BALANCE OF SUBSTRATE OXIDATION DURING SUBMAXIMAL EXERCISE IN LEAN AND OBESE PEOPLE

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SUMMARY - Objectives: To compare fat and carbohydrate oxidation at different exercise intensities between overweight and normal-weight subjects, in order to analyze the influence of muscular metabolic abnormalities in obese people on substrate utilization during exercise. Material and methods: 32 healthy sedentary overweight subjects (Body Mass Index (BMI): 30.8 ± 0.8 kg/m²; body fat: 37.4 ± 1.1%; mean ± SEM) and 26 controls (BMI: 23 ± 0.4 kg/m²; body fat: 22.7 ± 1.1%) matched for age and sex were examined. The test consisted in four six-min. submaximal steady-state workloads with calculation of substrate oxidation rates and derived quantitative parameters, i.e., crossover point (defined as the power at which carbohydrate-derived energy becomes predominant) and maximal fat oxidation rate point. In addition, the accuracy of the test was analyzed and was found to be satisfactory. Results: While exercise intensities were similar in both group, fat oxidation rates were significantly lower in overweight group (p < 0.05). The crossover and the maximal fat oxidation rate points were significantly lower in overweight subjects than in controls: 33.3 ± 2 vs 50.1 ± 3.4% and 30.5 ± 2.3 vs 44.6 ± 3.3% of maximal aerobic power, respectively (p < 0.001). Conclusion: Sedentary overweight subjects, compared to controls at the same exercise intensities, exhibited an alteration of the balance of substrate oxidation, reflected by lower rates of fat oxidation and a shift of quantitative parameters to lower intensities. The test appeared to be reliable and could be of interest to advise an individualized exercise prescription in obese people.

Key-words: fat oxidation, indirect calorimetry, exercise, overweight, crossover concept.

RÉSUMÉ - Balance de l’oxydation des substrats lors d’un exercice sous-maximal chez des sujets minces et obèses. Objectifs : Comparer les débits d’oxydation lipidique et glucidique à différentes intensités d’exercice chez des sujets en surpoids et des sujets de poids normal, afin de déterminer l’influence des anomalies métaboliques musculaires des sujets obèses sur la balance des substrats énergétiques à l’effort. Matériel et méthodes : 32 sujets sédentaires en surpoids sans pathologie associée (Index de Masse Corporelle (IMC) : 30,8 ± 0,8 kg/m² ; masse grasse : 37,4 ± 1,1 % ; moyenne ± SEM) et 26 sujets témoins (IMC : 23 ± 0,4 kg/m² ; masse grasse : 22,7 ± 1,1 %) appariés pour l’âge et le sexe ont réalisé un test d’effort sous-maximal comportant quatre paliers de six min. Les débits d’oxydation lipidique et glucidique ont été mesurés à chaque palier par calorimétrie indirecte et ont permis la détermination de paramètres quantitatifs reflétant la balance glucido-lipidique à l’effort : le point de croisement de l’utilisation des substrats (défini comme étant la puissance pour laquelle l’énergie provient majoritairement des glucides) et le point d’oxydation lipidique maximale. De plus, la fiabilité du test a été analysée et était satisfaisante. Résultats : À l’effort et à intensité d’exercice équivalente, les taux d’oxydation lipidique étaient significativement plus bas tout au long du test chez les sujets obèses que chez les sujets témoins (p < 0,05). Les paramètres quantitatifs de la balance de l’utilisation des substrats énergétiques étaient significativement abaissés chez les sujets obèses comparés aux témoins : point de croisement 33,3 ± 2 vs 50,1 ± 3,4 % et point d’oxydation lipidique maximale 30,5 ± 2,3 vs 44,6 ± 3,3 % de la puissance maximale (p < 0,001). Conclusion : Cette étude montre une altération de la balance glucido-lipidique à l’effort chez les sujets obèses sédentaires, caractérisée par une moindre oxidation lipidique par rapport aux témoins à même intensité relative d’exercice et par l’abaissement des paramètres quantifiant cette balance. Le test sous-maximal utilisé est simple et reproductible. Il pourrait constituer un moyen d’évaluation simple des altérations métaboliques musculaires et de leurs conséquences sur l’utilisation des substrats énergétiques à l’effort ainsi qu’un outil guidant la prescription individualisée de l’exercice dans cette population.

