Clinical Hemorheology and Microcirculation 30 (2004) 339–343
IOS Press

Blood fluidity is related to the ability to oxidize lipids at exercise

Jean-Frédéric Brun [a,*], Emmanuelle Varlet-Marie [b], Delphine Cassan [a], Jérôme Manetta [a] and Jacques Mercier [a]

[a] *Service Central de Physiologie Clinique, Centre d'Exploration et de Réadaptation des Anomalies du Métabolisme Musculaire (CERAMM), CHU Lapeyronie, Montpellier, France*
[b] *Laboratoire de Pharmacocinétique Clinique, Faculté de Pharmacie, Université Montpellier I, France*

Abstract. We previously reported in rugbywomen correlations between RBC deformability and the ability to oxidize at exercise more lipids. This surprising finding might of course be spurious, or reflect the importance of the balance of substrates at exercise on baseline parameters that regulate blood rheology. Actually, the capacity of skeletal muscle to utilize either lipid or carbohydrate as fuels strongly influences whole body metabolism both at rest and during exercise. While the healthy skeletal muscle has substantial metabolic flexibility and is able to switch from predominantly lipid o oxidation during fasting or endurance exercise to increased glucose oxidation in conditions of insulin stimulation, obese individuals and those with type 2 diabetes manifest higher lipid oxidation during insulin-stimulated conditions despite lower rates of lipid oxidation during fasting or prolonged exercise. A low ability to oxidize and to periodically deplete triglyceride in muscle is associated with raised blood lipids. In addition, high carbohydrate oxidation rates in the mitochondrion are likely to promote more free radical generation. An increase in either blood lipids or free radicals is likely to induce profound hemorheological effects. We present here hemorheological studies in various populations with the use of exercise calorimetry in order to assess this switch of substrates. These studies further evidence negative correlations between the ability to oxidize lipids at exercise and parameters of blood viscosity. Correlations found between RBC deformability and the ability to oxidize at exercise more lipids may be due to effects of endurance training on lipid oxidation which may in turn modify both lipid metabolism and free radical generation, thus influencing RBC rheology.

Keywords: Blood viscosity, hemorheology, erythrocyte deformability, erythrocyte aggregability, substrate oxidation, mitochondrion, crossover concept

1. Introduction

We previously reported in rugbywomen [1] correlations between RBC deformability and the ability to oxidize at exercise more lipids. In other terms, in these athletes, beside other more classical findings already reported in male rugby players [2], we observed that the more the athlete was able to burn lipids at exercise, the more deformable were her red blood cells. This surprising finding might of course be spurious, but it was not meaningless and one could speculate that it reflected the importance of the balance of substrates at exercise on baseline parameters that regulate blood rheology.

*Corresponding author: Dr J.F. Brun, MD, PhD, Service Central de Physiologie Clinique, Centre d'Exploration et de Réadaptation des Anomalies du Métabolisme Musculaire (CERAMM), CHU Lapeyronie, 34295 Montpellier-cédex 5, France. Tel.: +33 04 67 33 82 84; Fax: +33 04 67 33 89 86; Telex: CHR MONTP 480 766 F; E-mail: drjfbrun@dixinet.com.

Therefore in this paper we try to collect further evidence for this phenomenon in our recent databases and to review the current information available in the literature that may explain this finding.

2. The balance of substrate oxidation at exercise: an important issue in physiology and pathophysiology

Skeletal muscle substrate metabolism has been the matter of many recent studies [3]. Energy mostly derives from CHO and lipids, while the contribution of proteins is only marginal in normal conditions (e.g., well-fed subjects). Mitochondrial oxidative phosphorylation is a major source of ATP. The control of the balance between lipid and CHO remains incompletely understood. It has long been assumed that in resting muscle, the glucose-fatty acid cycle [4,5] was operative. Actually this conception is no longer accepted, and it is rather considered that fat inhibits glucose oxidation via its interference with intracellular insulin action at a post-receptor level [6].

