</td>
</tr>
<tr>
<td>LIPOXmax</td>
<td>47.00 ± 3.54</td>
<td>47.00 ± 3.76</td>
<td>48.70 ± 1.42</td>
</tr>
<tr>
<td>Crossover point</td>
<td>21.13 ± 1.00</td>
<td>21.13 ± 1.00</td>
<td>21.13 ± 1.00</td>
</tr>
</tbody>
</table>

All sportsmen answered a standardised questionnaire designed to assess this balance in athletes or patients referred for metabolic diseases have only recently been developed [3-4]. Since low intensity training increases lipid oxidation [5-6] while high intensity training improves the ability to oxidize carbohydrates (CHO) [7-8], this latter effect being reversed by overtraining [7], the individual assessment of this balance of substrates used at exercise may provide an evaluation of the metabolic effects of training protocols designed for improving lipid oxidation [4,6,9].

Values are expressed as mean ± SEM; BMI = Body Mass Index; * Units: VO₂max, ml/min/kg body weight. ** Units: %VO₂max

**Table 2:** Clinical characteristics of the 60 control subjects of the study.

<table>
<thead>
<tr>
<th>Source</th>
<th>Males</th>
<th>Females</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number</td>
<td>20</td>
<td>20</td>
</tr>
<tr>
<td>Age (yr)</td>
<td>30.13 ± 4.29</td>
<td>25.43 ± 1.27</td>
</tr>
<tr>
<td>Height (cm)</td>
<td>173.57 ± 3.02</td>
<td>164.78 ± 1.82</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>21.22 ± 1.30</td>
<td>21.86 ± 0.24</td>
</tr>
<tr>
<td>Waist circumference</td>
<td>79.38 ± 2.58</td>
<td>68.22 ± 1.44</td>
</tr>
<tr>
<td>Hip circumference</td>
<td>93.30 ± 0.96</td>
<td>90.69 ± 0.46</td>
</tr>
<tr>
<td>VO₂max</td>
<td>47.00 ± 3.54</td>
<td>48.00 ± 3.76</td>
</tr>
<tr>
<td>Crossover point</td>
<td>21.13 ± 1.00</td>
<td>21.13 ± 1.00</td>
</tr>
</tbody>
</table>

Prior to the exercise-test, subjects’ body composition was assessed with bioimpedance analysis with a six terminal imped-
Exercise Testing

The subjects performed an exercise testing on an electromagnetically braked cycle ergometer (Ergoline Bosch 500) connected to a breath by breath device (COSMED Quark CPET) for gas exchange measurements. The theoretical maximal aerobic power (Wmax th) was calculated for all sportsmen using Wasserman’s equations [18]. After an overnight fast, at 9 am, subjects underwent a standardized submaximal exercise-test [4-6,9] consisting of five 6-min submaximal steady-state workloads, with calculation of carbohydrate and lipid oxidation rates from gas exchange measurements at steady state at the 5th-6th min of every step according to the nonprotein respiratory quotient technique [19]. After smoothing of the curves, we calculated two parameters representative of the balance between fat and carbohydrate oxidation: the crossover point [20-22] which is the point where carbohydrate becomes the predominant fuel representing more than 70% of the total energy [20] and the point where lipid oxidation reaches a maximum [4-6,9,19].

Validity and reproducibility of this test were assessed in a previous publication. Coefficients of variation (CV) were calculated for RER, LIPOXmax and COP at four different intensities. CV of RER were between 2.8 and 4.75%. CV of LIPOXmax and COP was respectively 11.41% Pmax and 11.63%Pmax. VO2, CO2, RER, CHO and lipid oxidation rates were also compared during the incremental test and during single steady-state workloads of the same intensity performed at random order. These parameters were not significantly different.

Statistical Analysis

Significant differences among the various groups were determined with the non-parametric ANOVA (Kruskall-Wallis test). For all statistical analyses, values were expressed as mean (±SEM) and significance was accepted at p<0.05. All calculations were performed with the Sigmasstat package (Jandel Scientific, Erkrath, Germany).

Results

Characteristics of patients are shown on tables 1 and 2, which show that there were no significant differences for age among the studied groups. Male rugby players had a higher weight and higher waist and hip circumference than both control males and other athletes. All subjects had normal blood pressure at rest and exhibited no abnormality during exercise and recovery. When expressed as raw power values, the Power of maximal lipid oxidation and the crossover point ranked as follows: male rugby players > cyclists > male controls > female rugby players > female controls > male soccer players (Figure 1). When they were expressed as percentages of theoretical maximal power this ranking became: cyclists > male rugby players > male rugby players > female controls > male controls > soccer players (Figure 2). Raw lipid oxidation rates at the level of the Power of maximal lipid oxidation ranked as follows (Figure 3): male rugby players > cyclists > female rugby players > sedentary male controls > soccer. If lipid oxidation is expressed per kg of body weight this ranking becomes: cyclists > male rugby players > female rugby players > sedentary female controls > sedentary male controls > soccer players (Figure 4).