Mots-clés : oxydation lipidique, calorimétrie indirecte, exercice, obésité, point de croisement.

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With an increasing prevalence and high comorbidity, obesity is a major health problem. Both genetic and environmental factors are involved in its pathogenesis. The mechanisms underlying the development of obesity are still discussed. However, it is now recognized that skeletal muscle is largely involved, as, in obese subjects, it may exhibit several metabolic defects. Muscular insulin resistance is often marked, leading to a decrease in glucose uptake and utilization, and in glycogen storage ability [1]. In addition, it has been shown that free fatty acids (FFA) utilization is also altered in obese [2] as well as in subjects with a risk for obesity [3]. Some defects have been identified, such as a decrease in lipoprotein lipase [4], in carnitine palmitoyl transferase and in citrate synthase [2]. Moreover, carbohydrate (CHO) and lipid metabolism defects may interact, especially through substrate competition [5, 6].

In contrast, exercise training induces muscular metabolic changes, which can restore these defects [7]. Accordingly, for a long time, healthcare scientists and clinicians have been giving recommendations for regular physical activity and lifestyle modifications [8, 9]. However, while health benefits of physical activity are well known, it is still not clear whether the association of mode, intensity and frequency of exercise for optimal decreasing metabolic risks factors are the same than those which generally improve fitness. Regarding results of different training programs on glucose metabolism, weight and body fat reduction, blood lipid profile and substrate utilization, data are sometimes conflicting [7]. This may be related to the choice of training programs themselves which use standardized intensity, regardless from individual metabolic characteristics. The most common protocols and recommendations propose low to moderate intensity exercises [8, 9]. Only few studies have focused on benefits of resistance training [10] or high intensity exercise [11]. As obese subjects may exhibit many muscular defects, the metabolic bases for these various training programs remain poorly known. Presumably, given the heterogeneity of the obese population, an individualized exercise prescription taking into account potential muscular metabolic abnormalities could be of interest. Thus, this study was performed to compare CHO and lipid oxidation rates in overweight subjects and matched lean controls at various exercise intensities, in order to test the influence of muscular metabolic abnormalities in obese subjects on the balance of substrate utilization during exercise.

**PATIENTS AND METHODS**

Patients

32 healthy overweight patients (15 males and 17 females, with overweight defined according to the WHO as a Body Mass Index (BMI) > 25 kg/m²), who went to our unit for a metabolic and nutritional check-up, were recruited and compared to 26 healthy normal-weight volunteers (11 males and 15 females; BMI < 25 kg/m²). All overweight subjects were sedentary and reported not having participated to a structured exercise program for the six months prior to the test. Control subjects did not participate in intensive training, nor in competitions.

**Anthropometry**

Weight and height measurements were performed and BMI was calculated as weight in kilograms divided by height in meter squared (kg/m²). Impedance in body tissue to the flow of an applied alternative current was measured by bioelectrical impedance analysis and the values obtained were used to estimate body composition (body fat mass, percentage of body fat) [12]. All bioelectrical impedance measurements were performed by a multi-frequency (1, 5, 10, 50, 100 kHz) device (Human IM-Scan from Dietosystem, Milan, Italy). Analysis was performed with the software Master 1.0 provided by the manufacturer.

**Exercise testing**

As generally used to individualize the increment of exercise intensity during cardiopulmonary exercise testing [13, 14], the workload of each step was calculated from the theoretical maximal aerobic power (Wmax), i.e., power corresponding to the theoretical VO2max [14]. In consequence, the subjects underwent a test with the same relative incremental workload and were compared at the same percentage of their Wmax. The test consisted on a three-minutes warm-up at 20% of Wmax, followed by four six-minutes steady-states workloads at 30, 40, 50 and 60% of Wmax.

The subjects performed the test on an electromagnetically braked cycle ergometer (Ergoline Bosh 500). Heart rate was monitored continuously throughout the test by standard 12-lead procedures, and blood pressure (sphygmomanometer auscultation) was measured at rest, during each steady-state and after five-min. of recovery.

