Crossover and maximal fat-oxidation points in sedentary healthy subjects: Methodological issues

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Received 3 April 2011; received in revised form 7 July 2011; accepted 10 July 2011
Available online 25 September 2011

Abstract

Aim. – Our study aimed to assess the influence of protocol on the crossover point and maximal fat-oxidation (LIPOX_max) values in sedentary, but otherwise healthy, young men.

Methods. – Maximal oxygen intake was assessed in 23 subjects, using a progressive maximal cycle ergometer test. Twelve sedentary males (aged 20.5 ± 1.0 years) whose directly measured maximal aerobic power (MAP) values were lower than their theoretical maximal values (tMAP) were selected from this group. These individuals performed, in random sequence, three submaximal graded exercise tests, separated by three-day intervals; work rates were based on the tMAP in one test and on MAP in the remaining two. The third test was used to assess the reliability of data. Heart rate, respiratory parameters, blood lactate, the crossover point and LIPOX_max values were measured during each of these tests.

Results. – The crossover point and LIPOX_max values were significantly lower when the testing protocol was based on tMAP rather than on MAP (P < 0.001). Respiratory exchange ratios were significantly lower with MAP than with tMAP at 30, 40, 50 and 60% of maximal aerobic power (P < 0.01). At the crossover point, lactate and 5-min postexercise oxygen consumption (EPOC5min) values were significantly higher using tMAP rather than MAP (P < 0.001). During the first 5 min of recovery, EPOC5min and blood lactate were significantly correlated (r = 0.89; P < 0.001).

Conclusion. – Our data show that, to assess the crossover point and LIPOX_max values for research purposes, the protocol must be based on the measured MAP rather than on a theoretical value. Such a determination should improve individualization of training for initially sedentary subjects.

Keywords: Measured maximal aerobic power; Theoretical maximal aerobic power; Crossover point; Maximal fat oxidation; Metabolic evaluations; Obesity

Résumé

Détermination des points de croisement métabolique et d’oxydation maximale des lipides chez des sujets sédentaires sains : approche méthodologique.

Objectif. – L’objectif de notre étude était d’évaluer l’effet du protocole sur les points de croisement métabolique (COP) et d’oxydation maximale des lipides (LIPOX_max) chez des sujets sains mais sédentaires.

Méthodes. – Vingt-trois sujets ont réalisé initialement un test maximal progressif de mesure de la \( V_O_{2_{max}} \). Nous avons sélectionné parmi eux 12 hommes sédentaires âgés de 20,5 ± 1,0 ans ; ils présentaient une puissance maximale aérobie mesurée (PMA) inférieure à la PMA théorique (PMA théo). Ils ont réalisé ensuite dans un ordre aléatoire trois épreuves submaximales, séparées par un intervalle de trois jours : un protocole fondé sur la PMA théo et deux autres fondés sur la PMA mesurée. Le troisième test était réalisé pour étudier le critère de reproductibilité. La lactatémie, la fréquence cardiaque, les variables respiratoires, les points de croisement glucido-lipidique (COP) et d’oxydation maximale des lipides (LIPOX_max) ont été mesurés au cours des trois protocoles.

Résultats. – Les valeurs moyennes de COP et de LIPOX_max étaient significativement plus basses au cours du protocole fondé sur la PMA théo que celles obtenues avec le protocole fondé sur la PMA mesurée (P < 0.001). Les valeurs du quotien respiratoire à 30, 40, 50 et 60 % de la PMA étaient significativement plus basses avec le protocole fondé sur la PMA mesurée (P < 0.01). Au COP, les valeurs de lactates et celles de la consommation d’oxygène mesurées au cours des cinq minutes de récupération (EPOC5min) étaient significativement plus élevées avec le protocole...
fondé sur la PMA théo ($P<0.001$). Il existait une corrélation significative entre l’EPOC-5 min et les concentrations de lactates mesurées au cours de la récupération ($r=0.89; P<0.001$).

**Conclusion.**—Lors de l’évaluation de COP et LIPOX_{max}, nous recommandons de fonder le protocole sur la mesure de la PMA plutôt que sur sa valeur théorique. Cela permet d’individualiser les programmes d’entraînement en fonction de l’aptitude réelle du sujet.

