Substrate oxidation during exercise: type 2 diabetes is associated with a decrease in lipid oxidation and an earlier shift towards carbohydrate utilization

E Ghanassia, JF Brun, C Fedou, E Raynaud, J Mercier

SUMMARY

Objectives: Exercise is a recommended treatment for type 2 diabetes but the actual pattern of metabolic adaptation to exercise in this disease is poorly known and not taken in account in the protocols used. Metabolic defects involved in the pathways of substrate oxidation were described in type 2 diabetes. We hypothesized that type 2 diabetes, regardless of age, gender, training status and weight, could influence by its own the balance of substrates at exercise.

Methods: 30 sedentary type 2 diabetic subjects and 38 sedentary matched control subjects were recruited. We used exercise calorimetry to determine lipid and carbohydrate oxidation rates. We calculated two parameters quantifying the balance of substrates induced by increasing exercise intensity: the maximal lipid oxidation point (PLipoxMax) and the Crossover point (COP), intensity from which the part of carbohydrate utilization providing energy becomes predominant on lipid oxidation.

Results: Lipid oxidation was lower in the diabetic group, independent of exercise intensity. PLipoxMax and COP were lower in the diabetic group [PLipoxMax=25.3±1.4% vs. 36.6±1.7% %Wmax (P<0.0001)] – COP =24.2±2.2% vs. 38.8±1.9% %Wmax (P<0.0001).

Conclusions: Type 2 diabetes is associated with a decrease in lipid oxidation at exercise and a shift towards a predominance of carbohydrate oxidation for exercise intensities lower than in control subjects. Taking into account these alterations could provide a basis for personalizing training intensity.

Key-words: Type 2 diabetes · Training intensity · Substrate oxidation · Crossover point.

Ghanassia E, Brun JF, Fedou C, Raynaud E, Mercier J. Substrate oxidation during exercise: type 2 diabetes is associated with a decrease in lipid oxidation and an earlier shift towards carbohydrate utilization
Diabetes Metab 2006;32:604-610

CHU de Montpellier, Service central de Physiologie Clinique, Unité d'Exploration Métabolique (CERAMM), Hôpital Lapeyronie, 34000 Montpellier, France.

RÉSUMÉ

Oxydation des substrats lors de l'exercice : le diabète de type 2 est associé à une diminution de l'oxydation des lipides et à une utilisation prédominante des glucides pour une moindre intensité d'exercice

Objectifs : L'activité physique est recommandée dans le traitement du diabète de type 2 mais l'adaptation métabolique à l'exercice chez le diabétique est encore mal connue et n'est pas prise en compte dans les divers protocoles de réentraînement. Plusieurs anomalies des voies d'oxydation de substrats ont été décrites dans le diabète de type 2. Nous avons émis l'hypothèse que le diabète de type 2 est associé à des modifications de l'utilisation des substrats à l'exercice, indépendamment de l'âge, du sexe, du poids et du niveau d'activité physique.

Méthodes : 30 sujets sédentaires ayant un diabète de type 2 et 38 sujets non diabétiques appariés ont été recrutés. Nous avons mesuré les taux d'oxydation des lipides et des glucides à l'exercice par calorimétrie indirecte. Nous avons également calculé deux paramètres quantifiant l'utilisation respective de ces substrats en fonction de l'intensité croissante de l'effort : le point d'utilisation maximale des lipides (PLipoxMax) et le point de croisement (COP), niveau d'effort à partir duquel la contribution des glucides à la production d'énergie devient prédominante sur la contribution des lipides.

Résultats : l'oxydation des lipides était diminuée chez les sujets diabétiques à tous les paliers d'efforts. Le PLipoxMax et le COP étaient diminués chez les sujets [PLipoxMax = 25,3 ± 1,4 % vs 36,6 ± 1.7 % % Wmax (P < 0,0001)] – COP = 24,2 ± 2,2 % vs 38,8 ± 1,9 % % Wmax (P < 0,0001).