**Calculation of substrate oxidation balance and derived parameters during exercise**

Metabolic and ventilatory responses were assessed using a digital computer based breath to breath exercise analyzing system (CPX Medical Graphics, Minneapolis, Minnesota, USA). Calculation of CHO and lipid oxidation rates was assessed from gas exchange measurements according to the non-protein respiratory quotient (R) technique [15].
CHO rate oxidation (mg/min.) = 4.585 VCO2 – 3.2255 VO2

Lipid rate oxidation (mg/min.) = −1.7012 VCO2 + 1.6946 VO2

(with VO2 and VCO2 in ml/min.). VO2 and VCO2 were determined as the mean of measurements during the fifth and sixth min. of each state, according to Mac Rae [16]. This technique provided CHO and lipid oxidation rates at different levels of exercise. These values were then converted into Kcal.

Additionally, after smoothing the curves, we calculated two parameters representative of the balance between fat and CHO utilization induced by increasing exercise intensity: the crossover point of substrate utilization and the maximal fat oxidation point. According to the concept proposed by Brooks and Mercier [17], the crossover point of substrate utilization is defined as the power at which energy from CHO-derived fuels predominates over energy from lipids. Although the shift from fat to CHO during increasing intensity exercise occurs as a continuum, the crossover point can be identified when approximately 70% of the energy derive from CHO and 30% from lipids since the oxidation of one gram of lipids provides approximately nine Kcal, while the oxidation of one gram of glucose provides only four Kcal [17]. Thus, we used this power at which 70% derive from CHO as an index of substrate balance.

The maximal fat oxidation rate point is the power at which the increase in lipid oxidation induced by the increasing workload reaches a maximum, which will then be followed by a decrease as CHO becomes the predominant fuel. It is calculated from the above equations, considering that the empirical formula: fat = 1.6946 VO2 – 1.7012 VCO2 can be simplified as fat = 1.7 (1 − R) VO2, in which R is the respiratory quotient VCO2/VO2. Therefore fat oxidation rate appears to be the product of two different linear relationships: the decrease of (1 − R) and the linear rise in VO2 proportional to power. Derivation of this equation gives the maximal fat oxidation rate which is the point where the value of the derived equation is equal to zero. The crossover point and the maximal fat oxidation point were expressed either in absolute values (watts) or in percentage of the theoretical Wmax. Results for a control subject and an overweight patient. As an example, we give in Figure 1 two clinical results for a control subject and an overweight patient. Determination of the crossover point and maximal fat oxidation rate reflects a quantitative approach of substrate utilization during exercise.

Biochemical analyses

Plasma insulin was analyzed by radioimmunoassay (kit Insik-5, Sorin Biomedica France, Anthony, France). Plasma glucose and lactate were determined with a Vitros Product Chemistry analyzer (Johnson & Johnson, Clinical Diagnostics, Rochester, NY, USA).

Protocol

All subjects were asked to fast for 12 hours before each test. No dietary restriction was imposed during the days before exercise testing. The purpose of the study was explained to the subjects before they gave their written informed consent. Then, they underwent a medical examination designed to identify usual counter-indications for exercise testing. Anthropometric measurements were obtained before subjects started exercise testing. A cannula for blood sampling was set before the test in the cephalic vein at the level of the cubital fossa. Blood samples were taken twice at rest for plasma insulin, glucose and lactate and during the last minute of each steady-state for plasma glucose and lactate. This protocol has been approved by the Local Ethics Committee.

Accuracy of the test

To test the repeatability of the exercise testing, ten additional healthy male volunteers (age: 32.8 ± 4.2 years (mean ± S.E.M.); BMI: 22.7 ± 0.4 kg/m²) performed the same exercise test twice, within one week. In order to analyze the effect of the preceding step on substrate oxidation at a given step, five additional healthy male subjects (age: 28.2 ± 3 years; BMI: 23.1 ± 0.9 kg/m²) were asked to come to the laboratory on five separate days, to perform: 1 — the specific incremental exercise test (day one), 2 — four six-min. constant workload exercise tests, in random order, realized at the different workloads used during the initial specific test, i.e. 30, 40, 50 and 60% Wmax (days 2, 3, 4 and 5). The tests were separated by at least three days and never more than seven days. Metabolic and ventilatory responses were assessed continuously. The mean VO2, VCO2, R and derived substrate oxidation rates measured during the fifth and sixth min. of each constant workload exercise test were then compared to the corresponding values obtained during each step of the four six-min. incremental test. All the subjects who performed exercise testing twice or more were requested to have the same free meals and a similar physical activity during the two days before each test.

