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Abstract. We previously reported that low intensity endurance training in sedentary patients suffering from the metabolic syndrome improves blood rheology, mostly due to a decrease in plasma viscosity correlated with an increase in cardiorespiratory fitness. We investigated whether these findings can be extended to type-2 diabetics. 22 diabetics (11 women and 10 men, age: 52.00 ± 2.9 yr, BMI: 32.47 ± 1.17 kg/m^2) were tested before and after 2 months. Eight of them were trained (2 to 3×45 min/wk) at the power intensity where lipid oxidation reaches a maximum (LIPOX max) and thirteen served as controls. Over this period the only significant hemorheological effect of training was a decrease in RBC aggregation "M" (-1.25 ± 0.357 $p = 0.01$) in the trained group. Subjects who lost weight exhibited a decrease in plasma viscosity (from 1.46 ± 0.013 to 1.38 ± 0.02 $p < 0.01$). Changes in waist circumference are associated with changes in hematocrit ($r = -0.952$ $p = 0.01$); plasma viscosity ($r = -0.91$; $p = 0.03$); RBC aggregation ("M" $r = 0.940$; $p = 0.02$). Subjects can also be divided into those who improved their aerobic capacity VO_{2max} and those whose VO_{2max} decreased or remained unchanged. An increase in VO_{2max} is associated with a decrease in whole blood viscosity ($r = -0.79$ $p = 0.06$) explained by an improvement in RBC rigidity "Tk" ($r = -0.963$ $p = 0.002$). This study suggests that in Type 2 diabetic patients: (a) viscosity factors might be less responsive to training than in non diabetic individuals; (b) visceral fat loss is the main determinant of changes in hematocrit, plasma viscosity and RBC aggregation; (c) improvements in aerobic capacity improves blood viscosity via an increase in RBC deformability.

Keywords: Diabetes, exercise training, hemorheology, plasma viscosity

1. Introduction

Exercise training is a major tool in the management of obesity [36] and type 2 diabetes [38, 39], whose efficiency has been underestimated until very recently [2, 42]. It is able to improve body composition [14], carbohydrate homeostasis [1, 34], lipid profile [27, 37], inflammation [16] and endothelial function [3, 28].

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Since obesity and diabetes are both associated with hemorheologic abnormalities that are strongly related to inflammation and lipids [7], it is interesting to assess whether exercise improves blood rheology in these diseases and whether these improvements are related to metabolic and circulatory ones.

There are some reports of hemorheologic effects of training in athletes or in obese patients exhibiting metabolic disorders [5]. By contrast, despite the wideness of literature dealing with hemorheology in diabetes, whether regular exercise reverses diabetes-induced hemorheologic disturbances is poorly known.

Therefore, this study was undertaken in order to investigate whether an exercise protocol that has previously been shown to reverse hemorheologic alterations in obese patients exhibiting metabolic disorders has a similar effect in type-2 diabetes.

2. Experimental

2.1. Subjects

We recruited type 2 diabetic participants who met the following inclusion criteria: 1) type 2 diabetes duration of 1–10 years according to the primary care physician's diagnosis; 2) aged between 30 and 65 years; 3) body mass index (BMI) >25 kg/m^2; 4) HbA1C levels 7–10%, 5) plasma triglyceride levels 160–400 mg/dl; 6) creatinine <1.4 mg/dl; and 7) $\geq$3 months on the same medications before entering the study.

The sample comprised 21 diabetics (11 women and 10 men, age: 52.00 ± 2.9 yr, BMI: 32.47 ± 1.17 kg/m^2). Study subjects characteristics are shown on Table 1. All subjects were tested before and after 2 months. Eight of them were trained (3×45 min/wk) at a level defined by exercise calorimetry and corresponding to the power at which lipid oxidation reaches a maximum ($LIPOX_{max}$) as explained below and thirteen served as controls. The two groups were matched for age and body mass index as shown on Table 1.