Conclusions : Les sujets diabétiques sédentaires présentent une diminution de l'oxydation des lipides à l'exercice et une utilisation prédominante des glucides pour des niveaux d'exercice moindres que les sujets non diabétiques sédentaires appariés pour le poids, l'âge et le sexe. La prise en compte de ces spécificités pourrait fournir les bases théoriques nécessaires à l'individualisation de l'intensité d'effort optimale.

Mots-clés : Diabète de type 2 · Intensité d'effort · Oxydation des substrats · Point de croisement.

Address correspondence and reprint requests to:
E Ghanassia. Service central de Physiologie Clinique, CERAMM, CHU de Montpellier, Hôpital Lapeyronie, 371, avenue Gaston Giraud, 34295 Montpellier Cedex 5, France.
eghanassia@aol.com

Received: March 28th, 2006; accepted: June 12th, 2006.

Exercise training has been recently demonstrated to be a powerful treatment for the prevention of type 2 diabetes (T2D) [1,2] and appears to be also promising for correcting most metabolic defects such as insulin resistance [3] and lipid disorders [4].

However, some degree of controversy remains about its impact on glycaemic control [5] and may be related to differences in protocols applied by various investigators. In fact, these exercise protocols have generally been defined according to theoretical concepts of exercise physiology, while the actual pattern of metabolic adaptation to exercise in patients suffering from T2D appears to be uncompletely known.

Since specific metabolic defects in the pathways involved in carbohydrate (CHO) and lipid oxidation have been described in T2D, we hypothesized that, independently of obesity, sedentarity and aging, T2D could influence substrate balance at exercise by its own.

In obese patients, we have developed a specific protocol of exercise calorimetry, designed to target training at a level at which the ability to oxidize lipids reaches a maximum [6]. Such a targeted training protocol has been shown to improve the ability to oxidize lipids and to reduce fat mass in both adult [7] and adolescent subjects [8], resulting in metabolic improvements including an increase in insulin sensitivity which is expected to improve glycaemic control in subjects with T2D. However, in T2D, optimizing CHO oxidation could be another way of normalizing glycaemia.

So, before extending the protocol we successfully used in obese subjects to T2D, it was necessary to investigate the effect of diabetes by its own on the respective percentage of lipids and CHO oxidized at various exercise intensities. This study was thus undertaken in order to describe the specific pattern of balance of substrates at exercise in T2D, which would represent a basis to propose targeted exercise.

Using exercise calorimetry we aimed at investigating substrate utilization at various exercise intensities in a group of sedentary diabetic subjects and at comparing them with a group of sedentary control subjects matched for age, sex and BMI.

Subjects, materials and methods

Subjects

We recruited 30 subjects with non insulin-requiring T2D and 38 subjects with non impaired glucose tolerance who came to our unit for a metabolic check-up. They were aged 40 to 65, sedentary (<2 hours of physical activity per week, including the everyday life) and overweight (BMI>25 kg/m^2).

Subjects with coronary heart disease, retinopathy, lung or muscular disease and those suffering from a disability preventing them from performing exercise testing (peripheral vascular disease, arthropathy) were excluded. Subjects with no data available concerning these criteria were also excluded. T2D was diagnosed using the criteria of the American Diabetes Association [9] and all control subjects had an oral glucose tolerance test (OGTT) performed during the previous year or between inclusion and the day of the test. Diabetic subjects treated with insulin or glitazones were also excluded.

All patients received detailed printed and oral information and gave their informed consent. The protocol was approved by the local ethics committee according to French legislation (law of March 5, 2002, N° 2002-1138 describing the rights of patients and the quality of the French health care system, and modifying the "Huriet-Sérusclat" law (N° 88-1138) which regulates biomedical research protocols.).

Anthropometric measurements

The day of the test, we measured weight (W), size (S), waist and hip circumferences (WC, HC). Waist to Hip Ratio (WHR) and BMI (W/S^2 in kg/m^2) were calculated.

Body composition was assessed with a multifrequency bioelectrical impedancemeter (Dietosystem Human IM Scan) that uses low intensity (100-800 μA) at the following frequencies : 1, 5, 10, 50 and 100 kHz. Analysis was performed with the software Master 1.0.

