Methodological aspects of crossover and maximum fat-oxidation rate point determination

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Abstract

Aim. – Indirect calorimetry during exercise provides two metabolic indices of substrate oxidation balance: the crossover point (COP) and maximum fat oxidation rate (LIPOXmax). We aimed to study the effects of the analytical device, protocol type and ventilatory response on variability of these indices, and their relationship with lactate and ventilation thresholds.

Methods. – After maximum exercise testing, 14 relatively fit subjects (aged 32 ± 10 years; nine men, five women) performed three submaximum graded tests: one was based on a theoretical maximum power (tMAP) reference; and two were based on the true maximum aerobic power (MAP). Gas exchange was measured concomitantly using a Douglas bag (D) and an ergospirometer (E).

Results. – All metabolic indices were interpretable only when obtained by the D reference method and MAP protocol. Bland and Altman analysis showed overestimation of both indices with E versus D. Despite no mean differences between COP and LIPOXmax whether tMAP or MAP was used, the individual data clearly showed disagreement between the two protocols. Ventilation explained 10–16% of the metabolic index variations. COP was correlated with ventilation (r = 0.96, P < 0.01) and the rate of increase in blood lactate (r = 0.79, P < 0.01), and LIPOXmax correlated with the ventilation threshold (r = 0.95, P < 0.01).

Conclusion. – This study shows that, in fit healthy subjects, the analytical device, reference used to build the protocol and ventilation responses affect metabolic indices. In this population, and particularly to obtain interpretable metabolic indices, we recommend a protocol based on the true MAP or one adapted to include the transition from fat to carbohydrate. The correlation between metabolic indices and lactate/ventilation thresholds suggests that shorter, classical maximum progressive exercise testing may be an alternative means of estimating these indices in relatively fit subjects. However, this needs to be confirmed in patients who have metabolic defects.

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Résumé

Aspects méthodologiques de la détermination des points de croisement et d’oxydation maximale lipidique.

Objectifs. – La calorimétrie indirecte d’exercice permet de déterminer deux indices métaboliques qui reflètent la balance d’oxydation des substrats : le point de croisement glucidolipidique (COP) et le point d’oxydation maximale lipidique (LIPOXmax). Nous avons déterminé l’influence du système de mesure des échanges gazeux, du type de protocole et de la réponse ventilatoire sur la variabilité de ces indices. De plus, leur corrélation avec les seuils ventilatoire et lactique a été évaluée.

Méthodes. – Après un test d’exercice maximum, 14 sujets en bonne condition physique ont réalisé trois tests sous-maximaux : l’un fondé sur la puissance maximale théorique (tMAP) et deux sur la puissance maximale réelle (MAP). Les gaz ont été analysés simultanément par la méthode des sacs de Douglas (D) et sur ergospiromètre automatisé (E).

Résultats. – Seules les données déterminées avec D et le protocole MAP sont toutes interprétables. L’analyse de Bland et Altman montre une surestimation des deux indices par E comparativement à D. Bien que les valeurs moyennes de COP et LIPOXmax ne diffèrent pas suivant la

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1. Introduction

Regular endurance training has been shown to favorably modify the balance of substrate oxidation, especially in patients with metabolic defects such as obesity [1] and type 2 diabetes [2]. Indeed, exercise training helps to restore fat oxidation capacity [3], which can be linked to improvement in insulin sensitivity [2,4,5]. Moreover, fat oxidation is also related to exercise capacity in athletes [6–8]. Therefore, the follow-up of substrate oxidation – particularly fat oxidation – capacity during exercise is relevant.

Given its advantages, indirect calorimetry remains the most commonly used technique for assessing the balance between fat and carbohydrate (CHO) oxidation during exercise. During progressive exercise, it provides two simple indices: the crossover point of substrate utilization (COP) [9] and the maximum fat oxidation rate point (LIPOXmax) [8]. These indices can be obtained from gas exchange during submaximum graded exercise up to 60% of the theoretical maximum aerobic power (tMAP) [3], and are useful for prescribing an individualized exercise programme [10] in those with metabolic diseases such as obesity and type 2 diabetes. Indirect calorimetry can also be used to accurately determine fat and CHO oxidation at exercise intensities of up to 75% of maximum oxygen consumption (VO2peak) [8]. Although Perez-Martin et al. [10] confirmed COP and LIPOXmax reliability (test–retest), in 10 healthy subjects, there have been no systematic studies of the validity of the COP and LIPOXmax indices.