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**Mots clés :** Puissance maximale aérobie mesurée ; Puissance maximale aérobie théorique ; Point de croisement métabolique ; Oxydation maximale des lipides ; Évaluations métaboliques ; Obésité

1. **Introduction**

Submaximal steady-state cycle ergometer tests are commonly used to assess two important metabolic indices: the crossover point (COP), the intensity of effort at which energy is derived more from carbohydrate (CHO) than from fat metabolism [1]; and the point of maximal fat oxidation (LIPOX_{max}) [2]. Such data are of particular interest in patients with metabolic defects, such as obesity [3–5] and type 2 diabetes [6,7], but may also be useful when prescribing exercise and developing individualized training programmes for healthy subjects [8,9]. The corresponding intensities of effort can be expressed as either heart rate or power output values [10,11].

A variety of test protocols have been proposed to assess patterns of substrate oxidation during moderate-intensity exercise [2,3]. Usually, the work rates for a progressive exercise test have been set, using the equations of Wasserman et al. [12] to predict the individual’s theoretical maximal aerobic power (tMAP). However, a few authors have determined appropriate increments of work rate using the individual’s directly measured maximal aerobic power (MAP). Nevertheless, the individual data showed disagreement between protocols, and increments of work rate were larger for the tMAP protocol.

As metabolic testing is generally applied to relatively sedentary subjects, in cases of MAP < tMAP, there is clearly a need to establish the validity of the predictive approach in such a population. Thus, the COP and LIPOX_{max} values, using tMAP and MAP protocols, were compared in a group of untrained subjects who had a MAP that was significantly lower than their tMAP.

2. **Material and methods**

2.1. **Subjects’ characteristics**

After receiving a description of the protocol, risks and benefits of the study, all participants gave their written consent to a protocol approved by the Ethics Committee of the Faculty of Medicine of Sousse (Tunisia). Twelve sedentary, but otherwise healthy, male students were selected from an initial pool of 23 individuals on the basis of having a MAP < tMAP. All were also non-smokers, and none abused alcohol; their other characteristics are summarized in Tables 1 and 2.

2.2. **Anthropometric measurements**

Standing height and body mass were measured using standard techniques with a variability of 0.2 kg and 5 mm, respectively. Skinfold thickness was determined in triplicate at four standard sites (biceps, triceps, subscapular and suprailiac), using a recently calibrated Harpenden caliper (Holtain Instruments, Crosswell, Pembrokeshire, UK). The mean of the three values was recorded for each site.

Body density was calculated according to the equations of Durnin and Wormersley [15] for men aged 20–65 years: body density = $1.1765-0.0744 \times (\log_{10} \Sigma S)$—where $\Sigma S$ is the sum of the four skinfold readings (in mm)—and body fat = $(4.95/D-4.50) \times 100$—where $D$ is the body density as estimated from the summmed skinfolds.

Body mass index (BMI) was calculated as body mass divided by height squared (kg/m²).

2.3. **Experimental design**

Testing was undertaken consistently between 0800 h and 1030 h in the morning after a 12-h fast. At the first visit, maximal oxygen intake ($\dot{V}O_2\text{max}$) and MAP were determined for all 23 subjects, using a progressive maximal cycle ergometer test (Monark Ergometer 894E, Vansbro, Sweden). Twelve of the 23 had a measured MAP lower than the tMAP predicted by the equations of Wasserman et al. [12]. These 12 individuals performed three submaximal exercise tests in random sequence separated by three-day intervals. For the tMAP test, work rates were set at 20, 30, 40, 50 and 60% of the tMAP calculated from Wasserman et al.’s equations [12]. For the other two tests, work rates were set at 20, 30, 40, 50 and 60% of the previously measured MAP. The COP and LIPOX_{max} were estimated for each test, tMAP values were compared with values from the first MAP trial and reliability was assessed by comparing the two MAP tests.