Biochemical analysis

In addition to the tests asked by their physician, all subjects were screened for fasting glycaemia. Plasma glucose was determined with a Vitros Product Chemistry Analyzer (Johnson & Johnson, Clinical Diagnostics, Rochester, USA) with routine well-standardized procedures.

Exercise testing

All subjects were asked to come and perform the testing in the morning after an overnight fast (at least 12 hours).

As generally used to individualize the increment of exercise intensity during cardiopulmonary exercise testing, the theoretical maximal aerobic power (Wmax), corresponding to the power reached when theoretical VO_2max is reached, was calculated from Wassermann's equations modified for overweight subjects [10].

The test consisted on five six minute steady-state workloads at 20, 30, 40, 50, and 60% of Wmax. Consequently, they underwent a test with the same relative incremental workload and were compared at the same percentage of their Wmax.

The subjects performed the test on an electromagnetically braked cycle ergometer (Ergoline Bosch 500). Heart rate and electrocardiographic parameters were monitored continuously throughout the test by standard 12-lead

procedures. Metabolic and ventilatory responses were assessed using a digital computer based breath to breath exercise analyzing system (CPX Medical Graphics, Minneapolis, Minnesota, USA). Thus we could measure VO_2, VCO_2 (in ml/min) and calculate the non-protein respiratory quotient (RER=VCO_2/ VO_2).

Calculation of substrate utilization, COP and PLipoxmax

Lipid oxidation (Lipox) and carbohydrate utilization (Glucox) rates were calculated by indirect calorimetry from gas exchange measurements according to the non-protein respiratory quotient technique and using Peronnet and Massicote's equations [11]:

– Glucox (mg/min)=4,585xVCO_2 – 3,2255xVO_2

– Lipox (mg/min)=1,6946xVO_2 – 1,7012xVCO_2

VO_2 and VCO_2 were determined as the mean of measurements during the fifth and sixth minutes of each step, where CO_2 production from bicarbonates to compensate for lactate acidosis becomes negligible [12].

This technique provided carbohydrate and lipid oxidation rates at different exercise intensities. These values were then converted in Kcal/min.

Additionally, after smoothing the curves, we calculated the two parameters quantifying the balance between carbohydrates and lipids induced by increasing exercise intensity: the maximal lipid oxidation point (PLipoxmax) and the Crossover Point (COP).

The PLipoxMax is the exercise intensity at which lipid oxidation reaches its maximal level before decreasing while carbohydrate utilization further increases. It is calculated as previously reported after smoothing of the curve plotting lipid oxidation as a function of power [6].

The crossover point (COP) is the exercise intensity at which the part of carbohydrate utilization used to provide energy becomes predominant over lipid oxidation. Beyond this point, the subject is referred to as "glucodependent". It was calculated as the exercise intensity where 70% of the substrates used to provide energy are carbohydrates and 30% are lipids, according to Brooks [13].

The PLipoxmax and the COP were expressed either in absolute power values (Watts) or in percentage of the theoretical Wmax.

Validity and reproducibility of this test were assessed in a previous publication. Coefficients of variation (CV) were calculated for RER, PLipoxMax and COP at four different intensities. CV of RER were between 2.8 and 4.75%. CV of PLipoxMax and COP was respectively 11.41%Wmax and 11.63%Wmax. VO_2, CO_2, RER, CHO and lipid oxidation rates were also compared during the incremental test and during steady-state workloads of the same intensity performed isolately and at random order. These parameters were not significantly different [6].

Statistical analysis

Results are given as mean ± standard error of the mean (SEM). Normality each parameter's distribution was assessed using the Shapiro-Wilk test.

If normality was established, we used the Student's t test for unmatched series. If normality was not established, we used the non-parametric Mann-Whitney's U test. Significance was set at $P<0.05$.

All calculations were performed with the software Xlstat-pro 7.5 (Addinsoft Software, Paris, France).