Several factors can influence these indices and increase their variability and/or impair their validity even with a controlled diet (test performed after a 12-hour overnight fast). First, variability of gas-exchange determination may be due to the gas-analysis system. Second, the submaximum exercise test that allows determination of these metabolic indices has been validated in patients from a protocol based on the tMAP [10]. However, because the true maximum aerobic power (MAP) is often higher than the tMAP in trained subjects (healthy or not), it may be necessary to study the influence of the reference used to construct the protocol for these indices. Third, abnormally high or low ventilatory responses at rest or during moderate exercise, as seen in severely deconditioned patients or highly endurance-trained athletes, respectively, can modify carbon dioxide (CO2) output, leading to an altered respiratory exchange ratio (RER). This means that both COP and LIPOXmax may potentially be affected, as CO2 output is a component of COP and LIPOXmax determination.

Finally, although the test is submaximum to provide deconditioned patients with more comfortable conditions compared with progressive maximum exercise testing, the trial duration is long (30 min) and may be an obstacle for some. According to Brooks and Mercier [9], the COP may coincide with an increase in blood lactate, suggesting a relationship with the lactate threshold. However, to our knowledge, this possibility has never been verified, and metabolic indices have never been compared with other indices (lactate and ventilatory thresholds) obtained during cardiopulmonary exercise testing.

The aims of this methodological study were first to determine the influences of:

- the gas-analysis system;
- the reference chosen to construct the submaximum graded test protocol;
- the ventilatory response during exercise on the validity and variability of the COP and LIPOXmax.

In addition, these indices were compared with the lactate and ventilatory thresholds obtained during a maximum progressive exercise test.

2. Methods

2.1. Subjects’ characteristics

Fourteen healthy subjects (age: 32 ± 10 years; nine men, five women) who were moderately trained (except for two highly trained men) gave their written informed consent to participate in the study. No subject presented any contraindication to intense and prolonged exercise or severe disease. The participants’ characteristics are presented in Table 1.

Self-reported levels of leisure physical activity (SRPAL) were assessed from their disclosed structured activities (taking into account the intensity). The level of physical activity was calculated as the product of the duration and frequency of each activity (in hours per week), weighted by an estimate of the metabolic equivalent (MET) of that activity, then all activities performed were added up, with the result expressed as the average MET-hours per week for the previous year.
The study was approved by the local ethics committee (Comité de protection des personnes Sud-Est V) and performed according to the Declaration of Helsinki.

2.2. Experimental design

After a clinical examination and a 12-lead resting electrocardiogram (ECG), each subject underwent progressive maximum exercise testing to confirm the absence of cardiac or respiratory pathology and to determine the maximum oxygen consumption ($\dot{V}_{\text{O2peak}}$), and lactate and ventilatory thresholds (Fig. 1). Thereafter, all subjects performed three submaximum graded exercise tests, with each test separated by a three-day interval.

To assess the influence of gas measurements device on the validity and variability of the COP and LIPOXmax, each subject performed a submaximum graded test based on the tMAP. Gas-exchange measurements were performed simultaneously using two methods: the Douglas bag (D) method and the breath-by-breath automated ergospirometer (E; Ergocard, Medisoft, Dinant, Belgium). All subjects then underwent a submaximum graded exercise test based on the MAP to estimate the influence of the protocol on these indices. Finally, to test the influence of ventilation on these indicators and their reliability, all subjects performed a third submaximum graded exercise test based on the MAP.

All submaximum graded tests were performed in the morning after a 12-hour overnight fast, always at the same time (between 08:00 and 10:00 hours) and under identical laboratory conditions (temperature range: 20–21 °C; hygrometry: 25–35%; barometric pressure: 738–750 mmHg). The day before each of these tests, the subjects were asked to keep to the same diet and avoid physical activity.

2.3. Maximum progressive exercise testing

Each subject underwent a continuous maximum progressive test on a cycloergometer (Ergomedic 8/8, Monark, Sweden). The workload increase was 20 W (for men) and 15 W (for women) per minute until exhaustion, following a three-minute warm-up period (40 W for men and 30 W for women). The test was stopped when the following criteria were met:

- $\dot{V}_{\text{O2peak}}$ plateau;
- RER > 1.10;
- maximum heart rate (HRmax) close to the theoretical HRmax (220 – age);
- the subject was exhausted and could no longer maintain the imposed pedaling rate, despite verbal encouragements.

Gas exchange and HR (derived from an ECG; CardioLaptop AT-110, Schiller, Baar, Switzerland) were continuously recorded using an automated computerized analytical system (Ergocard, Medisoft, Dinant Belgium) that was carefully recalibrated before each test.

Microblood samples were taken from the earlobe at rest and during the last 15 s of each exercise stage for whole-blood lactate analysis (Microzym®, BioSenTec, Toulouse, France). Ventilatory and lactate thresholds were determined from graphs:

- the first ventilatory threshold (VT1) was an increase in $\dot{V}_E/\dot{V}_{\text{O2}}$ and PET$_{\text{O2}}$ with no concomitant increase in $\dot{V}_E/\dot{V}_{\text{CO2}}$;
- the first lactate threshold (LT1) was the first marked increase of blood lactataemia (at least 0.5 mmol/L) over resting values.