During exercise, the mechanisms of regulation have been extensively debated and some hypotheses have been purposed [7,8]. Briefly, during exercise, the balance between CHO and lipid oxidation depends on two opposite influences: the increase in CHO oxidation, proportional to the increase in exercise intensity, and the effect of training which increases the muscular capacity for lipid oxidation. While an increase in exercise intensity enhances contraction-induced muscle glycogenolysis and sympathetic nervous activity, endurance training increases lipid oxidation, and decreases sympathetic nervous responses. According to the concept proposed by Brooks and Mercier, the "crossover" point of substrate utilization is defined as the power at which energy from CHO-derived fuels predominates over energy from lipids. Further increases in power elicit a relative increment in CHO utilization and a decrement in lipid utilization. In normal subjects, the crossover point is reached at approximately 50 to 60% of maximal power (W_{max}). We have recently developed an exercise test allowing to determine substrate utilization during exercise [9] and this test has been employed to target exercise training in obese adults [10] and children [11]. During this test, glucose and lipid oxidation are determinated by indirect calorimetry, using non-protein respiratory exchange ratio (RER) during 4 steady-state exercise steps of 6 min duration. Other parameters of exercise calorimetry than the point of crossover can be calculated from this test in order to better describe the balance between carbohydrate and lipids: the LIPOXmax (i.e. the point where the lipid oxidation rate reaches a maximum), the maximum rate of lipid oxidation at the level of the LIPOXmax, and the glucidic cost of the watt [12] which is the slope of the almost linear relationship between power output and CHO oxidation. All these parameters provide a quantitative description, in standardized conditions, of body's metabolic adaptation to the level of exercise in terms of lipid and CHO oxidation. However, it is clear that different kinds of exercise result in different adaptations, and that, for example, when exercise prolongs at steady state there is a progressive adaptation of this balance of substrates that cannot be observed during our protocol with 6 min steps [13].

A recent sophistication of these concepts of balance of substrates is the notion of metabolic flexibility. Metabolic flexibility is the ability to switch from predominantly lipid oxidation during fasting or endurance exercise to increased glucose oxidation in conditions of insulin stimulation. This property is optimal in athletes and is lost in obese and diabetic subjects, for many genetic and acquired reasons, resulting in impairment in both lipid and CHO processing. In other terms, while the healthy skeletal muscle has thus a quite substantial metabolic flexibility and switch from predominantly lipid oxidation at fast (or during endurance exercise) to increased glucose oxidation under insulin stimulation, obese individuals and those with type 2 diabetes manifest higher lipid oxidation during insulin-stimulated conditions despite lower rates of lipid oxidation during fasting or prolonged exercise [14].

3. Evidence for relationships between the balance of substrates at exercise and hemorheology

We summarize here hemorheological studies in various populations with the use of exercise calorimetry in order to assess this switch of substrates.

In a study on 19 female rugby players [1] we observed that backward players (compared to forward) have a lower fat free mass and a lower fat mass, have a faster running speed during field testing as well as a higher $VO_{2\,max}$ (+17%), and that exercise calorimetry evidenced in a higher ability to oxidize lipids, as shown by a higher (+58%) "point of crossover" (70 vs. 43% of maximal power) and a higher LIPOXmax (59 vs. 40% max power). On the other hand their red cell rigidity was lower, resulting in a lower blood viscosity at high shear rate. Even more interestingly, there were correlations between hemorheological parameters and exercise metabolism. The LIPOXmax was correlated to whole blood viscosity and to the RBC rigidity index "Tk", while the crossover point was correlated to whole blood viscosity and to RBC rigidity.

Recently we performed a study on the opposite effects of endurance training and ageing [15,16]. This study evidenced very impressive training-related improvements in the GH-IGF-I axis, in glucose disposal, and in exercise calorimetry. Beside a host of parameters that were reported in the paper, we also measured (unpublished data) hemorheological parameters and, here again, we found some evidence regarding the relationships between the balance of substrates and hemorheology.