Results

Subjects characteristics

Anthropometric measurements are given in table I. There were no significant differences for age, BMI, fat-free mass, percentage of fat mass and Wmax (expressed in Watts or adjusted to fat-free mass and expressed in W/Kg of fat-free mass). WHR tended to be higher in the diabetic group but the difference was not significant. Sex ratio was

Table I
Characteristics of subjects (Values expressed as mean±SEM; BMI=Body mass Index; WHR=Waist/Hip circumferences Ratio; Wmax=Theoretical maximal aerobic power).

	Diabetic subjects			Control subjects		
	All (n=30)	Men (n=18)	Women (n=12)	All (n=38)	Men (n=23)	Women (n=15)
Age (years)	54±1.6	56.2±1.9	51.8±3	54±1.5	53.4±1.5	54.9±2.2
BMI (kg/m^2)	30.4±0.6	30.8±0.8	29.8±0.7	30.6±0.6	30.4±0.8	30.9±0.9
WHR	0.97±0.01	1±0.02	0.92±0.02	0.93±0.02	0.97±0.01	0.87±0.02
Fat mass (% of weight)	34.5±1.1	31±1.2	39.7±1.1	33.3±1.3	28.7±1.2	40.4±1.6
Fat-free mass (kg)	55.4±2.1	63.8±1.6	44±1.1	58.3±1.8	65.9±1.2	46.6±1.3
Wmax (W)	149±8.1	174.7±7.5	106.2±3.8	151.2±6.3	177.1±5.6	111.5±2.6

identical in both groups and no differences in any parameters were reported when gender was taken into account. Every subject completed the entire exercise protocol.

Fasting glycaemia was 8.44±0.37 mmol/l in the diabetic subjects group and 5.18±0.07 mmol/l in the control subjects group.

Ventilatory parameters

Results are given in table II.

VO_2 was not significantly different between the two groups, at rest or during any workload step, even after adjustment to fat-free mass.

RER was not significantly different between the two groups at rest but was significantly higher in the diabetic group, at every workload step (P<0.05).

Parameters of substrate utilization

Lipid oxidation rate (Lipox) at rest was not significantly different between the two groups. In contrast, during exercise, whatever the exercise intensity, Lipox was significantly lower in the diabetic group (figure 1A). The difference remained significant even when expressed in $ml^{-1}.min^{-1}.kg_{\text{fat-free mass}}^{-1}$ (figure 1B).

The COP was significantly lower in the diabetic group: 24.2±2.2% vs. 38.8±1.9% %Wmax (P<0.0001). The PLipoxMax was also significantly lower in the diabetic group: 25.3±1.4% vs. 36.6±1.7% %Wmax (P<0.0001). These differences remained significant even when gender was taken into account (figures 2 and 3).

Fasting glycaemia was positively correlated with COP (r=0.54, P<0.05) and PLipoxMax (r=0.5, P<0.05).