Figure 1: Comparison of the power at which occur the crossover point and the Power of maximal lipid oxidation in control subjects and in various groups of athletes. *p<0.05; **p<0.0001 (male athletes vs. male control subjects); ##p<0.0001 (male rugby players vs. soccer players); ∆∆p<0.0001 (cyclists vs. soccer players); ◊p<0.05 (female rugby players vs. female control subjects); ++p<0.0001 (female rugby players vs. male rugby players).

Figure 2: Comparison of the crossover point and the Power of maximal lipid oxidation, expressed in % of Wmax, in control subjects and in various groups of athletes. *p<0.05; **p<0.0001 (male athletes vs. male control subjects); ##p<0.0001 (male rugby players vs. soccer players); ∆∆p<0.0001 (cyclists vs. soccer players); ◊◊p<0.0001 (female rugby players vs. female control subjects); ++p<0.0001 (female rugby players vs. male rugby players).

Figure 3: Lipid oxidation rates in control subjects and in various groups of athletes, expressed in mg/min. **p<0.0001 (male athletes vs. male control subjects); ##p<0.0001 (male rugby players vs. soccer players); ∆∆p<0.0001 (cyclists vs. soccer players); ◊◊p<0.0001 (female rugby players vs. female control subjects); ++p<0.0001 (female rugby players vs. male rugby players).
intensity exercise, respiratory gases are mostly a reflect of the measured by stable isotope labelling [27]. Clearly, even at high [26], so that these calculations closely predict oxidation rates in CO2 has no measurable effect on calorimetric calculations production which can be assumed to interfere with the calcu- perform above the lactate threshold, there is an extra CO2 etry [13]. The theoretical concern was that, when exercise is to support the validity of such protocols of exercise calorimetrically. In this case it can be rapidly performed after a short duration steady-state workloads [7-8,24] we devel- patients [4,23]. Based on our previous studies on calorimetry the balance of substrates at exercise in either athletes [10] or for oxidation during muscular activity, this pattern may reflect the exercise [23] both increase the ratio between CHO and fat used for oxidation during muscular activity, this pattern may reflect the exercise [23] both increase the ratio between CHO and fat used since exercise training at high intensity [7-8] and intermittent exercise [23] both increase the ratio between CH0 and fat used for oxidation during muscular activity, this pattern may reflect a peak value of fat oxidation at 230 mg/min and 300 mg/min). Romijn [32] reported a level of maximal lipid oxidation at 40.1% of VO2max in women and 35.9 % in men (with respectively a peak value of fat oxidation at 200 mg/min and 310 mg/min). In long duration steady-state workloads [7-8,24] we developed a test [4] consisting of five 6-min submaximal steps, in which a steady-state for gas exchanges was obtained during the 2 last minutes. Actually, there is now a large body of literature to support the validity of such protocols of exercise calorimetry [13]. The theoretical concern was that, when exercise is performed above the lactate threshold, there is an extra CO2 production which can be assumed to interfere with the calculations [25]. In fact, below 75% of the VO2max, this increase in CO2 has no measurable effect on calorimetric calculations [26], so that these calculations closely predict oxidation rates measured by stable isotope labelling [27]. Clearly, even at high intensity exercise, respiratory gases are mostly a reflect of the balance of substrate oxidation. Even more, despite the theoretical uncertainty about the stability of gas exchanges during short bouts, protocols of graded exercise calorimetry with only 3-min duration steps have been carefully validated and successfully used [3,11,28], further supporting the accuracy of our protocols based on 6-min steps. Interestingly, results given by the 3-min protocol and results given by the 6-min protocol are very similar, if one compares the description of the balance of substrates in endurance athletes in the current study and to that of Achten’s previous paper [10]. On the whole, we think that graded exercise-tests for exercise calorimetry can nowadays be considered as validated. Two findings suggest that such explorations may provide useful information in sports medicine. First, the fact that training markedly influences the balance of substrates as assessed with this method [5-6,9]. Then, the fact that there are very different patterns of substrate oxidation among athletes, which seem to indicate that there are to some extent specific metabolic profiles.

The finding of a high ability to oxidize lipids in athletes submitted to regular endurance training, like cyclists, is consistent with previous literature [10]. There are some papers assessing the balance of substrates in healthy subjects, for example Haufe [29] reports a level of maximal lipid oxidation at 43% of VO2max in women and 42% in men (with respectively a peak value of fat oxidation at 230 mg/min and 300 mg/min). Similarly Bogadanis [30] reports a level of maximal lipid oxidation at 40.1% of VO2max in women and 35.9 % in men (with respectively a peak value of fat oxidation at 200 mg/min and 310 mg/min). In trained endurance athletes the level of maximal lipid oxidation is generally found to be higher, at 63-65 % of VO2max [3,31]. More recently Gonzalez-Haro [32] reported a level of maximal lipid oxidation at 52% of VO2max in male road cyclists (with a peak value of fat oxidation at grossly 400 mg/min). Romijn [33] reported in such athletes a level of maximal lipid oxidation ranging between 57 and 75 % of VO2max. Most of these papers employ Achten’s protocol [3] with 3 min steps, which in our experience slightly overestimates exercise lipid oxidation levels [34], but it is clear that a large body of evidence shows that endurance athletes oxidize more lipids at exercise and that this oxidation peaks at a higher %VO2max than sedentary subjects.