The exercise intensity at which these parameters occurred was visually identified by two independent, experienced observers. If there was disagreement, the opinion of a third investigator was obtained.

2.4. Submaximum graded exercise testing

All participants were tested after a 12-hour overnight fast. A two-minute recording of resting parameters was made to obtain ventilation and RER stabilization. The continuous exercise test consisted of a three-minute warm-up at 20% of tMAP [10] or MAP, followed by four six-minute steady-state workloads, at

![Fig. 1. Experimental design.]
30, 40, 50 and 60% of the tMAP or MAP. To ensure that the subjects did not hyperventilate before exercise, they were not allowed to start pedalling if their RER was greater than 0.85. HR was recorded throughout the entire test.

To compare the influence of the gas-exchange analytical device, a Pitot pneumotachograph was connected to a unidirectional Rudolph valve. The expiratory port of the Rudolph valve was directed toward a Douglas bag using a low-resistance conducting tube. Expiratory gases were collected during the last 2 min of each exercise stage, and only after the conducting tube had been rinsed to eliminate “dead spaces” (45 s before collection in resting conditions, 15 s before collection during exercise). It was then analyzed for expiratory fractions in O₂ (paramagnetic gas analyzer, Ergocard) and CO₂ (infrared cell gas analyzer, Ergocard), and with a Tissot spirometer for ventilation. The time for gas analysis was 10 s at a sampling rate of 300 mL/min. The final ventilation volume was corrected accordingly.

2.5. Calorimetry

Calculation of CHO and lipid oxidation rates was made from gas-exchange measurements using the nonprotein respiratory quotient technique [11], where:

\[
\text{CHO oxidation rate (mg/min) = } 4.585 \dot{V}_{CO_2} - 3.2255 \dot{V}_{O_2}
\]

Lipid oxidation rate (mg/min)

\[= -1.7012 \dot{V}_{CO_2} + 1.6946 \dot{V}_{O_2}
\]

(with \(\dot{V}_{O_2}\) and \(\dot{V}_{CO_2}\) in mL/min).

\(\dot{V}_{O_2}\) and \(\dot{V}_{CO_2}\) were averaged over the last 2 min of each exercise stage. This technique provided CHO and lipid oxidation rates at different levels of exercise with the assumption that urinary nitrogen excretion rate was negligible. These values were then converted into Kcal, assuming that 1 g of lipid provides approximately 9 kcal, while the oxidation of 1 g of glucose provides only 4 kcal. Hence, the percentage of CHO or lipid participating in the total energy expenditure could be determined. According to the theory proposed by Brooks and Mercier [9], the COP of substrate utilization is defined as the intensity at which energy from CHO-derived fuels predominates over energy from lipids. The point at which approximately 70% of the energy is derived from CHO and 30% from lipids can be identified. To assess this point, we smoothed out the curve between the percentage of oxidized CHO and power output over four points to dampen variations that eventually may be linked to a point departing from the general fitting curve. The maximum fat oxidation rate point (LIPOXmax) is the exercise intensity at which the increase in lipid oxidation induced by the increasing intensity reaches maximum (maximum fat oxidation rate [MFO]), and is then followed by a decrease as CHO becomes the predominant fuel. This is calculated from the above equations according to the empirical formula in which fat = 1.6946 \(\dot{V}_{O_2}\) – 1.7012 \(\dot{V}_{CO_2}\), which can be simplified as fat = 1.7(1–RER) \(\dot{V}_{O_2}\). This equation gives the MFO, the point at which the value of the derived equation is equal to zero. For LIPOXmax determination, we smoothed the relationship between RER and power output. All smoothing procedures were performed using custom-made software developed by Perez-Martin et al. in Montpellier. COP and LIPOXmax were expressed as a percentage of maximum aerobic power (% MAP) [10].

2.6. Statistical analysis

Data are expressed as means ± S.D. Paired t-tests or Wilcoxon’s tests (depending on data distribution) were used to assess the influence of the gas-exchange analytical device (E versus D) on the COP and LIPOXmax. Men and women were compared using the Mann-Whitney U-test. The influence of the devices on RER was assessed by repeated-measures analysis of variance (ANOVA). In addition, E and D were compared using the Bland and Altman method [12].

All of the comparative tests cited above were applied to assess the influence of the protocol used (tMAP or MAP), and the reliability (test–retest) of gas exchange and metabolic indices (COP and LIPOXmax). For the lattermost point, the coefficient of variation (CV) was calculated [13]. The influence of ventilation on RER and metabolic indices, and the relationship between these indices and the lactate/ventilatory thresholds were assessed using Pearson or Spearman regression (depending on data distribution). Statistical significance was set at \(P < 0.05\) and type I risk \(\alpha\) at 5%.