2.2. Bioelectrical impedance measurements

Prior to the exercise-test, subjects' body composition was assessed with bioimpedance analysis with a six terminal impedance plethismograph BIACORPUS RX 4000 Biacorpus RX4000, (Healthnesslink, 8 avenue Jean-Jaurès 92130 Issy-les-Moulineaux, France) with data analysis with the software BodyComp 8.4. This device measures total resistance of the body to an alternative electric current of 50 kHz [11]. Body fat mass, fat-free mass were calculated in each segment of the body according to manufacturer's database-derived disclosed equations, and total water with published equations using the classical cylindric model and Hanai's mixture theory [25].

Table 1

Anthropometry, body composition, and maximal oxygen consumption (VO_{2max}) of the 21 study patients, showing the matching of the two subgroups

	Age (years)	BMI (kg/m^2)	WHR	VO_2max ml·min^{-1}·kg^{-1}	Hb_{A1c} (%)
Trained ($n = 8$) 6F/2M	53.78 ± 4.51	31.59 ± 1.57	0.94 ± 0.04	17.63 ± 2.66	7.00 ± 0.82
Untrained ($n = 13$) 5F/8M	50.77 ± 4.01	33.07 ± 1.69	0.92 ± 0.03	17.85 ± 2.69	7.63 ± 0.61

All subjects were asked to come and perform the testing in the morning after an overnight fast (at least 12 hours). Exercise testing was performed on an electromagnetically braked cycle ergometer to a breath by breath device for gas exchange measurements (VO_2 and VCO_2). Before exercise, we calculated the theoretical maximal aerobic power (W_{maxth}) using Wasserman's equations [40]. As generally used to individualize the increment of exercise testing, the workload of each step was calculated from the theoretical maximal aerobic power (W_{maxth}), *i.e.*, power corresponding to the theoretical VO_{2max}. In consequence, the subjects underwent a test with the same relative incremental workload and were compared at the same percentage of their W_{maxth}.

The test consisted of a progressive five six-minute steady-state workloads corresponding to 20, 30, 40, 50, 60% of W_{maxth}. Pedal frequency was maintained between 60 and 70 rpm throughout the test. Heart rate and electrocardiographic parameters were monitored continuously throughout the test by standard 12-lead procedures. Blood pressure was measured before, during and at the end of the exercise testing. The test finishes at the end of the thirtieth minute by an active recovery.

Since the exercise test did not reach the maximum, aerobic capacity was calculated from the submaximal workloads according to the American College of Sports Medicine (ACSM) with a linear extrapolation of VO_2 at the theoretical maximal heart rate [23, 26].

Whole body substrate oxidation was calculated from the measurement of VO_2 and VCO_2 during last three-minutes of each workload with classical stoichiometric equations:

$$Glucox(mg/min) = 4.585 \times VCO_2 – 3.2255 \times VO_2 \tag{1}$$

$$Lipox(mg/min) = 1.6946 \times VO_2 – 1.7012 \times VCO_2 \tag{2}$$

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 ($Lipox_{max}$) [6] and the Crossover Point (COP) [4]. The $Lipox_{max}$ is the exercise intensity at which lipid oxidation reaches its maximal level before decreasing as carbohydrate utilization increases. It is calculated from the above equations, considering that the Lipox formula can be simplified as:

$$Lipox = 1.7 \times (1 - R) \times VO_2 \tag{3}$$

This formula is easy to derivate and the point where its derivative is equal to zero is assumed to be the $Lipox_{max}$. This point is used for targeting according to Brun et al. [8, 10].

2.4. Exercise training

Exercise training was targeted on lipid oxidation according to the protocol we previously reported [1, 21].

Training was carried out over 10 weeks. The first session included a blood test, as well as an exercise test as described above. Then subjects were asked to come three times a week to exercise for a duration of two months. Actually the mean attendance was rather twice a week on the average. The training protocol was initiated on the second week in out laboratory; under the control of a physician and a nurse. Its total duration was eight weeks. It took place at 1 p.m. to 4 p.m. in the afternoon. During the exercise bout,

heart rate was monitored by using a cardiofrequency measurement. During the tenth week, the second exercise testing and blood check up were carried out.