2.4. **Progressive maximal exercise test**

The progressive maximal cycle ergometer test followed the protocol of Hansen et al. [16], with measurement of the ventilatory threshold ($\dot{V}T$), $\dot{V}O_2\text{max}$ and MAP. A pedal frequency of 60 rpm was paced by metronome, and the belt load was increased progressively every minute. The test was stopped when one or more of the following criteria were met: attainment of a $\dot{V}O_2\text{max}$ plateau < 2.2 mL·kg\(^{-1}\)·min\(^{-1}\); respiratory exchange ratio (RER) > 1.10; maximal heart rate ($HR_{\text{max}}$) close to the theoretical value of 220–age (in years); or the subject was
2.5. Submaximal graded exercise tests

The submaximal exercise tests followed the protocol of Brandou et al. [3]. After a 3-min rest period, subjects underwent five bouts of submaximal exercise, each lasting for 6 min and corresponding to 20, 30, 40, 50 and 60%, respectively, of the individual’s tMAP or MAP [3]. Further data were collected over a 5-min period of passive recovery.

2.6. Physiological parameters

Metabolic responses were assessed using a breath-by-breath analysis system (Quark b2, COSMED, Rome, Italy), which measured $\dot{V}O_2$ and $\dot{V}CO_2$ (in mL min$^{-1}$) and calculated the non-protein respiratory gas-exchange ratio (RER = $\dot{V}CO_2/\dot{V}O_2$). The VT was determined according to the method of Beaver et al. [17].

Samples of arterialized capillary blood were collected after gentle fingertip massage. Lactate concentrations were determined after 3 min of passive recovery using a Lactate Pro analyzer (Arkray Inc., Kyoto, Japan).

2.7. Substrate oxidation during submaximal exercise

CHO and lipid oxidation rates were calculated from gas-exchange measurements, using the non-protein respiratory quotient (RER) technique [18], wherein $CHO = 4.585 \times VCO_2 - 3.2255 \times VO_2$, and lipids $= -1.7012 \times VCO_2 + 1.6946 \times VO_2$, where $VO_2$ and $VCO_2$ (mL min$^{-1}$) were mean values for the final 3 min of each exercise stage.

After smoothing the curves, using software developed by the Montpellier team, the absolute power outputs (W) corresponding to two parameters were determined: the substrate metabolic COP beyond which CHO utilization predominates [1]; and the point of LIPOX$\text{max}$ [2]. The fat-oxidation rate was calculated as $-1.7012 \times VCO_2 + 1.6946 \times VO_2$, which can be simplified to $1.7 (1-\text{RER}) \times VO_2$.

In other words, the fat-oxidation rate is reflected by two different linear relationships: a progressive decrease ($1-\text{RER}$); and a linear increase of $\dot{V}O_2$ as power output is increased. Fat oxidation is maximal when the solution to the second equation yields a value of zero.

2.8. Calculation of excess postexercise oxygen consumption (EPOC$\text{5min}$)

Excess postexercise oxygen consumption during the first 5 min of passive recovery (EPOC$\text{5min}$; in L) was computed by trapezoidal integration. The resting $\dot{V}O_2$ was subtracted from the measured $\dot{V}O_2$ at each 5-s interval, and the EPOC$\text{5min}$ was calculated according to the formula:

$$EPOC(l) = \frac{t_n - t_0}{2n} \left[ f(t_0) + f(t_n) + 2 \sum_{k=1}^{n-1} f(t_k) \right]$$

where $t_0$ = the peak of $\dot{V}O_2$ at the end of the exercise, $t_n$ = the final $\dot{V}O_2$ at the end of the 5-min recovery period and $n$ = the number of 5-s periods during the 5-min recovery period.

2.9. Statistical analysis

Data analyses were performed using SPSS version 13 for Windows® (SPSS Inc, Chicago, IL, USA). The normality of the data was checked using Kolmogorov–Smirnov statistics. Means and standard deviations (SD) were calculated for all variables. Student’s $t$ test for dependent samples evaluated the effect of the protocol (tMAP vs MAP) on the two metabolic indices (COP and LIPOX$\text{max}$), EPOC$\text{5min}$ and blood lactate concentrations, and the tMAP and MAP protocols were further compared using Bland–Altman plots [19]. Relationships between selected parameters were tested by calculation of Spearman’s correlation coefficients. Statistical significance was fixed at $P<0.05$ throughout.