3. Results

3.1. Subjects’ characteristics

The characteristics of the subjects enrolled in the present study are presented in Table 1. Based on \(\dot{V}_{O_2}\)peak (44.7% greater than the predicted value), these subjects were physically fit. However, the heterogeneity of their fitness should be mentioned (range: 112–169% of predicted value). The metabolic indices obtained with reference D method, using a protocol based on the true MAP, correlated with \(\dot{V}_{O_2}\)peak (COP in W: \(r = 0.92, P < 0.0001\); COP in % MAP: \(r = 0.54, P = 0.048\); LIPOXmax in W: \(r = 0.94, P < 0.0001\); LIPOXmax in % MAP: \(r = 0.56 P = 0.039\) and SRPAL (COP in W: \(r = 0.60, P = 0.02\); LIPOXmax in W: \(r = 0.80, P < 0.001\); no correlation when COP and LIPOXmax were expressed as % MAP).

3.2. Influence of gas-exchange measurement device

RER did not significantly differ between the two devices (ANOVA: \(F = 1.4; P = 0.7\); Table 2). However, RER obtained with E did not strongly correlate to that obtained with D (\(r = 0.5; P < 0.01\)). Moreover, the Bland and Altman analysis showed a large random error (0.135) despite the small bias (−0.006) (Fig. 2A).

No difference was observed between E and D for COP and LIPOXmax determinations (COP: 144 ± 93 W and 148 ± 85 W; LIPOXmax: 136 ± 81 W and 123 ± 68 W; for E and D, respectively). Bland and Altman analysis revealed important systematic errors for COP and LIPOXmax (both were...
Fig. 2. Bland and Altman plot agreement between RER (A), COP (B) and LIPOXmax (C) values obtained with Ergocard device (E) versus Douglas bag (D).
Table 2
Respiratory exchange ratios obtained simultaneously with Douglas bag method (D) and Ergocard Medisoft device (E)

<table>
<thead>
<tr>
<th>Exercise intensity (% tMAP)</th>
<th>20%</th>
<th>30%</th>
<th>40%</th>
<th>50%</th>
<th>60%</th>
</tr>
</thead>
<tbody>
<tr>
<td>E: RER test 1</td>
<td>0.75 ± 0.06</td>
<td>0.86 ± 0.05</td>
<td>0.88 ± 0.05</td>
<td>0.90 ± 0.06</td>
<td>0.92 ± 0.05</td>
</tr>
<tr>
<td>E: RER test 2</td>
<td>0.77 ± 0.05</td>
<td>0.88 ± 0.04</td>
<td>0.90 ± 0.04</td>
<td>0.92 ± 0.04</td>
<td>0.94 ± 0.04</td>
</tr>
<tr>
<td>CV (%)</td>
<td>4.3</td>
<td>2.9</td>
<td>2.6</td>
<td>2.4</td>
<td>1.6</td>
</tr>
<tr>
<td>D: RER test 1</td>
<td>0.75 ± 0.06</td>
<td>0.88 ± 0.04</td>
<td>0.89 ± 0.04</td>
<td>0.92 ± 0.04</td>
<td>0.94 ± 0.04</td>
</tr>
<tr>
<td>D: RER test 2</td>
<td>0.74 ± 0.07</td>
<td>0.87 ± 0.04</td>
<td>0.89 ± 0.04</td>
<td>0.92 ± 0.03</td>
<td>0.96 ± 0.04</td>
</tr>
<tr>
<td>CV (%)</td>
<td>6.2</td>
<td>2.5</td>
<td>2.7</td>
<td>1.7</td>
<td>2.0</td>
</tr>
</tbody>
</table>

Values are expressed as means ± S.D.
CV: coefficient of variation; RER: respiratory exchange ratio.

overestimated with E), and random errors for COP and LIPOX-
max, as indicated by the limits of agreement (bias ± random
error = 17.6 ± 74.7 W for COP and 30.3 ± 78.8 W for LIPOX-
max; Fig. 2B, C). In addition, for both parameters, a proportional
systematic error (as the slopes of the relationships were not zero:
COP: slope = 0.41, P < 0.05; LIPOXmax: slope = 0.55, P < 0.05)
and a uniform random error (Fig. 2B, C) were observed. Furthermore,
the COP and LIPOXmax obtained with E were weakly or
not correlated to those obtained with D (r = 0.6, P = 0.01 and
r = 0.3, P = 0.14, respectively).