First of all, there was higher red cell rigidity and aggregability (assessed with laser retro diffusion with the SEFAM-Affibio device), and a lower RBC disaggregability at middle age than 25 yrs, regardless training status. We found no age-related difference in blood or plasma viscosity, hematocrit, and the Myrenne aggregation parameters. Training was found to reduce hematocrit in middle age subjects but not in 25 year old ones, and had no clear effect on the other hemorheologic parameters. The respiratory exchange ratio (RER $= VCO_2/VO_2$) at rest was strongly correlated to "M1". Since the higher is the RER, the more one oxidizes CHO, in terms of calorimetry, this correlation means "the more you burn CHO, the more your red cells aggregate". Besides, the maximal rate of lipid oxidation (at the point called above "LIPOXmax") is correlated to "M" aggregation index.

4. Hemorheology and the balance of substrate oxidation at exercise: working hypotheses

These correlations between RBC rheology (deformability and aggregability) and the ability to oxidize at exercise more lipids (i.e. a parameter of endurance) need to be further studied and confirmed on other groups of subjects. However, they seem to mean that:

- the more you burn lipids at exercise, the more your red cells are deformable and the less they aggregate;
- the more you preferently burn CHO, the more your red cells are rigid and aggregable.

Several metabolic consequences of the balance of substrates are likely to provide an explanation to these findings.

First of all, an increased use of lipids as a fuel rapidly reduces the body's stores of fat, as observed in our two recent studies that evidenced in adults and children that the earliest effect of endurance training is to increase lipid oxidation at exercise and to decrease body fatness. Body fat stores are by their own a strong regulator of blood rheology, due to the fact that their release fatty acids, cytokines, PAI-1, etc. as reviewed recently [17]. In addition, a low ability to oxidize and to periodically deplete triglyceride in muscle is associated with raised blood lipids [18].

However, another interesting aspect should be considered. High carbohydrate oxidation rates in the mitochondrion are known to promote more free radical generation [19]. High glucose fluxes increase production of electron donors from the tricarboxylic acid (TCA) cycle (NADH and $FADH_2$). This increases the membrane potential ($\Delta\mu_{H^+}$), because protons are pumped across the mitochondrial inner membrane in proportion to electron flux through the electron-transport chain. Inhibition of electron transport at Complex III by increased $\Delta\mu_{H^+}$ increases the half-life of free radical intermediates of coenzyme Q, which reduce O_2 to superoxide [20]. Oxygen free radicals can markedly affect RBC aggregability, as fairly demonstrated by Baskurt and Meiselman [21].

On the whole, altered balance of substrates may result in an increase in either blood lipids or free radicals that is likely to induce profound hemorheological effects. The more an individual oxidizes lipids, the better his red cell rheology is likely to be.

This hypothesis has still a weak experimental support, but needs to be now extensively studied in patients and athletes, by studies involving situations stat shift the balance of substrates.

5. Conclusion

We propose thus, on the basis of some recent findings of our team that require more extensive research, that correlations found between RBC deformability and the ability to oxidize at exercise more lipids may be due to effects of endurance training on lipid oxidation which may in turn modify both lipid metabolism and free radical generation, thus influencing RBC rheology. Experiments in order to further test this hypothesis are in progress in our department.

References

[1] D. Cassan, J.F. Brun, J.P. Micallef, M.J. Alemany, C. Sagnes and M.C. Granat, Relationships between hemorheological and metabolic adaptation to exercise in female rugby players: importance of erythrocyte rheology, in: *IIIrd international conference on Haemorheology (Proceedings)*, A. Muravyov, I. Tikhomirova, V. Yakusevitch, L.G. Zaitsev, A.G. Gushin, I.A. Bakanova, D.V. Borisov and O.N. Semenova, eds, Yaroslavl, Russia 2003, p. 58.

[2] D. Bouix, C. Peyreigne, E. Raynaud, J.F. Monnier, J.P. Micallef and J.F. Brun, Relationships among body composition, hemorheology and exercise performance in rugbymen, *Clin. Hemorheol. Microcirc.* **19** (1998), 245–254.

[3] A. Perez-Martin, E. Raynaud and J. Mercier, Insulin resistance and associated metabolic abnormalities in muscle: effects of exercise, *Obes. Rev*. **2**(1) (2001), 47–59.