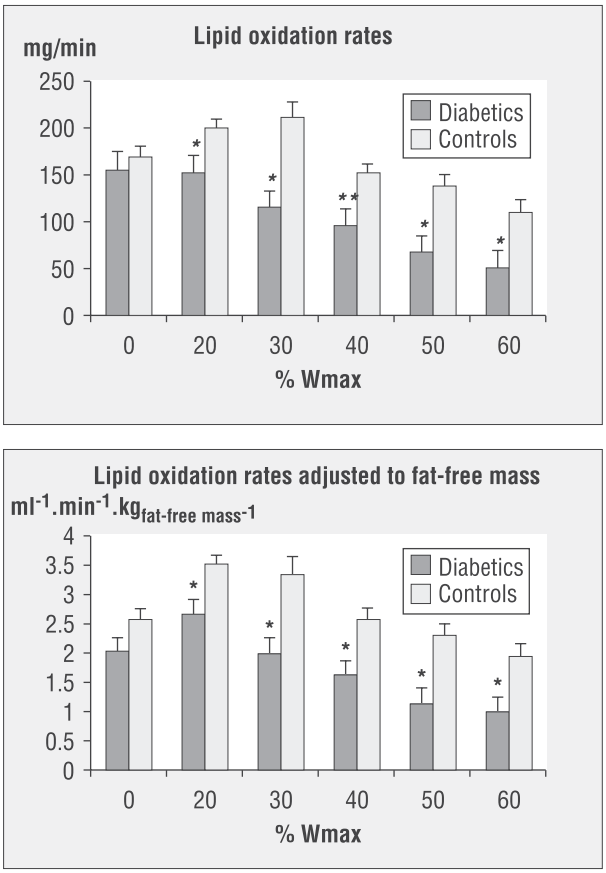

Figure 1
A: Lipid oxidation rates at rest and during exercise in diabetics and controls (Values expressed in mean ±SEM; * P<0.05; ** P<0.005 – Student's t test). B: Lipid oxidation rates at rest and during exercise in diabetic and control (Values expressed in mean ±SEM; * P<0.05; ** P<0.005 – Mann-Whitney's test).

Discussion

This study aimed at determining the parameters of substrate utilization at various exercise intensities in a group of sedentary subjects with T2D and at comparing them with a group of sedentary subjects without impaired glucose tolerance matched for age, sex and weight. Our results demonstrate that subjects with T2D exhibit a decrease in lipid oxidation and a predominance of carbohydrate utilization occurring at lower intensities when compared to control subjects.

Table II
Comparisons of VO_2 and R between diabetic subjects and control subjects at rest and during each workload step (Values expressed in mean ±SEM; *P<0.05; **P<0.01 – Mann-Whitney's test).

	VO_2 (ml/min)		VO_2 (ml/min/kg fat-free mass)		RER	
	Diabetic subjects	Control subjects	Diabetic subjects	Control subjects	Diabetic subjects	Control subjects
Rest	341.3±5.6	321.8±17.5	6.2±0.2	5.8±0.3	0.83±0.01	0.83±0.01
20%Wmax	843.6±43.7	832.1±29.2	15.2±0.6	14.3±0.3	0.9±0.01**	0.85±0.01
30%Wmax	1021.7±59.2	979.5±34.2	18.3±0.7	17.1±0.4	0.94±0.02**	0.9±0.008
40%Wmax	1143.9±78.5	1205.5±49.3	21.1±1	20.4±0.6	0.95±0.01*	0.92±0.007
50%Wmax	1390.5±90.5	1388±54.5	25.2±1.1	23.3±0.7	0.99±0.02**	0.94±0.004
60%Wmax	1601±110.5	1574±72	29.2±2.6	26.6±0.7	1±0.02**	0.95±0.006

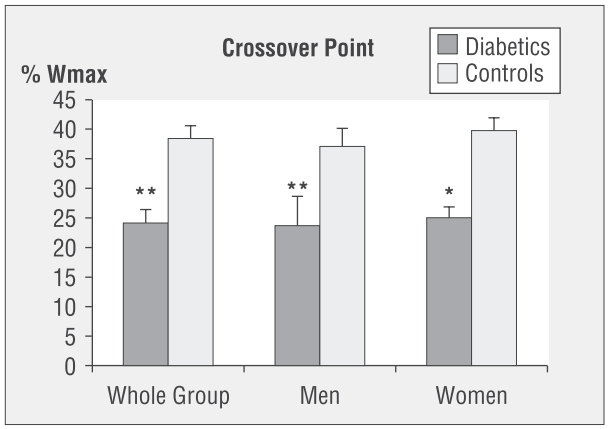

Figure 2
Comparison of the crossover point expressed in %Wmax chez in diabetic and control subjects (Values expressed in mean ±SEM; * P<0.05; ** P<0.0001 – Student's t test).

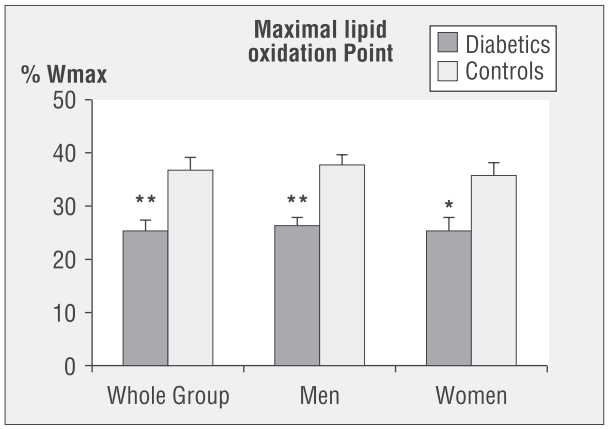

Figure 3
Comparison of the maximal lipid oxidation point expressed in %Wmax chez in diabetic and control subjects (Values expressed in mean ±SEM; * P<0.05; ** P<0.0001 – Student's t test).