3.3. Influence of exercise protocol

Individual data related to the influence of the exercise pro-
tocol (tMAP versus MAP) used to calculate metabolic indices
assessed by the D method are shown in Fig. 3. On average, the

Fig. 3. Individuals values and means ± S.D. of COP (A) and LIPOXmax (B) obtained with protocols built either from MAP or tMAP. Continuous line: men. Broken line: women.
exercise intensity at which the COP and LIPOXmax occurred was not significantly different between the two protocols (COP: \(P = 0.50\); LIPOXmax: \(P = 0.33\)). However, on inspection, the individual data clearly showed disagreement between the two protocols, with a maximum difference of 150 W for the fitter subjects (Fig. 3). Indeed, 13 of the 14 subjects presented either higher \((n = 8)\) or lower \((n = 5)\) COP values with the protocol based on the tMAP compared with MAP (Fig. 3A). As for the LIPOXmax, an effect of protocol was observed here as well, although only six subjects presented with significantly different values \(>(20 \text{ W})\) with the two approaches. For four subjects, COP and LIPOXmax values were increased at least two- to threefold with the tMAP compared with the MAP protocol. COP and LIPOXmax values were decreased by an average of 30% in four other subjects when comparing the tMAP with the MAP protocol. The variation in COP and LIPOXmax between the two protocols (tMAP and MAP) was not significantly different according to gender (COP: men, 50 ± 6 W; women, 55 ± 9 W \(P = 0.20\); LIPOXmax: men, 53 ± 7 W; women: 52 ± 9 W \(P = 0.40\)). Fig. 3 also shows that two \((40\%)\) of the five women had marked variations \(>(20 \text{ W})\) in workload at COP \(+153, +80 \text{ W}\), and two others at LIPOXmax \(+39, +82 \text{ W}\) between MAP and tMAP, compared with seven \((50\%, \text{NS})\) and two \((22\%, \text{NS})\) men out of 14, respectively.

A COP greater than 90% of MAP was not considered a valid value as there were no high-level endurance athletes included in the present study. In addition, the exercise intensity at which we considered LIPOXmax as noninterpretable was a value greater than 75% of MAP as it is thought that, when exercise intensity exceeds 75% of \(\dot{V}_\text{O}_2\max\), calculation of fat oxidation by indirect calorimetry becomes inaccurate \[8\].

With the submaximum graded exercise test protocol based on MAP, individual analyses revealed that, with the reference D method, all COP determinations (range: 29–83% of MAP) were interpretable. With Ergocard (E), one \((7\%)\) COP value was abnormally high \((>100\%\) of MAP; range: 33–91%). Individual analyses of the LIPOXmax data obtained with the D reference method revealed that all values were interpretable (range: 30–83% of MAP) whereas, with Ergocard (E), one \((7\%)\) value was abnormally high \((>90\%\) of MAP; range: 33–91%).

For the submaximum graded exercise test protocol based on tMAP, 30% of the measured COP values using D and 46% using E were abnormally elevated \((>90\%\) of MAP). For LIPOXmax, the number of noninterpretable values reached 30% with the D method and 54% using E.

Finally, the variations of COP and LIPOXmax between MAP and tMAP did not correlate with weight (COP: \(r = 0.2, P = 0.07\); LIPOXmax: \(r = 0.04, P = 0.93\)), BMI (COP: \(r = 0.02, P = 0.94\); LIPOXmax: \(r = 0.02, P = 0.94\)), age (COP: \(r = 0.22, P = 0.44\); LIPOXmax: \(r = 0.02, P = 0.94\)), \(\dot{V}_\text{O}_2\max\) (COP: \(r = 0.15, P = 0.62\); LIPOXmax: \(r = 0.05, P = 0.86\)) or SRPAL (COP: \(r = 0.12, P = 0.7\); LIPOXmax: \(r = 0.02, P = 0.94\)).

### 3.4. Reliability study

The reproducibility of RER, COP and LIPOXmax with D and E was determined using a submaximum graded exercise test based on MAP (Tables 2 and 3). RER did not significantly differ between tests 1 and 2 with either D or E (Table 2), and RER CV of 20–60% of MAP in the submaximum exercise test ranged from 1.7 to 6.2%, regardless of which device was used. Also, the exercise intensity at which COP and LIPOXmax occurred did not differ significantly between tests 1 and 2, whichever method was used (D or E) (Table 3). However, there was greater variability in COP (CV: 14 and 21% for D and E, respectively) compared with LIPOXmax (CV: 7 and 12% for D and E, respectively).

### 3.5. Influence of ventilation on calculation of metabolic indices

The coefficient of determination \(r^2\) of the relationship of ventilation difference between tests 1 and 2, and the same difference for RER, was \(0.17 (P < 0.01)\), suggesting that ventilation was the explanation of 17% of the RER variance. The mean differences in ventilation between tests 1 and 2 over the entire submaximum exercise protocol explained 16% \((P < 0.01)\) of COP variance and 10% of LIPOXmax variance \((P < 0.01)\).

