Partially opposite hemorheological effects of aging and training at middle age.

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1. Abstract

Aging impairs blood rheology while various training protocols improve it. The purpose of this study was to delineate the respective role of aging and endurance training on blood rheology. Thirty-two subjects [16 middle-aged men: 8 cyclists (MAcy) and 8 sedentary men (MAsed) and 16 young men : 8 cyclists (Ycy) and 8 sedentary men (Ysed)] were compared in this study. Results showed higher red blood cell (RBC) rigidity and aggregability (AFFIBIO), lower RBC disaggregability (AFFIBIO) at middle age than at 25 yr, regardless of training status. However there was no age-related difference in whole blood viscosity at either native or corrected hematocrit, plasma viscosity, hematocrit, and Myrenne aggregation indexes M and M1. Training was associated with a reduced hematocrit in middle age subjects but not in 25 yr old ones. We

evidenced no effect of trainingon red cell rigidity (Dintenfass's Tk index), in whole blood viscosity at either native or corrected hematocrit, and plasma viscosity. Thus, regular cycling at middle age maintains a low hematocrit but does not prevent aging-related increase in red cell rigidity and aggregability. Specific effects of cycling among other sports may explain this specific pattern.

Key words: exercise training, hemorheology, aging, viscosity, hematocrit, erythrocyte aggregability, cyclism

2. Introduction

Aging impairs blood rheology [1]. On the other hand, various training protocols improve it [2]. In a recent study, we compared the effects of aging and training on glucose disposal [3], somatotropic axis [4] and the balance of substrates oxidized at exercise [5]. This study extends this comparison to blood rheology, based on data measured on the same selected samples of subjects. Basically, this study aimed thus at delineating the respective roles of aging and endurance training on blood rheology by comparing four matched groups of subjects: 1) middle-aged (ca 50 yr) cyclists; 2) middle-aged sedentary men; 3) young (ca 25 yr) trained cyclists; young sedentary men.

3. **Materials and Methods**

Subjects.

Sixteen male cyclists, [eight young (24.7 ± 1.4 yr) elite cyclists (Ycy) and eight middle-aged (51.6 ± 1.2 yr) cyclists (MAcy)] and sixteen sedentary males, [eight young (23.9 ± 0.8 yr) men (Ysed) and eight middle-aged (52.3 ± 1.1) men (MAsed)] participated in the study. None had a family history of diabetes or hypertension. Smokers or those currently using medication for the control of blood pressure and lipid or carbohydrate metabolism were excluded. No subject exhibited electrocardiogram abnormalities at rest or during a maximal ergocycle test. Physical characteristics of all subjects are shown in Table 1. The training program for the middle-aged

cyclists was carried out as a group activity and amounted to almost 10 hours of cycling per week. These cyclists had been following this training schedule for the past 10 ± 1.5 (SEM) years. The training program for the young elite cyclists was also carried out in a group and amounted to almost 17 hours of training per week. Subjects had followed this training schedule for the past 7 ± 1.2 (SEM) years. None of the sedentary subjects participated in competitive sports or organized leisure time activities. After a complete and accurate verbal description of the procedure, risks and benefits associated with the study, subjects provided their written consent.

Protocol

The subjects came to the laboratory on two separate occasions for an intravenous glucose tolerance test and a maximal aerobic capacity test. All subjects were requested to refrain from exercise for the three days before the glucose tolerance test. Methods and results concerning the measurements of glucose disposal are reported elsewhere [3].

The subject's VO_{2max} was measured during 8-12 min of exercise performed on an electronically-braked cycle ergometer (550 ERG, Bosch, Germany). Fractions of oxygen and carbon dioxide in the expired air were measured by a mass spectrometer (Marquette MGA 1100, Blagnac, France). Heart rate was monitored throughout the exercise test. Exercise testing was started with a 3-min warm-up at 40 W. The workload was increased by steps of 20 W for the sedentary group and 30

W for the trained group every minute until maximal exercise was reached. This was evaluated in terms of maximal heart rate, RER values (>1.15) and O_2 consumption (VO_2) stability.

Body composition was assessed with a four-terminal impedance plethysmograph Dietosystem Human IM-Scan.

Hemorheological 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 high shear rate (1000 s^{-1}) with a falling ball viscometer (MT 90 Medicatest, F-86280 Saint Benoit) [6]. The coefficient of variation of this method ranged between 0.6 and 0.8% [6]. With this device we measured apparent viscosity of whole blood at native hematocrit, plasma viscosity, and blood viscosity at corrected hematocrit (0.45) according to the equation of Quemada [7]. Dintenfass' 'Tk' index of erythrocyte rigidity was calculated [8]. RBC aggregation was assessed with the Myrenne aggregometer [9] 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}).