Weight and training status interfere with the balance of substrate oxidation [6]. Our two groups were matched for age, BMI and training status. Thus, these parameters cannot explain our results. The exercise testing we used has already been described and its validity and reproducibility are assessed in a previous study [6].

Lipid oxidation rates were significantly lower at any exercise intensity in the diabetic group and the difference remained significant even after adjustment for fat-free mass. The PLipoxMax was also lower in the diabetic group. Consequently, maximal capacity of lipid oxidation is decreased in subjects with T2D. Our results contrast with those of previous studies [14,15] which did not conclude to a significant difference in lipid oxidation rates between diabetic and control subjects. However, the number of subjects was likely too small for a significant difference to appear. Moreover, those protocols were realized at one or two exercise intensities and subjects were not always matched for weight. As a matter of fact, the effects of exercise intensity on substrate utilization could not be studied.

The COP was significantly lower in subjects with T2D. Consequently, these subjects become glucodependent for exercise intensities lower than matched control subjects with normal glucose tolerance.

We conclude that T2D is associated with an alteration of substrate utilization during exercise with a decrease in lipid oxidation and an earlier shift towards a predominance of CHO oxidation for exercise intensities lower than subjects without impaired glucose tolerance. These results cannot be explained by either sex, age, weight or training status. Thus, we assume that metabolic defects specific to T2D can be responsible for these alterations.

Considering published data about substrate metabolism during exercise, it is noticeable that they often show discordant results. Two facts are likely to explain this: first, protocols designed for these studies use different frequencies, durations, intensities, exercise types and subjects are not always matched for their sex, age or training status. Next, phenomena well established in the resting state are sometimes extrapolated to the exercising state.

Two mechanisms resulting from exercise seems to initiate metabolic adaptations: muscular contraction and catecholamines production [16]. They cause a decrease in insulin secretion and a variable increase in glucagon production through neural afferences and α-adrenergic action. Then, energy substrate availability is increased with an increase in EGP and glucose uptake the synchronization of which leads to a remarkable maintain in glycaemia whereas decreased insulin-induced lipolysis provides a flux of non-esterified fatty acids. Inside muscle, substrate interactions, certainly different from what is described at rest such as Randle's glucose / fatty acid cycle or Winders' "Reverse Randle's cycle", modulate the proportion of CHO and lipids getting to mitochondria [17,18].

Defects in insulin secretion and hyperglycaemia, specific to T2D, are likely to interfere with all those mechanisms.

Defect in insulin secretion is a major characteristic of T2D. Its effects on substrate utilization during exercise in T2D remain poorly known. Muscular glucose uptake is decreased in subjects with type 1 diabetes despite normalized blood glucose and insulin levels [19]. However, in contrast with type 1 diabetes, insulinopenia is not absolute in T2D. Insulin secretion slowly deteriorates at various speeds depending of many factors such as the exposition of beta cells to glucotoxicity or lipotoxicity [20]. Further studies should be designed to quantify insulin secretion and kinetics during exercise and to determine their influence on substrate utilization.

Muscular substrate utilization is also influenced by the availability of these substrates. As shown in normal subjects, an increase in glucose availability causes a relative increase in glucose oxidation compared with lipid oxidation

[21]. Similarly, an increase in lipid availability causes an increase in muscular fatty acid oxidation [22]. Hyperglycaemia and lipid disorders are frequently associated in T2D and are both likely to interfere with substrate utilization with regard to their blood levels. According to our results, fasting glycaemia before exercise is positively correlated to COP and PLipoxMax in subjects with T2D.