3. Results

The physical characteristics of the present study subjects are summarized in Tables 1 and 2. The observed values for HR$\text{max}$, RER and blood lactate show that all the participants made good

Table 1

<table>
<thead>
<tr>
<th>Physical characteristics of the subjects ($n=12$).</th>
<th></th>
</tr>
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<tbody>
<tr>
<td>Age (years)</td>
<td>Height (m)</td>
</tr>
<tr>
<td>20.5 ± 1.0</td>
<td>1.82 ± 0.1</td>
</tr>
</tbody>
</table>

Data are expressed as means ± SD; BMI: body mass index; tMAP: theoretical maximal aerobic power; MAP: maximal aerobic power; W: watts.

Table 2

<table>
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<tr>
<th>Peak values obtained during testing of maximal aerobic power.</th>
<th></th>
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<tbody>
<tr>
<td>$n$</td>
<td>HR$\text{max}$ (bpm)</td>
</tr>
<tr>
<td>12</td>
<td>197 ± 6</td>
</tr>
</tbody>
</table>

Data are expressed as means ± SD; HR$\text{max}$: maximal heart rate; RER$\text{max}$: maximal respiratory exchange ratio; L$\text{a}\text{max}$: maximal blood lactate concentration; $\dot{V}O_2\text{max}$: maximal oxygen intake.
maximal efforts (Table 2), although their absolute $\dot{V}O_2^{\text{max}}$ values were relatively low for young men. In confirmation of this, tMAP values estimated by the equations of Wasserman et al. [12] were substantially larger than the directly measured MAP values ($276 \pm 54$ W vs $219 \pm 39$ W, respectively; $P < 0.001$). However, the directly measured $\dot{V}O_2^{\text{max}}$ was 8.5% higher than the predicted value in our subjects.

Because of this difference, COP and LIPOX$_{\text{max}}$ values (Fig. 1A and B, respectively) were significantly lower with the tMAP rather than MAP protocol ($P < 0.001$). However, the respiratory gas-exchange ratios were significantly lower for the MAP vs tMAP protocol at 30, 40, 50 and 60% of maximal aerobic power ($P < 0.001$). Bland–Altman plots revealed the systematic errors of the tMAP for COP, LIPOX$_{\text{max}}$ and RER values; bias ± random error values were $12.9 \pm 16.3$ W for COP, $18.1 \pm 12.7$ W for LIPOX$_{\text{max}}$ and $-0.037 \pm 0.08$ for RER (Fig. 2A–C). Blood lactate concentrations sampled during the third minute of recovery and the EPOC$_{\text{5min}}$ values were both significantly higher with the tMAP vs MAP protocol ($P < 0.001$; Figure S1 A and S1 B respectively) (see supplementary material associated with this article online), and the EPOC$_{\text{5min}}$ was linearly related to lactate recovery ($r = 0.89; P < 0.001$; Fig. 3).

When using the MAP protocol, COP and LIPOX$_{\text{max}}$ measurements showed relatively low coefficients of variation (CV = 6.0% and 5.0% for COP and LIPOX$_{\text{max}}$, respectively), although both fell well within the commonly accepted 10% criterion of absolute reliability (Table S1 see supplementary material associated with this article online). Intraclass correlation coefficient (ICC) values ranged from 0.967 for COP to 0.978 for LIPOX$_{\text{max}}$; again, both data sets amply met the commonly accepted reliability criterion of 0.90.

4. Discussion

Directly measured MAP values were substantially lower than the corresponding tMAP in the present group of sedentary,
but otherwise healthy, young men, and the COP and LIPOXmax were reached at lower intensities of effort with tMAP than with MAP protocols. In agreement with our observations, Aucouturier et al. [14] found little agreement between datasets when LIPOXmax values expressed as a percentage of the directly measured VO2max were compared with values expressed as a percentage of the predicted VO2max; whether estimated according to American College of Sports Medicine (ACSM) guidelines [20] or using the predictive equations of Wasserman et al., these predictions underestimated directly measured values by an average of 6% and 14%, respectively. According to Aucouturier et al. [14], acceptance of an estimated VO2max led to wide interindividual variations (33–74% VO2max) and significant underestimation of the LIPOXmax value [14]. In contrast, Michallet et al. [13] found no significant differences in either COP or LIPOXmax values according to protocol. They were, however, testing relatively fit subjects whose MAP was > tMAP. Nevertheless, even they observed large intraindividual variations, and agreed that the optimal approach to the assessment of COP and LIPOXmax was to measure MAP directly. Of course, errors in the determination of the COP and LIPOXmax values can have substantial practical implications when assessing an individual’s metabolic response to any given intensity of exercise.