### 3.6. Comparison of metabolic indices with lactate/ventilatory thresholds

Expressed in terms of metabolic cost (\(\dot{V}_\text{O}_2\)), COP occurred at the same exercise intensity (1.6 ± 0.5 L/min) as \(V_T_1\) (1.7 ± 0.6 L/min; \(P = 0.26\)) and \(L_T_1\) (1.6 ± 0.4 L/min; \(P = 0.99\)), whereas LIPOXmax occurred at a lower exercise intensity (1.4 ± 0.4 L/min) compared with \(V_T_1\) (1.7 ± 0.6 L/min; \(P < 0.01\)) and \(L_T_1\) (1.6 ± 0.4 L/min; \(P < 0.01\)). A relationship was observed between \(V_T_1\) and both COP \((r = 0.96; P < 0.01)\) and LIPOXmax \((r = 0.95; P < 0.01)\). In addition, COP \((r = 0.79; P < 0.01)\), but not LIPOXmax \((r = 0.52; P = 0.09)\), correlated with the \(m_2\) coefficient of the modeling equation of plasma lactic

### Table 3

<table>
<thead>
<tr>
<th>D</th>
<th>E</th>
</tr>
</thead>
<tbody>
<tr>
<td>Test 1</td>
<td>Test 2</td>
</tr>
<tr>
<td>RER</td>
<td>0.75–0.94</td>
</tr>
<tr>
<td>COP (% MAP)</td>
<td>48 ± 17</td>
</tr>
<tr>
<td>LIPOXmax (% MAP)</td>
<td>42 ± 10</td>
</tr>
</tbody>
</table>

CV: coefficient of variation (%); RER is expressed as a range obtained over the whole protocol for exercise intensities (20, 30, 40, 50 and 60% of tMAP); COP and LIPOXmax are expressed as means ± S.D. in percentage of MAP.

* \(P < 0.05\) versus D.
acid concentration from metabolic cost as proposed by Maltais et al. [14], where: \[ \text{[lactate]} = m_1 + m_2\dot{V}_\text{O}_2, \]
with \( m_2 \) representing the rate of blood lactate increase.

4. Discussion

Indirect calorimetry is the method of choice for assessing the balance between fat and CHO oxidation during exercise through calculation of the COP and LIPOXmax. However, to our knowledge, validation studies of how these indices are determined are scarce, with only one reliability study in the literature [10]. The present study aimed to specify the conditions of application of these indicators in healthy subjects in routine clinical settings. As recommended for determining COP and LIPOXmax [3], four six-minute steady-state bouts of exercise at 30–60% of the theoretical maximum workload were used, as this allows calorimetric calculations to be safely made, especially with sedentary patients [15]. To dampen ventilation effects on gas exchange, the last 3 min of each bout of exercise were averaged, after first confirming that such a procedure had no effect on COP and LIPOXmax calculations (results not shown).

The present methodological study of healthy subjects highlights:

- the impact of the gas-exchange measurement device on COP and LIPOXmax;
- the significant influence of maximum aerobic capacity reference (either the predicted tMAP or true MAP) in constructing the submaximum graded exercise protocol;
- the significant, though weak, influence of external ventilation on the determination of these metabolic indices;
- the relationship between these metabolic indices and the ventilatory and lactate thresholds.

4.1. Influence of gas-exchange measurement device

Bland and Altman analysis revealed disagreement between the two devices used to assess COP and LIPOXmax, despite the lack of difference between means. This result and the larger proportion of noninterpretable values of COP (7%) and LIPOXmax (14%) obtained with E questions the validity of such gas-exchange measures (see the large random error with the Bland and Altman analysis of RER) using some commercial devices, despite careful calibration procedures and rigorously following the recommendations of the manufacturer. Ventilation tended to be underestimated with E (results not shown), an intriguing result as ventilation was measured using a Pitot tube (one of the most reliable devices available for ventilation measurement during exercise).

One explanation may be related to the incorrect linear table of ventilation included in the software. However, an error in ventilation cannot explain the discrepancy in RER, as the latter is the ratio between \( V\text{CO}_2 \) and \( V\text{O}_2 \), and ventilation is part of the calculation of these variables. Discrepancies in \( V\text{O}_2 \) and \( V\text{CO}_2 \) (results not shown) measures between the two devices suggest an error in gas analysis (\( \text{O}_2 \) as well as \( \text{CO}_2 \)). Because the error was already evident during the first stage of the exercise (for low RER, Fig. 1A), it cannot be attributed to gas-analyzer drift over the duration of exercise, as suggested by Atkinson et al. [16]. An error in the time alignment between gas fractions and flow-rate signals may have occurred, but such a hypothesis has not been confirmed. On the other hand, it may be worth noting that the automated E used in the present experiment was not the latest version of the instrument. Whatever the cause(s) of the error, our results confirm the influence of gas-exchange measurement devices on metabolic indicators assessed by indirect calorimetry. Therefore, our study reinforces the need to systematically validate gas-exchange devices before routine clinical assessment of these metabolic indices during exercise.