2.5. *Hemorheological ex vivo measurements*

Blood samples for hemorheological measurements (7 ml) were drawn with potassium EDTA as the anticoagulant in a vacuum tube (Vacutainer). Viscometric measurements were done at very high shear rate (1000 s^{-1}) with a falling ball viscometer (MT 90 Medicatest, F-86280 Saint Benoit) [19]. The coefficient of variation of this method ranges between 0.6 and 0.8%. We measured with this device apparent viscosity of whole blood at native hematocrit, plasma viscosity, and blood viscosity at corrected hematocrit (45%) according to the equation of Quemada [32].

$$\eta = \eta_p(1 - 1/2k\phi)^{-2} \tag{4}$$

- where ϕ is hematocrit, η_p is plasma viscosity, and $k(\gamma)$ is a shear-dependent parameter quantifying the contribution of erythrocyte rheological properties to whole blood viscosity.

- At the high shear rate used here $k(\gamma)$ is representative of red cell rigidity (*i.e.*, the lower $k(\gamma)$, the higher is erythrocyte deformability).

With this equation it is possible to standardize η for hematocrit 45% after calculating k:

$$k = 2 \cdot (1 - \eta r^{-0.5})\phi^{-1} \tag{5}$$

This value of k is reintroduced in Equation (4) with ϕ set at 0.45. Dintenfass's 'Tk' index [18] was also calculated as an index of red cell rigidity.

RBC aggregation was assessed with the Myrenne aggregometer [35] which gives two indices of RBC aggregation: "M" (aggregation during stasis after shearing at 600 s^{-1}) and "M1" (facilitated aggregation at low shear rate after shearing at 600 s^{-1}). It was also measured with laser backscattering (erythroagregometer SEFAM – AFFIBIO) [20]. Hematocrit was measured with microcentrifuge.

2.6. *Statistics*

Values are presented as mean ± standard error of the mean (SEM). Normality of samples was checked with the Kolmogorov-Smirnov test. After verification of normality, we used ANOVA followed by post-hoc Student's t test for paired samples. Correlations were assessed with Pearson's procedure (least square fitting). A value of $p < 0.05$ was considered as significant.

2.7. *Ethical approval*

This study was performed in accordance of the Helsinki declaration and in agreement with the local ethical guidelines.

3. Results

3.1. Effects of training on selected parameters

Training did not significantly improve VO_{2max} (before: 16.1 ± 1.9; after: 17.9 ± 2.05 ml/min/kg NS) in this sample, but markedly increased the ability to oxidize lipids during exercise as measured by the crossover point (before: 43.77 ± 8.75; after: $69.38 \pm 10.05\%$ P_{max} $p < 0.01$), the $LIPOX_{max}$ (before: 38.5 ± 6.04; after: $63.8 \pm 9.4\%$ P_{max} $p < 0.01$), and the maximal lipid oxidation rate at exercise (before: 135.06 ± 24.63; after: 179.27 ± 29.25 mg/min $p < 0.01$). Besides, there was a significant weight loss in the trained group (-3.0 ± 0.78 kg; $p < 0.01$) while in the untrained group there was a non significant trend to weight gain ($+1.28 \pm 0.45$ $p < 0.10$). Over this period lipid parameters and HbA1c were not significantly changed, despite a tendency to improvement in the trained group.

3.2. Effects of training on on hemorheological parameters

Over this period the only significant effects of training on hemorheological parameters was a decrease in RBC aggregation "M" (-1.25 ± 0.357 $p = 0.01$) in the trained group, while other hemorheologic effects previously reported in non diabetic subjects were not evidenced (Table 2).

3.3. Hemorheological effects of weight loss

Actually, regardless they were trained or not, 13 of the 22 subjects lose weight (-0.2 to -9 kg) while 8 did not lose (or sometimes gained) weight (0 to 3.5 kg). As shown on Fig. 1., those who lose weight exhibited a decrease in plasma viscosity (from 1.46 ± 0.013 to 1.38 ± 0.02 mPa.s; $p < 0.01$) while it did not change when weight did not decrease (from 1.45 ± 0.03 to $1.39 \pm 0{,}04$ mPa.s; NS).

3.4. Hemorheological effects of improvement in aerobic capacity

Subjects can also be divided into those who improved their aerobic capacity VO_2max and those who VO_{2max} decreased or remained unchanged. An increase in VO_{2max} is associated with a decrease in whole blood viscosity ($r = -0.7936$ $p = 0.0595$) explained by an improvement in Tk ($r = -0.96332$ $p = 0.00199$) while neither plasma nor hematocrit change, and resulting in an improvement in the hematocrit/viscosity ratio ($r = 0.94156$ $p = 0.00502$).