For years, the Montpellier laboratory has studied metabolic responses using five 6-min steady-state bouts of exercise set at 20% through to 60% of the individual’s tMAP calculated from the prediction equations of Wasserman et al. [12], applying such tests with apparent success in both healthy and pathological subjects, with the main goal of making simple clinical evaluations. The present study suggests the need for caution if such an approach is adopted, particularly when evaluating individuals who have a MAP < tMAP. In such cases, the intensity of exercise is likely to be significantly lower with the MAP protocol and, although use of the tMAP protocol may serve clinical needs, it is not appropriate for research investigations. Where the tMAP protocol is used, it is suggested that the ergometer loading, based on the patient’s response as observed during testing, be modified, while keeping in mind the need for several stages with an RER both slightly below and slightly above 0.9 to obtain clear definitions of the COP and LIPOXmax. Indeed, Michallet et al. [13] have emphasized that, particularly with fit subjects, test stages set at 20, 30, 40, 50 and 60% of the individual’s tMAP may not satisfy these requirements.

One possible explanation of the problems seen with the tMAP is that the predictive equations of Wasserman et al. [12] were developed and validated in fit healthy subjects. Also, they take into account only the individual’s body mass, which may lead to substantial overestimation of VO2max in overweight and obese subjects. Goran et al. [27] pointed out that the most important variable influencing VO2max is fat-free mass (FFM) rather than total body mass. To avoid any possible effects of increased body fat content or low physical fitness on the determination of metabolic indices, we recommend that, wherever possible, metabolic testing should be based on the directly measured MAP.

Although the EPOC5min and blood lactate recovery concentrations were lower with the MAP protocol, in agreement with previous studies [21,22], there was a close linear relationship between these two values postexercise [23] (r = 0.89; P < 0.001).

One striking feature of our present data was the greater reproducibility of COP and LIPOXmax values compared with some previous reports [9,24,25], with CVs of 6 and 5%, respectively. This may reflect our careful standardization of the test conditions. Observations were always made at the same time of day under carefully controlled conditions of temperature, humidity and aeration. The subjects had also been habituated to the laboratory and its equipment (cycle ergometer and oxygen mask), and all followed a consistent regimen of physical activity and diet, fasting for a minimum of 12 h before testing. Brun et al. [26] recently pointed out that the measurement of LIPOXmax by progressive metabolic testing [3] is potentially reproducible, although it is easily modified by various factors, such as diet, training and previous exercise. Meyer et al. [9] also emphasized the importance of rigorous standardization of procedures to obtain reliable estimates of LIPOXmax. Their Bland–Altman plots showed widely dispersed 95% limits of agreement, and they concluded that the interindividual variability in LIPOXmax values was too large to recommend the use of this parameter when prescribing training intensity. Others have also found a discouragingly large CV even within supposedly homogeneous groups of individuals. Michallet et al. [25] studied the reproducibility of COP and LIPOXmax values in 14 healthy subjects, using two techniques to measure oxygen consumption (a Douglas bag and an Ergocard Medisoft gas analyzer). They found no systematic differences between the two approaches, although the CV was 17% for COP and 8.7% for LIPOXmax. Achten and Jeukendrup [24] have reported a CV of 9.6% for LIPOXmax.

5. Conclusion

The present study confirms that, in sedentary subjects, a submaximal exercise test based on a directly measured MAP is necessary to obtain accurate estimates of metabolic indices and that this approach is clearly desirable for research protocols.
However, use of the tMAP is simpler and may provide adequate information for routine clinical studies of exercise metabolism.

Disclosure of interest

The authors declare that they have no conflicts of interest concerning this article.

Acknowledgements

The authors are grateful to Prof J. Mercier of the University of Montpellier, who invited us to his laboratory to learn how to use the metabolic tests. We also thank our study subjects for their participation. This study was supported by the Minister of Higher Education and Scientific Research of Tunisia.

Appendix A. Supplementary data

Supplementary data (Fig. S1) (Table S1) associated with this article can be found, in the online version, at doi:10.1016/j.diabet.2011.07.004.

References