Statistics

Results are given as mean ± S.E.M. Between groups comparisons were made using the Mann-Whitney test. Statistical significance was set at p < 0.05. Paired data were analyzed with a Wilcoxon test. Mean repetability was evaluated by the calculation of the coefficient of variation, with standard deviation calculated as the squared root of the ratio \( \Sigma d^2/n \), where d represented the difference between the two values obtained for a same subject, and n the number of subjects [18]. All calculations were performed with the Sigmastat package (Jandel Scientific, Erkrath, Germany).
RESULTS

Subjects characteristics

The characteristics of overweight and control subjects are given in Table I. The two groups were compared for age, BMI, WHR, body fat percentage and theoretical Wmax. There were no significant differences for age and theoretical Wmax, expressed in W or in W/kg of fat-free mass. The same results were found when comparing overweight and control females and overweight and control males. All subjects had normal blood pressure at rest and physiological evolution during exercise and recovery.

Biochemical analyses

Plasma glucose concentrations did not differ between the two groups, neither at rest nor during exercise. Plasma lactate concentration was higher in overweight group during the second exercise step but was similar at the other times (Table II). Resting plasma insulin concentration was significantly higher in overweight than in control subjects: 13.7 ± 1.2 vs 8.9 ± 0.4 µU/ml (p < 0.005).

Substrate utilization during exercise in overweight and lean subjects

Mean VO2 were compared in overweight and control group and did not show significant difference, neither at rest nor during each step of the test (Table III). When fat-free mass was considered, mean
VO2 (in ml/min./kg of fat-free mass) were also similar in the two groups, at rest and during exercise (Table III). R was similar in the two groups at rest, but higher during exercise, whatever the intensity level, in overweight than in control group (p < 0.005) (Table III). Lipid oxidation rates did not differ at rest, but...
were significantly lower in overweight group during exercise (p < 0.005) (Fig. 2a). When expressed in mg/min./kg of fat-free mass, the difference remained significant (Fig. 2b).

The crossover point of substrate utilization was significantly lower in the overweight than in control group: 33.3 ± 2% vs 50.1 ± 3.4% of Wmax (56.4 ± 4.8 vs 82.9 ± 5.7 W) (p < 0.001) (Fig. 3). The difference remained significant when gender was taken into account. The heart rate at the crossover point did not differ between the two groups: 113.3 ± 3.3 and 120.3 ± 4.6 bpm in overweight and control groups respectively. The maximal fat oxidation rate point was also significantly lower in the overweight group compared with controls, whether expressed as a percentage of Wmax or as absolute power values: 30.5 ± 2.3 vs 44.6 ± 3.3% Wmax (p < 0.001) (Fig. 4) and 51.2 ± 5 vs 69.5 ± 6.1 W (p < 0.001) respectively. Additionally, the maximal fat oxidation rate point was significantly lower than the crossover point (Wilcoxon test: p < 0.05 in overweight group and p < 0.005 in control group).

Accuracy of the test

The ventilatory responses at each exercise intensity (VO2, VCO2 and R) and the derived parameters (crossover and maximal fat oxidation rate points) did not differ when subjects underwent the test twice (Wilcoxon test for paired values). The coefficients of variation of R during each of the four tested intensities were respectively 2.8, 3.68, 2.23 and 4.75%. The coefficients of variation of the crossover, maximal fat oxidation rate point, both expressed in % of theoretical Wmax were 11.63 and 11.41 respectively. In the group of five volunteers who performed five separate exercise tests, VO2, VCO2, R, fat and CHO oxidation rates were compared during the incremental test and during randomized separate corresponding intensities tests. These parameters were not significantly different (Wilcoxon test for paired values).
DISCUSSION

The main result of this study is that sedentary overweight subjects, compared to controls matched for age and Wmax, exhibited a highly significant alteration of the balance of substrate oxidation during exercise. This was evidenced by: 1— the decrease of fat oxidation rates during each intensity tested, and 2— the shift to lower intensities of the crossover and of the maximal fat oxidation rate points. This could reflect an impairment of fat oxidation ability and an early CHO dependency during exercise in sedentary overweight people.