4.2. Influence of exercise protocol

Despite the lack of any statistical difference in the metabolic indices calculated with a protocol based on either the tMAP (as recommended by Brun et al. [3] for patients with metabolic defects) or the MAP, inspection of the individual data showed large differences between the two approaches. The reasons for these are not clear. It may be that, for some physically fit subjects, the exercise intensities calculated on the basis of tMAP were not high enough to raise the RER during the exercise test, inducing a need to extrapolate COP with a greater risk of error. Thus, it appears that the level of each exercise step needs to be modified in the case of fit subjects, whose power capacity is higher than the theoretical one.

On the other hand, in the case of very unfit patients, adaptation of the workload may be required to avoid underestimating the metabolic indices. Indeed, as the COP (70% CHO/30% fat) corresponds to an RER of 0.9, the theoretical protocol should be modified to include steps below and above 0.9 as, otherwise, the test cannot be interpreted.

However, if a marked increase in COP and LIPOXmax using a tMAP protocol may be explained by insufficient stimulation, the marked decrease observed in several of our healthy subjects is then especially difficult to explain. A too high stimulation is unlikely as none of our subjects had a level of physical fitness lower than could be predicted. Other variables might account for these results, as gender, age, fat mass, \( V\text{O}_2\text{peak} \), level and type (walking or running versus cycling) of physical activity and diet have all been reported to be potential factors influencing fat oxidation [18].

We can partially confirm these findings as we found a good correlation between \( V\text{O}_2\text{peak} \), self-reported physical activity and our metabolic indices. However, the lack of relationship with the variations in metabolic indices induced by the exercise protocols (tMAP or MAP) does not support any influence of these factors. As we controlled the diet (identical on the day before each trial), we do not believe that this factor can account for the variations induced by the protocol. Gender and, in particular, the phase of the menstrual cycle may have some effect. Unfortunately, we did not assess the phase of menstrual cycle in our five female subjects. However, the fact that only three days separated the two exercise protocols and that the mean variation in metabolic indices induced by the protocol was identical between the men and women does not support an influence of gender.
For research purposes especially, we advise constructing a protocol using the true MAP, determined from a previous maximum exercise test, to obtain the most interpretable results. For routine clinical examination of patients, we recommend taking into account the level of patients’ fitness (on the basis of clinical interview) if they are expected to be less or more fit than average. The exercise protocol based on the tMAP as recommended by Brun et al. [3] should be adapted to include the transition (from fat to CHO) that occurs at an RER of 0.9.

4.3. Validity of metabolic index measurements

The traditional D method is still considered the gold standard for indirect calorimetry [8], as more modern, automated systems have not always been reported to provide accurate and valid results [16]. This technique was first applied to assess the validity and reliability of COP and LIPOXmax. Knowing the number of noninterpretable results is important and should be taken into account when evaluating these indices as part of a routine clinical examination. Although the D method is not free of errors, whichever the index considered, all values were interpretable with a protocol based on a true maximum aerobic power. The present study shows that, with careful methodology (tests performed after a 12-hour overnight fast and after stabilization of RER at rest at less than 0.85, and ensuring the minimal influence of food and ventilation on gas exchange), the protocol recommended by Brun et al. [3] – a six-minute steady-state workload based on MAP – provides interpretable results despite weak values in approximately 29–36% (five subjects’ COP and four subjects’ LIPOXmax were less than 30% of MAP) of subjects. When we first started using this metabolic evaluation during exercise, we were surprised to find such low values, especially in trained individuals. However, in some high-intensity trained athletes or in those who take only intermittent exercise, Brun et al. [3] reported profiles of glucose dependency occurring at COP and LIPOXmax intensities of less than 30% of MAP. Thus, we consider these values to be accurate.

Also, unlike using a commercial device or inappropriate protocol (see above section) for which the rate of noninterpretable values is high (30–54%, depending on the metabolic index being considered), determining metabolic indices with a protocol based on MAP is accurate in healthy subjects, provided that certain recommendations are adhered to (such as fasting) and that the device used to assess gas exchange is valid.

4.4. Reliability and impact of ventilation on metabolic indices

With the D method, LIPOXmax reproducibility (CV = 7%) was comparable to that reported by Perez-Martin et al. (11.4%) [10]. Also, LIPOXmax did not differ between the two tests performed separately (Tables 2 and 3). However, reproducibility was not as high for COP (CV = 14%). The consistently greater variability with the Ergocard device (CV = 12 and 21% for LIPOXmax and COP, respectively) suggests that methodological errors cannot account for the poor reproducibility, but that it may be related to subjects’ behavior during the test. Whichever device was used for the gas-exchange measurements, the reproducibility of the two tests separated by no more than three days was better for LIPOXmax than COP. Therefore, LIPOXmax appears to be a more stable indicator than COP. One explanation for this may be related to how COP is calculated: interpolation or extrapolation (depending on the kinetics of RER) of the relationship between RER and workload. In our experience, one or two points that departed from linearity were frequently observed. The impact of RER on LIPOXmax determination is probably less important as that parameter is the product of two different linear relationships: the decrease (1 – RER) and the linear rise in $V_{\text{O}_2}$ proportional to power [10].