By contrast, it is interesting to notice in soccer players, a pattern of “glucodependence” that implies a reduced reliance on lipids at exercise. Although in our study we can only present data on soccer, this pattern is likely to occur in several sports. Since exercise training at high intensity [7-8] and intermittent exercise [23] both increase the ratio between CH0 and fat used for oxidation during muscular activity, this pattern may reflect an adaptation of muscle metabolism to short repeated bouts of high intensity. Daussin [35] has shown that 5 sessions per week of high intensity training increases the ability to oxidize carbohydrates with molecular adaptations at the mitochondrial level. Interestingly, such a “glucodependence” is also found in obesity [4] and type 2 diabetes [36]. In this case it can be rap-
idly reversed by a few weeks of targeted exercise training at the level of the Power of maximal lipid oxidation [5-6, 37]. A recent randomized control trial on obese patients has shown that endurance training targeted at the level of maximal oxidation strongly increases the maxima lipid oxidation rate at exercise [38]. Since physical inactivity rapidly shifts the balance of substrates at rest towards a lower ratio of lipid/CHO used for oxidation [39] it can be assumed that sedentarity explains at least in part the glucodependence of these patients, while endurance training explains a reversal of this profile toward more lipid oxidation. Bruce [40] has shown that 5 sessions of one hour per week of endurance training in obese subjects induces a twofold increase in mitochondrial fat oxidation and a four-fold increase in carnitine palmitoyl transferase 1 (CPT1) activity.

Bruce [40] and Sahlin [41] have demonstrated that exercise-induced lipid oxidation is a reflect of mitochondrial fat oxidation. Accordingly, in a sample of diabetics we reported [42] that low intensity endurance training twice a week improves lipid oxidation during exercise parallel to an increase in mitochondrial fat oxidation. Therefore the bell-shaped curve of lipid oxidation at exercise is a reflect of the potential of fat oxidation by mitochondria in (mostly type-I) muscle fibers.

Although not tightly matched for age, all athletes were between 20-35 years old and thus age is not likely to interfere with our results. By contrast, there are differences in body composition, since male rugby players are both heavier and taller than the other athletes and exhibit a higher BMI and waist circumference. Actually, we found no correlation in our sample of athletes between these markers of body composition and the balance of substrates. Although it is clear that adiposity in sedentary subjects is associated with an alteration in muscular substrate metabolism, this relationship does not clearly appear in athletes. Moreover, sedentary subjects with a higher BMI exhibit a lower ability to oxidize lipids at exercise when explored by exercise calorimetry [4,9] while in this study male rugby players have both a higher BMI and a maximal lipid oxidation occurring at a higher power intensity. Thus, it is not likely that our results are explained by a higher adiposity.

Actually, concerning the ability to oxidize lipids, there is also a wide range of variability among the populations studied here, as already reported by the team of Jeukendrup [10-11, 28]. These authors found an influence of two factors: fat-free mass and gender [11]. The repeatedly reported influence of gender [43-44] is not significant in our study, but Figure 2 shows that the shift towards CHO seems to occur at a slightly higher level in female than male subjects, either for sedentary controls or for rugby players. However, the magnitude of this difference does not seem to be very important, in comparison to the specific pattern of each sport, and to the marked increase in lipid oxidation induced by training in obese adults [5-6, 9, 45]. Our maximal lipid oxidation rates close from 500 mg/min in male rugby players and cyclists are consistent with previous reports [10] of a value of 1000 mg/min on treadmill, reduced by 50% during cycling [46]. However, there are also groups of trained subjects exhibiting a markedly lower ability to oxidize lipids, suggesting that beside the already reported factors that are gender [11-12, 43-44] fat-free mass [10-12, 28] and the training status [47], the variety of sport is probably an important determinant of the ability to oxidize lipids during exercise. Besides, the aging-induced decline in CHO oxidation [8] does not appear in this study on young, fit subjects. Whether our findings can be extended to larger populations of athletes trained for cycling, rugby or soccer remains of course to be demonstrated. Presumably, various training regimens may induce a different profile. We have observed that some soccer players that had initially a high ability to oxidize lipids exhibited a progressive shift toward glucodependence when submitted to the same training procedure as the others of the team presented here. Since the balance of substrates appears to be training-sensitive [6-9, 24, 45], it is likely that changes in training protocol will modify the profiles presented here. In addition, overarching may reverse some training-induced modifications of the balance of substrates, as demonstrated in a follow-up study of cyclists [24].

In summary, our study shows that in some athletes, like the soccer players studied here, there is a physiological pattern of balance of substrates at exercise that mimics the “glucodependence” already reported in obese and type-2 diabetic patients. The mechanism of this adaptation remains to be further studied. However, this finding further suggests that the balance of substrates assessed with exercise calorimetry is a rather flexible physiological characteristics of an individual which may be interesting to explore in athletes submitted to various training protocols.

Acknowledgments

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