The SEFAM aggregometer was used for a more precise assessment of RBC aggregation. This device measures the changes in backscattered light which are observed when sheared RBC suspensions are abruptly brought to a full stop. The decrease in the optical signal reflects the formation of RBC aggregates [10]. Some parameters are derived from the curve of light intensity

as a function of time. The aggregation time (TA) is the reciprocal of the initial slope (calculated between 0.5 and 2 sec after the shear has stopped). The aggregation index at 10 sec (S10) is a measurement of the extent of erythrocyte aggregation and is the relative surface area above the curve calculated over the first 10 seconds and the aggregation index at 60 sec (S60) is a measurement of the extent of erythrocyte aggregation and is the relative surface area above the curve calculated over the first 60 seconds. This device measures also disaggregation thresholds, by submitting blood to a succession of shear rates from 600 s^{-1} to 7 s^{-1}. The total disaggregation threshold (γS) is the shear rate below which the backscattered light intensity starts to decrease, indicating that the shear stress applied to aggregates is no longer sufficient for allowing complete dispersion of RBC aggregates. The partial disaggregation shear rate (γD) is defined as the shear rate corresponding to the intersection point of the two asymptotes drawn from the extremes (maximum and minimum shear rate).

Statistics

Data are expressed as means ± SE. To detect differences between parameters represented by a single measurement, non-parametric tests for unpaired (Mann-Whitney) and paired (Wilcoxon) data were used as appropriate. Correlations were performed by Pearson analysis and multiple regression analysis. Normality of parameters was assessed with the normality test of Kolmogorov and Smirnov. This test gives a K-S Distance and a p value that allow to conclude that the test "passes" or "fails". A test that fails indicates that the data varies significantly from the pattern expected if the data was drawn from a population with a normal distribution. A test

that passes indicates that the data matches the pattern expected if the data was drawn from a population with a normal distribution. $P < 0.05$ was considered significant.

4.Results.

Subjects were matched for height, weight, BMI and fat (%) in each category of age (Table 1). VO_{2max} was higher in the cyclists than in the sedentary subjects independently of age and it was higher in the younger than in the older subjects (Table 1).

Parameters of glucose disposal in these four subgroups have been described elsewhere [3]. To summarize, very striking differences in insulin sensitivity (SI) among the subgroups were evidenced. SI was lower (- 165 %, $P< 0.05$) in MAsed than in Ysed and was higher in Ycy than in Ysed (+100 %, $P<0.05$. It was higher (+31 %, $P<0.05$) in MAcy than in MAsed. It was correlated with the VO_{2max} ($R= 0.76$, $P<0.01$, $n=16$). Thus endurance training improved SI and Sg in all subjects regardless of the age ($P<0.05$), but an increase in GEZI was only found in young men ($P<0.05$). An effect of aging was found in sedentary subjects (who exhibited a lower SI ($P<0.05$) when older). However this effect disappeared with training since SI was almost similar in trained young and middle-aged subjects.

Results for hemorheologic parameters are shown on Table 2. There was a higher red cell rigidity at middle age than at 25 yr in the sedentary subjects but not in the cyclists. There was a higher RBC aggregability (AFFIBIO) and a lower RBC disaggregability (AFFIBIO) at middle age than

at 25 yr, regardless of the training status. However there was no age-related difference in whole blood viscosity at either native or corrected hematocrit, plasma viscosity, hematocrit, and Myrenne aggregation indexes M and M1. Training was associated with a reduced hematocrit in middle age subjects but not in 25 yr old ones. We evidenced no significant training effect on red cell rigidity (Dintenfass's Tk index), in whole blood viscosity at either native or corrected hematocrit, and plasma viscosity. In trained cyclists regardless age a surprising non significant tendency towards a higher RBC rigidity index "Tk" compared to sedentary subjects was observed.

3. Discussion

This study is a part of a wider protocol which has investigated the correction of aging's effects on metabolism by regular training. In comparison with the marked improvements induced by training on glucose disposal [3], growth hormone and IGF-I axis [4] and fuel oxidation at exercise [5], the effects of training on the aging-related effects of blood rheology are not striking. This study shows that regular cycling at middle age maintains a low hematocrit but does not prevent aging-related increase in red cell rigidity and aggregability.

This study is cross-sectional in nature, so that one could theoretically suggest that rather than demonstrating the effects of training at middle age, it rather indicates that the subjects who train have a specific biological profile that explain their ability to exercise. This

assumption is very unlikely, put together with the whole body of international literature. On the whole, the findings of the other parts of this study have demonstrated that training reverses most of the aging-related decline in physiological functions [3-5]. Even more, its interest is to show the effects of a heavy training at 25 and 50 yr, and it seems rather unrealistic to imagine that a longitudinal study could be performed to investigate this issue. In totally untrained individuals, it would be extremely difficult to reach such levels of intense regular training.