Ventilation output is a potential factor that might explain variability in RER. Indeed, the relationship between ventilation and RER on the one hand, and between ventilation and COP on the other hand, confirm the potential role of ventilation in the calculation of these indicators, although its impact is relatively small (ventilation explained 10 and 16% of the variance in LIPOXmax and COP, respectively). It is worth noting that the variance of RER explained by ventilation is in the same order of magnitude as the variance of COP explained by ventilation.

4.5. Relationship between metabolic indices and anaerobic threshold

The results of the present study confirm that metabolic intensity (expressed as the level of oxygen consumption) is similar between COP, VT1 and LT1. Moreover, the relationship between COP and the coefficient of modeling equation of plasma lactic acid proposed by Maltais et al. [14] confirms the link between COP and lactate production kinetics. Interestingly, when exercise intensity was expressed in terms of mechanical power output, we observed lower exercise intensity for COP compared with VT1 and LT1 (results not shown). This finding was probably due to differences in the protocol for determining the different indices. Indeed, the duration of each step of the sub-maximum graded exercise was long (allowing calculation of COP and LIPOXmax), whereas determination of the anaerobic thresholds was assessed during short-step (one-minute) maximum progressive testing. In this case, because of the rapidity of the increments, the slope of the linear relationship between oxygen consumption and workload is decreased due to a greater deficit in oxygen consumption at each step [17]. Therefore, oxygen consumption is proportionally slower than the change of workload, resulting in a systematic overestimation of workload for each given metabolic cost using this kind of protocol. This means that, when comparing metabolic indices obtained during exercise with two different protocols, the best approach is to compare the metabolic intensity expressed as oxygen consumption.

LIPOXmax occurred at a lower exercise intensity compared with VT1 and LT1. For VT1, our results are consistent with those reported by Venables et al. [18]. However, it has already been reported that LIPOXmax occurred at the same exercise intensity (relative to $V_{\text{O}_2}$ peak) as LT1 – an increase in lactate concentration above baseline (LIAB) [16]. The reasons for such differences are not clear, but they may be due to protocol dif-

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ferences. Indeed, for LIPOXmax determination, Achten and Jeukendrup [19] used an exercise protocol with a three-minute per stage duration that may have overestimated LIPOXmax, according to Bordenave et al. [15]. However, in agreement with these authors [19], we found a correlation between LIPOXmax and LT, owing to the influence of acidosis and lipid oxidation rate. The relationship between these metabolic indices and thresholds may provide an estimate of these variables, and the application of such findings is of clinical interest. If our results are confirmed, then determination of LT1 or VT1 (expressed as a percentage of maximum oxygen consumption) may be alternative estimations of COP and LIPOXmax, respectively, in healthy subjects undergoing standard maximum progressive exercise testing. Such an approach would integrate all of the indices (LT1 and 2, VT1 and 2, \(V_{O_2}\text{peak}\), estimations of COP and LIPOXmax) necessary for planning or assessing the effects of an exercise-training programme. Moreover, given the long duration (30 min) of the exercise protocol needed for COP and LIPOXmax determination, and the limited capacity of severely deconditioned patients (because of long-standing and severe type 2 diabetes, heart failure or COPD, for example), a shorter maximum progressive exercise may be more suitable for purposes of evaluation. However, this idea needs to be confirmed in sedentary as well as in healthy glucodependent subjects whose metabolic indices appear to be dissociated from the ventilatory threshold.

5. Conclusion

The present study reassessed the methodology for determining metabolic indices as applied to healthy and physically fit subjects. Using the reference method (D) for indirect calorimetry and a protocol based on a true, previously determined, maximum aerobic capacity, all determined metabolic indices were interpretable. For such subjects and particularly for research purposes, and to obtain interpretable values of COP and LIPOXmax, we recommend constructing a protocol based on the true MAP of the subject or adapting the protocol to include the transition (from fat to CHO) that occurs at an RER of 0.9. In the routine clinical examination of patients, attention should be paid to those whose levels of fitness are expected to be greater than those predicted.

The relationship we found between metabolic indices and the lactate and ventilatory thresholds suggests that a shorter classical maximum progressive exercise test may be a useful alternative means of estimating these indices in healthy subjects. This method, however, needs to be confirmed in patients presenting with metabolic defects.

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