3.5. Correlations

Correlations are shown on Table 3. Changes in BMI exhibit correlation on the edge of significance with changes in plasma viscosity and RBC aggregation. Changes in waist circumference are correlated with changes in hematocrit, plasma viscosity, and RBC aggregation. Changes in waist to hip ratio (WHR), are associated with changes in hematocrit and RBC aggregation "M". Changes in plasma viscosity were related to weight changes. Changes in waist circumference were correlated to: hematocrit, plasma viscosity and red cell aggregation. Changes in WHR were correlated to those of hematocrit and red cell aggregation "M".

Table 2
Hemorheologic parameters before and after training in type 2 diabetic subjects. $^{*}p = 0.01$

	Hct		Whole blood viscosity (mPa.s)		Plasma viscosity (mPa.s)		Whole blood viscosity at Hematocrit 45% (mPa.s)		RBC aggregation Myrenne index 'M'		RBC aggregation Myrenne index 'M1'		RBC viscometric index of rigidity 'Tk'		Hematocrit/ viscosity ratio	
	before	after	before	after	before	after	before	after	before	after	before	after	before	after	before	after
Trained ($n = 8$)	44.00	45.50	3.34	3.18	1.48	1.48	3.39	3.17	5.25	4.00	10.20	9.78	0.63	0.58	13.19	14.44
6F/2M	±3.34	±0.96	±0.28	±0.14	±0.02	±0.05	±0.05	±0.20	±0.54	±0.58*	±0.82	±0.53	±0.01	±0.03	±0.20	±0.95
Untrained ($n = 13$)	43.25	41.10	2.71	2.70	1.46	1.35	3.10	2.80	6.65	5.60	12.25	11.51	0.54	0.60	16.63	15.60
5F/8M	±1.80	±1.06	±0.45	±0.20	±0.07	±0.04	±0.37	±0.20	±0.39	±0.58	±0.49	±0.56	±0.12	±0.07	±2.54	±0.98

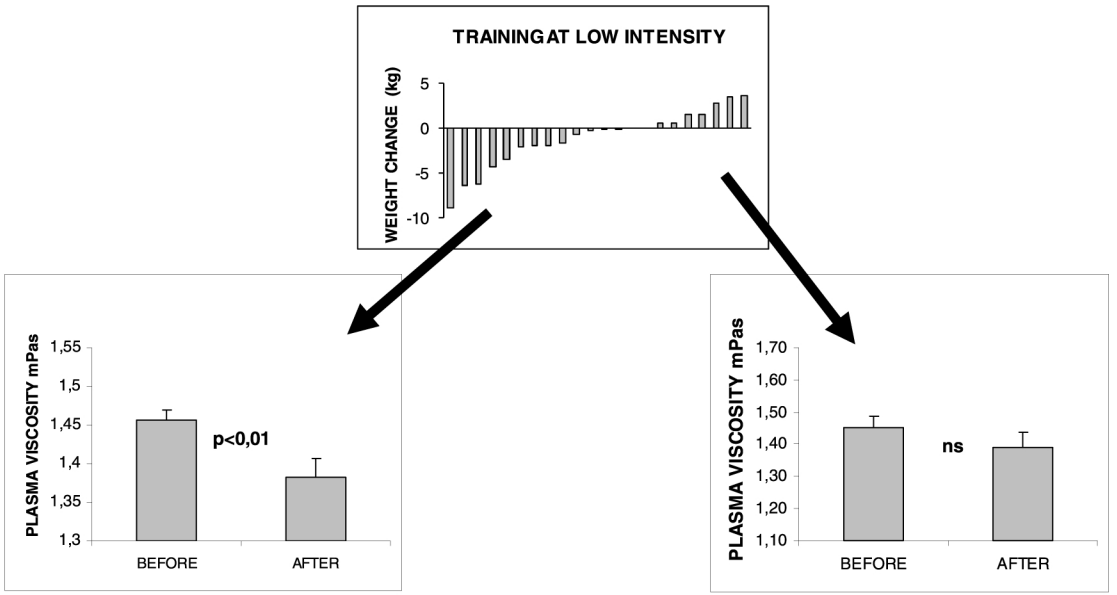

Fig. 1. Upper panel: individual modifications of body weight, allowing to split into two subgroups the sample of subjects. Plasma viscosity decreases only in the subgroup of subjects that lose weight and is not significantly modified in the subgroup of subjects who don't.