The choice of the exercise test was made in order to fulfill several conditions: 1— indirect calorimetry technique had to be applicable; 2— the protocol had to be standardized in terms of exercise duration and relative incremental workload and substrate utilization had to be analyzed at comparable relative exercise intensity for all subjects; 3— in addition of comparison of CHO and fat oxidation rates at different intensities, we needed quantifiable parameters to objective the shift from lipid to CHO.

Regarding the first point, indirect calorimetry is an usual method to quantify substrate oxidation rates at rest and during exercise. Nevertheless, during exercise above the lactate threshold, the accuracy of the technique has been discussed, as bicarbonate kinetics and thus, CO2 production can be markedly altered. Actu-

nique has been discussed, as bicarbonate kinetics and the accuracy of the respiratory quotient technique during exercise performed at 25, 65 or 85% of VO2max in trained subjects and have concluded that, even at high intensity, VCO2 can be considered as a reflection of working muscle production [20]. The validity of the respiratory quotient technique during 6-min. steps can also be discussed. Using a similar protocol, Mac Rae et al. [16] showed that during the fifth and sixth minutes of each step, the VCO2 varied by less than 0.1 L/min. and that the ventilation (VE) varied by less than 0.5 L/min. The relative constancy of these parameters during the last minutes of each step suggests that, at this time, respiratory compensation for metabolic acidosis, i.e. lactatemia and thus VCO2 increase, is negligible. The measurements of steady-state gas exchanges in our study had the same low variability (data not shown). Moreover, the blood lactate concentrations were similar in both groups. It could be assumed that the more rapid changes of R throughout the different steady-state workloads in overweight subjects were not explained by lactate accumulation, and were directly linked to the shift in substrate utilization. In addition, we had to determine the influence of immediately preceding step on substrate utilization at a given intensity. This influence seems to be negligible, as ventilatory responses and derived oxidation rates exhibited a good concordance between the continuous incremental test and each exercise intensity tested separately. Furthermore, the analysis of repeatability for VO2, VCO2, R, HR, crossover point and maximal fat oxidation rate point has shown CV < 12% which is a satisfactory result for biological variables [18]. Therefore one can reasonably assume that this test is reliable to determine differences of balance of substrate utilization between normal and overweight subjects in fasting conditions. At least, even then R may be strongly influenced by energy balance and dietary macronutrient composition, we voluntary asked all subjects to avoid any change of their usual dietary habits prior the test, in order to reproduce usual conditions. All obese subjects were analyzed before weight loss started to prevent fat oxidation decline induced by weight reduction.

As far as the second point is concerned, the standardization of the test was necessary in order to compare subjects at similar relative exercise intensity. With this aim in view, we used the predictive equations of theoretical VO2max and Wmax recommended by Wasserman et al. [14]. These equations take into account age, sex and anthropometric data. In normal weight individuals, theoretical VO2max is predicted from the weight, while in overweight individuals, it is predicted from height, and then is generally not over-predicted. We made sure that both overweight and normal subjects were tested at similar relative intensities, by comparing Wmax/kg of fat-free mass and VO2 (in ml/min. and in ml/min./kg of fat-free mass) during the test. None of these parameters differed between the two groups.