Many abnormalities are described in the skeletal muscle of subjects with insulin resistance [23]. However, effects of insulin resistance on substrate utilization during exercise have been poorly studied. Previous works are very few, with too small a number of subjects and were not designed to delineate the effects of obesity, T2D and insulin resistance by their own on substrate utilization. Once more, they used only one exercise intensity and cannot establish a pattern of substrate utilization at various exercise intensities.

Recent guidelines recommend, in T2D, endurance training, at least 3 times a week, during 45 minutes (including a 5 minutes warm-up) at exercise intensities between 50 and 70% of Wmax [24,25]. These values, used by most studies referred to in these guidelines, appear to have been arbitrarily set. There seems to be no clear scientific evidence of a specific efficiency of training at these intensities rather than others in T2D.

Since the UKPDS, we know that an optimal glycaemic control with an HbA_{1c} <7% is crucial for preventing microangiopathic and neuropathic complications and greatly contributes to decrease cardiovascular events in T2D [26]. If physical activity is used as a specific therapy, it is mandatory to individualize an exercise intensity aiming at an optimal glycaemic control and the already proven benefits on cardiovascular events, other metabolic defects and general well-being.

Overweight status can contribute to impaired glucose tolerance and cardiovascular events in T2D. The use of parameters allowing the individualization of an exercise intensity at which lipid oxidation is maximal (such as the PLipoxMax) or at which there is a compromise between carbohydrate and lipid oxidation (such as the COP) seems logical. According to a recent study from our group, a 2-months training at the PLipoxMax of subjects with the metabolic syndrome results in a decrease in BMI, waist circumference and improves insulin sensibility, lipid oxidation with an increase in PLipoxmax and COP [7].

Moreover, efficiency of physical activity on T2D is dependent on the subject's long-term adherence to training. Many factors, specific to the patient or his environment can explain the lack of adherence. The use of PLipoxmax or COP as training intensity has two advantages likely to improve adherence. First, the feeling of the activity as strenuous or painful can result in training discontinuation. On the other hand, the intensities proposed by the guidelines are particularly high for such subjects cumulating factors of unconditionning as sedentarity or muscular abnormalities associated to insulin resistance. As far as the PLipoxMax and the COP are at lower ranges of values, the initial intensity of training would make it much easier to perform. Next, individualizing the training, whatever the way of choosing intensity, has proven effective for improving long-term adherence, whatever the behaviour asked to be changed [27].

However, improving insulin sensitivity and lipid oxidation is obviously not the only way of improving glycaemic control and the use of the COP or the PLipoxMax to set training intensity must prove its efficiency compared to the guidelines. A randomized controlled prospective study assessing the short-term and long-term effects of an individualized training using the COP, the PLipoxMax or perhaps another workload individually determined and compared to a training using the guidelines' intensities is necessary. Such a study should also assess the effects on long-term adherence to individualized training compared to training based on general theoretical guidelines.

References

1. Laaksonen DE, Lindstrom J, Lakka TA, Eriksson JG, Niskanen L, Wikstrom K, et al. Physical activity in the prevention of type 2 diabetes: the Finnish diabetes prevention study. Diabetes 2005;54:158-65.
2. Knowler WC, Barrett-Connor E, Fowler SE, Hamman RF, Lachin JM, Walker EA, et al. Reduction in the incidence of type 2 diabetes with lifestyle intervention or metformin. N Engl J Med 2002; 346:393-403.
3. Eriksson J, Taimela S, Koivisto VA. Exercise and the metabolic syndrome. Diabetologia 1997;40:125-35.
4. Lehmann R, Vokac A, Niedermann K, Agosti K, Spinas GA. Loss of abdominal fat and improvement of the cardiovascular risk profile by regular moderate exercise training in patients with NIDDM. Diabetologia 1995;38:1313-9.
5. Boule NG, Haddad E, Kenny GP, Wells GA, Sigal RJ. Effects of exercise on glycemic control and body mass in type 2 diabetes mellitus: a meta-analysis of controlled clinical trials. JAMA 2001;286:1218-27.
6. Perez-Martin A, Dumortier M, Raynaud E, Brun JF, Fedou C, Bringer J, et al. Balance of substrate oxidation during submaximal exercise in lean and obese people. Diabetes Metab 2001;27:466-74.
7. Dumortier M, Brandou F, Perez-Martin A, Fedou C, Mercier J, Brun JF. Low intensity endurance exercise targeted for lipid oxidation improves body composition and insulin sensitivity in patients with the metabolic syndrome. Diabetes Metab 2003;29:509-18.
8. Brandou F, Dumortier M, Garandeau P, Mercier J, Brun JF. Effects of a two-month rehabilitation program on substrate utilization during exercise in obese adolescents. Diabetes Metab 2003;29:20-7.
9. American Diabetes Association: clinical practice recommendations 1997. Diabetes Care. 1997;20 Suppl 1:S1-70.
10. Wasserman K, Hansen JE, Sue DY. Principles of exercise testing and interpretation: Including Pathophysiology and Clinical Applications. Eds Philadelphia, Lea & Febiger, 1986, 50-80.
11. Peronnet F, Massicotte D. Table of nonprotein respiratory quotient: an update. Can J Sport Sci 1991;16:23-9.