Table 3
Correlations among changes in the various parameters measured before and after the study period

	Whole blood viscosity	Hematocrit	Plasma viscosity	RBC deformability	RBC aggregation "M"
BMI	$r = -0.5510$	$r = -0.7270$	$r = -0.8782$	$r = -0.1180$	$r = 0.8670$
	NS	NS	$p = 0.05$	NS	$p = 0.06$
Waist	$r = -0.5540$	$r = -0.9523$	$r = -0.9066$	$r = 0.6610$	$r = 0.9399$
	NS	$p = 0.01$	$p = 0.03$	NS	$p = 0.02$
Waist to Hip Ratio	$r = -0.6560$	$r = -0.9671$	$r = -0.7760$	$r = 0.2740$	$r = 0.9828$
	NS	$p = 0.007$	NS	NS	$p = 0.003$
VO_{2max}	$r = -0.7936$	$r = -0.2950$	$r = -0.3410$	$r = -0.9633$	$r = 0.5290$
	$p = 0.06$	NS	NS	$p = 0.002$	NS
LDL cholesterol	$r = -0.0325$	$r = -0.0534$	$r = 0.2360$	$r = -0.3110$	$r = 0.2940$
	NS	NS	NS	NS	NS
Triglycerides	$r = 0.7890$	$r = 0.1680$	$r = 0.1140$	$r = 0.8078$	$r = 0.5360$
	$p = 0.06$	NS	NS	$p = 0.05$	NS

4. Discussion

This study shows that 10 weeks of low intensity exercise training targeted on the level of maximal lipid oxidation in diabetic subjects decreases erythrocyte aggregation. Other hemorheological changes are observed when training is associated with changes in other parameters. A decrease in plasma vis-

cosity is only found in patients that lose weight. Changes in hematocrit are related to changes in waist circumference. Changes in VO_{2max} are associated with a hemorheologic improvement consisting of an increase in red cell deformability and hematocrit/viscosity ratio.

The main limitation of this pilot study is the low number of subjects. It needs therefore to be extended to a higher number of patients. Nevertheless it clearly evidences effects of this kind of training on blood viscosity.

The exercise protocol requires some comments. Current guidelines for physical activity from numerous public health agencies recommend in type-2 diabetes at least 150 min per week of moderate to vigorous aerobic-based exercise [15]. Such guidelines are based on incontrovertible evidence from observational and randomised clinical trials demonstrating that regular physical activity contributes to the primary and secondary prevention of type-2 diabete, coronary heart disease and all-cause mortality [17, 29, 31]. Even more, the improvement in blood glucose control as reflected by HbA_{1c} levels is greater if exercise is performed on a structured manner, and is proportional to exercise volume [38, 39]. The interest of high intensity protocols and resistance exercise remains controversial while endurance even at low intensity has unequivocally proven its efficiency [13].

In our team since fifteen years we propose an alternative paradigm based on the ability to oxidize lipids at exercise [10, 33]. The rationale for this approach is that low intensity endurance is the most natural variety of exercise performed by humans since prehistory, and thus adapted to the "thrifty genotype" [11]. In addition both type-2 diabetes and obesity are due to excess lipid accumulation so that it is logic to target on lipid oxidation. Moreover, lipid burning exercise exerts satiating effects that improve their weight-reducing efficiency [9]. Therefore, in diabetics, this approach yields better metabolic effects on blood glucose than endurance interval training although the latter is more efficient on blood pressure and cholesterol [30].