Third, in addition of CHO and fat oxidation rates, quantitative parameters of the balance of substrate utilization appeared to be of interest to objective potential modifications. For this purpose the crossover concept provides an interesting background, whereas it has been first described to quantify relative changes in substrate oxidation as a result of exercise training [17]. In fact, this concept implies that the balance between CHO and lipid can be quantified by the crossover point of substrate utilization. This point is defined as the power at which energy provided by CHO becomes predominant over fat-derived fuels, with further increases in power eliciting an increment in CHO utilization while fat oxidation declines. Obviously, when exercise intensity increases, substrate utilization shifts as a continuum from a greater dependence on fats at low intensity toward a preferential use of CHO at high intensity. However, the smoothing of the curves of oxidation rates and of relative contribution of lipid and CHO to total energy expenditure provided a modelization leading to the determination of crossover and maximal fat oxidation rate points. Although this approach seems caricatural, it could be useful in practice. Both the crossover point and the maximal fat oxidation rate point were expressed either in absolute values or as a percentage of the theoretical
Wmax. Indeed, we had to select adequate reference values to establish patterns of normal or abnormal response. The test performed was submaximal and did not allow a direct determination of actual Wmax. Moreover, in their guidelines for interpretation of cardiopulmonary exercise testing, many authors recommend to express submaximal parameters, such as lactate threshold, as a percentage of theoretical VO2max [14, 21]. Theoretical and actual VO2max and Wmax are quite similar in normally active healthy individuals, whereas they differ in many diseases. Therefore, predicted VO2max or Wmax are usual reference values for submaximal parameters in patients who exhibit altered maximal aerobic power and impaired muscular function [22].

We first hypothesized that in obese, muscular abnormalities resulting in both CHO and lipid metabolism defects, could alter the balance of substrate utilization during exercise. Actually, overweight subjects exhibited an earlier shift from lipid to CHO-derived fuels during exercise than matched controls. Fat oxidation rates were similar in both groups at rest, but were significantly lower during exercise whatever the intensity in overweight group. This decrease remained significant when total body weight or fat-free weight were taken into account. Thus, the maximal fat oxidation rate point and the crossover point occurred at lower intensity in overweight subjects. This could reflect the changes in substrate utilization and muscular metabolic abnormalities. Whether these defects occur as a potential cause or as a consequence of obesity, the matter remains to be clarified. We still have to determine which component is of a greatest importance for impaired muscle substrate utilization: body mass index, absolute amount of body fat, relative fat distribution, visceral fat content, insulin efficiency, dietary macronutrient composition, degree of physical inactivity. The contribution of the latter to increasing prevalence of obesity appears to be of importance [23] and could enhance pre-existent muscle metabolic disturbances. In this study, all overweight subjects were sedentary, while none of the control subjects had regular endurance training.

Nevertheless, whatever the mechanisms of the decline in fat oxidation, we may speculate that an optimal prescription should take individual metabolic abnormalities into consideration and thereby be individualized. The better understanding of substrate utilization during exercise and of the mechanisms regulating fuel selection provides an interesting background to define individual optimal exercise intensity. In fact, in many usual programs of exercise training obese subjects exercise above their crossover point and their maximal fat oxidation rate point, i.e. at an intensity level where fat oxidation rate is quite low. Recently, Anderson et al. [24] have shown that lifestyle modifications are as efficient as structured aerobic program on short-term weight loss and more efficient on long-term weight-maintenance. In addition to better individuals complying, similar health benefits can be due to the low-intensity of lifestyle activity. Accordingly, when compared to high intensity structured program with equal total energy expenditure, 12-wk low-intensity training induces a significantly higher fat oxidation increase during moderate intensity exercise session [25]. In normal weight men, Thompson et al. [26] have shown that low-intensity long duration exercise result in a greater total fat oxidation than does moderate intensity exercise of similar expenditure. We suggest that in obese, individual exercise intensity prescription could be done either at the crossover point, or at the maximal fat oxidation rate point. At these power outputs, fat utilization remains sustained, whereas it breaks down at higher intensities. To analyze if individual exercise management is more successful than standardized exercise prescription, we shall have to compare the effects of each strategy: individualized training programs at the crossover point level or at the maximal fat oxidation rate point level, versus standardized training programs.

## CONCLUSION

In conclusion, the comparison of substrate oxidation at different exercise intensities showed that overweight people exhibited a lower fat oxidation ability and an earlier shift from lipid towards CHO-derived fuel than lean people. The submaximal exercise test we used allowed the analyze of the balance of substrate oxidation during exercise and the determination of two parameters representative of this balance with a satisfactory reliability. Moreover, this exercise test appears to be practical and simple enough to be posed in routine programs. It could be helpful to investigate the consequences of muscular metabolic disorders on substrate utilization during exercise in obese subjects and to advise individualized exercise training prescription.

## REFERENCES

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