12. MacRae HS, Noakes TD, Dennis SC. Role of decreased carbohydrate oxidation on slower rises in ventilation with increasing exercise intensity after training. Eur J Appl Physiol Occup Physiol 1995;71:523-9.

13. Brooks GA, Mercier J. Balance of carbohydrate and lipid utilization during exercise: the "crossover" concept. J Appl Physiol 1994;76: 2253-61.

14. Blaak EE, van Aggel-Leijssen, Wagenmakers AJ, Saris WH, van Baak MA. Impaired oxidation of plasma-derived fatty acids in type 2 diabetic subjects during moderate-intensity exercise. Diabetes 2000;49:2102-7.

15. Borghouts LB, Wagenmakers AJ, Goyens PL, Keizer HA. Substrate utilization in non-obese Type II diabetic patients at rest and during exercise. Clin Sci (Lond). 2002;103:559-66.

16. Viru A. Plasma hormones and physical exercise. Int J Sports Med 1992;13:201-9.

17. Frayn KN. The glucose-fatty acid cycle: a physiological perspective. Biochem Soc Trans. 2003;31:1115-9.

18. Kiens B. Skeletal muscle lipid metabolism in exercise and insulin resistance. Physiol Rev 2006;86:205-43.

19. Riddell MC, Bar-Or O, Hollidge-Horvat M, Schwarcz HP, Heigenhauser GJ. Glucose ingestion and substrate utilization during exercise in boys with IDDM. J Appl Physiol. 2000;88:1239-46.

20. Poitout V, Robertson RP. Secondary beta-cell failure in type 2 diabetes – a convergence of glucotoxicity and lipotoxicity. Endocrinology 2002;143:339-42.

21. Coyle EF, Jeukendrup AE, Wagenmakers AJ, Saris WH. Fatty acid oxidation is directly regulated by carbohydrate metabolism during exercise. Am J Physiol 1997;273(2 Pt 1):E268-75.

22. Horowitz JF, Mora-Rodriguez R, Byerley LO, Coyle EF. Lipolytic suppression following carbohydrate ingestion limits fat oxidation during exercise. Am J Physiol 1997;273(4 Pt 1):E768-75.

23. Perez-Martin A, Raynaud E, Mercier J. Insulin resistance and associated metabolic abnormalities in muscle: effects of exercise. Obes Rev 2001;2:47-59.

24. Gautier JF, Berne C, Grimm JJ, Lobel B, Coliche V, Mollet E. Physical activity and diabetes. Diabetes Metab 1998;24:281-90.

25. Report of the Expert Committee on the Diagnosis and Classification of Diabetes Mellitus. Diabetes Care 1997;20:1183-97.

26. UK Prospective Diabetes Study (UKPDS) Group. Intensive blood-glucose control with sulphonylureas or insulin compared with conventional treatment and risk of complications in patients with type 2 diabetes (UKPDS 33). Lancet 1998;352:837-53.

27. Lerman I. Adherence to treatment: the key for avoiding long-term complications of diabetes. Arch Med Res 2005;36:300-6.