Clearly, the effects of heavy training in leisure cyclists are impressive: they include massive improvements in insulin sensitivity, growth hormone release, catecholamines, and fuel oxidation at exercise. On the whole, 50-yr aged cyclists have for these functions a physiological response which is almost the same as 25-yr athletes, ie markedly above that of 25-yr sedentary subjects and even more of 50-yr sedentary people.

More recently, a separate study on the same topic [11] has emphasized the pivotal role of adrenal response. Training-induced changes in catecholamine release are likely to explain most of the previously described phenomena. At 25-yr and at 50-yr, a stronger catecholamine response during exercise is probably an important mechanism for the improvements described above. Since catecholamines are well known to modify the rheologic properties of erythrocytes [12], they may explain some of our findings. For example, the lack of improvement in RBC flexibility in the group of young trained subjects may be related to this phenomenon. However, intense oxidant stress in these subjects submitted to heavy and prolonged workloads may also play a role in this process. Presumably, specific effects of cycling amond other sports may also explain at least in part

this specific pattern. In this study, we cannot delineate the role of exercise-induced changes in the lipid profile, but it is clear that circulating lipoproteins are major regulators of blood rheology [12] and are probably involved in age-related alterations in blood fluidity [13] so that this question deserves further consideration.

On the whole, this study shows that regular cycling at middle age maintains a low hematocrit but does not prevent aging-related increase in red cell rigidity and aggregability, despite the drammatic improvements it induces in other physiological functions. The reason for this paradox remains to be more specifically investigated.

4. References

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Table 1: Baseline characteristics for cyclists and sedentary subjects

	Sedentary Subjects		Cyclists	
	Middle-aged (n=8)	**Young (n=8)**	**Middle-aged (n=8)**	**Young (n=8)**
Age, yr	52.3 ± 1.1	23.9 ± 0.8**	51.6 ± 1.2	24.7 ± 1.4&
Height, cm	173.3 ± 1.9	179.2 ± 1.6	173.6 ± 1.3	178.4 ± 1.9
Weight, kg	75.4 ± 2.4	70.9 ± 2.6	72.1 ± 1.1	69.2 ± 2
BMI, kg/m²	25.1 ± 0.6	22.1± 0.4	23.9 ± 0.3	21.7 ± 0.6&
Fat, %	22.8 ± 1.5	15.1 ± 2.8	20.3 ± 0.8	12.8 ± 0.5&
VO_{2max}, ml/min/kg	33.3 ± 1.1	47.4 ± 2.2**	50.41± 2.3#	64.1 ± 3.2*,&

Values are means ± SE; BMI: Body mass index, * Significant difference between trained vs sedentary in young group, $P<0.05$. # Significant difference between trained vs sedentary in middle-aged group, $P<0.05$. **Significant difference between young vs middle-aged in sedentary group, $P<0.05$. & Significant difference between young vs middle-aged in trained group, $P<0.05$.

Table 2: Hemorheological parameters in cyclists and sedentary subjects

	Sedentary Subjects		Trained Cyclists	
	Middle-aged (n=8)	**Young (n=8)**	**Middle-aged (n=8)**	**Young (n=8)**
η blood	2.82 ± 0.21	2.62 ± 0.04	2.78± 0.13	3.09 ± 0.08
η pl	1.2 ± 0.06	1.35 ± 0.02	1.27 ± 0.02	1.50 ± 0.09
η45	2.91± 0.12	2.96 ± 0.06	3.05 ± 014	3.54 ± 0.1
Hct (%)	42.7 ± 1.2	39.9 ± 1.2	41 ± 0.63 *	40.5 ± 1.5
TK	0.65 ± 0.06	0.57 ± 0.02 #	0.59 ± 0.12	0.68 ± 0.1
TA	2.9 ± 0.2	3.54 ± 0.55#	2.55 ± 0.21	3.54 ± 0.35
TF	41.78 ± 2.2	46.4 ± 5.9	41.33 ± 3.62	47.4 ± 4.66
γS (s^{-1})	106.6 ± 8.9	86.47 ± 12.4 #	107.5 ± 9.6	84.18 ± 7.8
γS (s^{-1})	54.6 ± 2.6	51.5 ± 5.04	47.4 ± 1.54	44.87 ± 1.8
S10	22.15 ± 0.8	22.27 ± 1.8	23.34 ± 1.5	20.15 ± 1.7
S60	39.7 ± 1	39.25 ± 1.7	42.1 ± 1.66	40.27 ± 1.3
M	4.84 ± 0.8	3.8 ± 0.4	4.76 ± 0.44	3.87 ± 0.5
M1	8.32 ± 1.6	7.78 ± 0.27	8.07 ± 0.65	7.92 ± 0.5

Values are means ± SE; * Significant difference between trained vs sedentary, $P<0.05$. # Significant difference between young vs middle-aged, $P<0.05$.