In preceding studies we reported that this variety of exercise training applied to non diabetic sedentary subjects exhibiting the metabolic syndrome decreases plasma viscosity with no change in either hematocrit, red cell rigidity or red cell aggregation. Aloulou et al. [1] further investigated this issue and reported that changes in RBC rigidity appeared to reflect weight loss and decrease in LDL cholesterol. Plasma viscosity was related to cholesterol. Changes in plasma viscosity were related to those of VO_{2max}. Red cell aggregation reflected both the circulating lipids (Cholesterol, HDL and LDL) and the ability to oxidize lipids at exercise. Factors associated to a post-training decrease in aggregation ("M1") were weight loss and more precisely decrease in fat mass, improvement in lipid oxidation, rise in HDL-Cholesterol, and decrease in fibrinogen. On the whole the major determinant of hemorheologic improvement was an increase in cardiorespiratory fitness (VO_{2max}), correlated with a decrease in plasma viscosity, rather than an improvement in lipid metabolism, although RBC aggregation and deformability exhibited clear relationships with lipid metabolism. In that study we noticed that Hct increased in 30% of the patients during training and this finding was reported as intriguing.

In our sample of type-2 diabetics the picture is not completely different but there are clear discrepancies. The first discrepancy is that plasma viscosity does not decrease unless subjects lose weight. Since it is more difficult to induce a weight loss in diabetics than in non diabetic subjects [41], this may account for the lesser effect of exercise training on hemorheologic parameters in these patients. In diabetics plasma viscosity may have direct relationships with vascular hindrance if microvascular vasodilatory response to viscous resistance is impaired. Therefore it is interesting to know that this important determinant of blood viscosity is not easy to change during such exercise protocols in type-2 diabetics and that a weight loss seems to be required to significantly reduce it. Actually, there is a nonsignificant tendency to a decrease in plasma viscosity even in the group who does not lose weight, suggesting that in a larger series of patients

a significant improvement of plasma viscosity due to training could be evidenced even if there were no weight loss. This issue remains to be investigated.

Another difference that is interesting to point out deals with the hemorheological determinants of aerobic capacity. Changes in VO_{2max} are negatively associated with those of whole blood viscosity, but in contrast to what is found in metabolic syndrome this effect is not related to changes in plasma viscosity but those of RBC rigidity index "Tk" ($r = -0.963$ $p = 0.002$) while neither plasma viscosity nor hematocrit change, and resulting in an improvement in the hematocrit to viscosity ratio ($r = 0.942$ $p = 0.005$).

Maximal oxygen consumption (VO_{2max}) is an individual's measurement of physical working capacity representing the point where the linear relationship between oxygen uptake (VO_2) and mechanical power attains a plateau which cannot be overcome despite further increases of power. The mechanism explaining this limitation was classically described by the "oxygen cascade theory", which indicates that oxygen transfer follows a series of steps from ambient air to the mitochondria and that one or several of them can become limiting. Limitation can come from the cardiovascular oxygen transport capacity, from the size of muscle mass, or from intrinsic muscle oxidative capacity [22]. In the case of this study, a combination of muscle mass and metabolic efficiency, together with vascular factors (capillary network, viscosity factors) is likely to be involved.

In this study, we observe that changes in hematocrit are related to those of waist circumference. This finding extends our previous reports of correlations between hematocrit and abdominal size in subjects with the metabolic syndrome [24] and also in athletes. This study shows that training-related changes in abdominal size are correlated to changes in hematocrit. This is in agreement with our previous speculation that the relationship between abdominal fatness and hematocrit is actually not due to obesity but to this 'android' morphotype even in lean and regularly exercising subjects [12].

5. Conclusions

In summary this study suggests that in type-2 diabetics: (a) viscosity factors might be less responsive to training than in non diabetic individuals; (b) visceral fat loss is the main determinant of changes in hematocrit, plasma viscosity and RBC aggregation; (c) improvements in aerobic capacity is associated with an improvement in blood viscosity via an increase in RBC deformability.

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List of symbols: ACSM: American College of Sports Medicine; BMI: body mass index; COP: Crossoverpoint of substrate oxidation according to Brooks and Mercier; HbA1C: glycated haemoglobin; Hct: hematocrit; HDL: high density lipoprotein; $LIPOX_{max}$: level of maximal lipid oxidation during exercise; LDL: low density lipoproptein; Pmax: maximal power output; RBC: red blood cell; SEM: standard error on the mean; VO_{2max}: maximal oxygen cosumption; WHR: waist-to hip ratio.

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