[4] P.J. Randle, C.N. Hales, P.B. Garland and E.A. Newsholme, The glucose-fatty acid cycle. Its role in insulin sensitivity and the metabolic disturbances of diabetes mellitus, *Lancet* **1** (1963), 785–789.

[5] P.J. Randle, E.A. Newsholme and P.B. Garland, Regulation of glucose uptake by muscle. Effects of fatty acids, ketone bodies and pyruvate, and of alloxan-diabetes and starvation, on the uptake and metabolic fate of glucose in rat heart and diaphragm muscle, *Biochem. J.* **93** (1964), 652–665.

[6] G.I. Shulman, Cellular mechanisms of insulin resistance, *J. Clin. Invest.* **106** (2000), 171–176.

[7] G.A. Brooks, Importance the "crossover" concept in exercise metabolism, *Clin. Exp. Pharmacol. Physiol.* **24** (1997), 889–895.

[8] G.A. Brooks and J. Mercier, Balance of carbohydrate and lipid utilization during exercise: the "crossover" concept, *J. Appl. Physiol.* **76** (1994), 2253–2261.

[9] A. Perez-Martin, M. Dumortier, E. Raynaud, J.F. Brun, C. Fédou, J. Bringer and J. Mercier, Balance of substrate oxidation during submaximal exercise in lean and obese people, *Diabetes Metab.* **27** (2001), 466–474.

[10] M. Dumortier, F. Brandou, A. Pérez-Martin, C. Fédou, J. Mercier and J.F. Brun, Low intensity endurance exercise targeted for lipid oxidation improves body composition and insulin sensitivity in patients with the metabolic syndrome, *Diabetes Metab.* **29**(5) (2003), 509–518.

[11] F. Brandou, M. Dumortier, P. Garandeau, J. Mercier and J.F. Brun, Effects of a two-months rehabilitation program on substrate utilization during exercise in obese adolescents, *Diabetes Metab.* **29** (2003), 20–27.

[12] I. Aloulou, J.F. Brun and J. Mercier, Le coût glucidique du watt: une constante biologique?, *Sci. Sports* **17** (2002), 315–317.
[13] J. Manetta, A. Perez-Martin, J.F. Brun, A. Callis, C. Préfaut and J. Mercier, Fuel oxidation during exercise in middle-aged men: effect of training and glucose disposal, *Med. Sci. Sports Exerc.* **34**(3) (2002), 423–429.
[14] D.E. Kelley and B.H. Goodpaster, Skeletal muscle triglyceride. An aspect of regional adiposity and insulin resistance, *Diabetes Care* **24**(5) (2001), 933–941.
[15] J. Manetta, J.F. Brun, A. Callis, J. Mercier and C. Prefaut, Insulin and non-insulin-dependent glucose disposal in middle-aged and young athletes versus sedentary men, *Metabolism* **50**(3) (2001), 349–354.
[16] J. Manetta, J.F. Brun, L. Maimoun, A. Callis, C. Prefaut and J. Mercier, Effect of training on the GH/IGF-I axis during exercise in middle-aged men: relationship to glucose homeostasis, *Am. J. Physiol. Endocrinol. Metab.* **283**(5) (2002), E929–E936.
[17] J.F. Brun, Hormones, metabolism and body composition as major determinants of blood rheology: potential pathophysiological meaning, *Clin. Hemorheol. Microcirc*. **26**(2) (2002), 63–79.
[18] B.H. Goodpaster, R.R. Wolfe and D.E. Kelley, Effects of obesity on substrate utilization during exercise, *Obes. Res.* **10**(7) (2002), 575–584.
[19] X.M. Leverve and E. Fontaine, Role of substrates in the regulation of mitochondrial function in situ, *IUBMB Life* **52**(3–5) (2001), 221–229.
[20] M. Brownlee, A radical explanation for glucose-induced beta cell dysfunction, *J. Clin. Invest.* **112**(12) (2003), 1788–1790.
[21] O.K. Baskurt and H.J. Meiselman, Activated polymorphonuclear leukocytes affect red blood cell aggregability, *J. Leukoc. Biol.* **63**(1) (1998), 89–93.