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Post-Exercise Decrease of Plasma Hyaluronan: Increased Clearance or Diminished Production?

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Summary

The exercise-induced increase and post-exercise decrease of plasma hyaluronan concentration were studied in human subjects. Six well trained men performed incremental exercise until exhaustion (MAX), intensive (submaximal, SUB) and extensive exercise (moderate, MOD) on a bicycle ergometer, defined as work at 100, 77 and 50 % of maximal oxygen consumption. Hyaluronan was analyzed using a high-sensitivity, proteoglycan-dependent time-resolved immunoassay and hemoglobin, hematocrit and plasma protein levels were assessed using standard laboratory procedures. Compared to resting control levels, the plasma hyaluronan concentration (pHA) increased ($p<0.05$) by 76 % (65.0±6.1 vs. 37.0±1.0 µg/l) during 15 min MAX, by 44% (56.4±2.6 vs. 39.2±3.8 µg/l) during 30 min SUB and by 27 % (46.3±7.8 vs. 36.4±4.3 µg/l) during 90 min MOD. The increase with time averaged 4.03 %.min^{-1} during MAX, 1.35%.min^{-1} during SUB and 0.35 %.min^{-1} during MOD. After exercise (15 and 30 min), pHA decreased by 43 % below resting levels after MAX ($p<0.05$) and by 36 % after SUB, respectively. In conclusion, pHA steadily rose with time during physical exertion, with a non-linear increase of concentration/time slope with exercise intensity; second, the magnitude of the post-exercise pHA decrease was proportional to the exercise-induced pHA increase, suggesting elevated hyaluronan clearance with rising plasma levels after physical exertion.

Key words
Hyaluronan • Hyaluronic acid • Lymph flow • Physical exercise • Plasma volume

Introduction

Hyaluronan is a widely distributed extracellular polysaccharide which serves in bone formation, cartilage structural maintenance, lubrication, wound healing, tissue transport, and is able to interact with the immune system (Laurent *et al.* 1992, 1996). Extracellular hyaluronan concentration far exceeds that in the bloodstream because of rapid degradation by hepatic and other endothelium after lymphatic discharge (Erikson *et al.* 1983, Tengblad *et al.* 1986, Reed and Laurent 1992, Reed *et al.* 1994, Laurent *et al.* 1996). Thus, the intravascular hyaluronan pool is being constantly removed, whereas the extravascular pool serves its physiological tissue functions (Fraser and Laurent 1989, Levick 1996).

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Increased lymph propulsion during exercise fosters hyaluronan transport into the circulation (Rowell 1993), and some effects of physical exercise on plasma hyaluronan concentration (pHA) have been reported (Engström-Laurent and Hällgren 1987, Tulamo *et al.* 1990, Piehl-Aulin *et al.* 1991). However, no clear dose-response relations have been indicated, particularly not those concerning in the decrease of pHA after muscular activity. Previous studies have shown increases in pHA with exercise; however, our study has shown a regimented and incremental increase of pHA with the workload at three levels of exercise.

The purpose of this study was to use exercise of different intensity as a model for elevating pHA by physiological means, and to quantitate the consecutive decrease during recovery. We hypothesized that there is indication of altered hyaluronan clearance from the intravascular compartment after muscular activity of different intensities. We present evidence of increased hyaluronan removal from the macrocirculation after higher exercise loads.

Methods

Subjects

Six healthy, non-smoking well trained men (29±1 yr, 76±3 kg, 181±3 cm, maximal oxygen intake 63±1 ml/kg/min) participated in the study. They were free from medication and gave their informed consent after having been explained the experimental procedure. The project was approved by the University Review Board.

Protocol and study design

Exercise was performed on a Monark Ergomed (Sweden) bicycle ergometer. O_2 intake/CO_2 output was determined using an Oxycon (Jaeger, Germany) open system and the heart rate was assessed with a Sporttester (Polar Electro, Finland). For MAX, an incremental test was applied; the work load started at 40W and was increased by 20W every minute until voluntary exhaustion (average test duration approx. 15 min). Both endurance loads started as the incremental test at 40 W and the work load was increased by 20W per minute until the target load (77 % maximum oxygen intake for SUB, 50 % for MOD) was reached. Exercise lasted 30 (SUB) and 90 min (MOD), respectively, to achieve sufficient increases in pHA. The tested persons were allowed to drink 250 ml water during the final phases of MOD and SUB, respectively. An interval of at least 4 days was chosen in-between the tests. Six days before and during the experimental period (total 2 weeks), the subjects received an isocaloric diet consisting of 55 % carbohydrate, 30 % fat, and 15 % protein. All experiments were carried out at 22-24 °C room temperature (40-70 % relative humidity).

Blood sampling and analysis

15 ml of blood were withdrawn from an antecubital vein at each sampling time with a 20-gauge teflon catheter. Blood was taken every 5 min during MAX, every 10 min during SUB and every 30 min during MOD; plus 15 and 45 min after MAX, and 30 and 60 min after SUB and MOD, respectively. Samples were drawn into vacutainer tubes containing dry disodium EDTA (1 mg/ml) and cooled in ice. After centrifugation at 3000 rpm for 10 min, plasma was stored at –20 °C.

pHA was determined using a proteoglycan dependent time resolved immunoassay of high sensitivity (Rössler 1998). Duplicate measurements were made to determine hemoglobin concentrations (Hb) using the cyanohemoglobin method, and fractional hematocrit (Hct) by microcentrifugation without correcting for trapped plasma. Total plasma protein concentration was assessed using the Biuret method (Merck). Initial plasma volume was estimated according to Sawka *et al.* (1992). Since microvascular fluid filtration influences macromolecular plasma concentrations *per se*, pHA was corrected for hemoglobin/hematocrit changes on a sample-to-sample basis during and after exercise.

Statistics

Results are presented as means ± S.E.M. A repeated-measures ANOVA was applied to test for overall significance of pHA changes with time, and Tukey's honest significant difference test to compare between points in time. The null hypothesis was rejected if $p<0.05$.

Results

MAX elevated pHA by 76 % from 37±1 to 65±6 µg/l in 15 min, corresponding to a 4.03 %.min^{-1} rise according to a linear best-fit. 15 and 45 min after finishing the exercise, pHA was below pre-exercise control values (-43±5 and -32±9 %, respectively). With SUB, the values were +43 % (from 39±4 to 56±3 µg/l) in 30 min, corresponding to 1.35 %.min^{-1}; 30 and 60 min after exercise, pHA was 35±9 and 30±2 % below control values, respectively. The 27 % pHA rise with 90 min MOD (from 37±4 to 42±8 µg/l) corresponded to a 0.35

%.min^{-1} increase in time (Table 1). Figure 1 depicts the average pHA time course, Fig. 2a the linear fits of pHA increase with time during exercise, and Fig. 2b the resulting slopes as a function of exercise load. Table 1 also shows changes in total plasma protein concentration, hemoglobin and hematocrit.

Table 1. pHA, plasma TP, hemoglobin concentration, and hematocrit values (mean±S.E.M.) before and at end of exercise, and during recovery.

	Rest	**End of exercise**	**Recovery**	
	0 min		**30 min**	**60 min**
			15 min (MAX)	**45 min (MAX)**
Plasma hyaluronan concentration (µg/l)				
Max	37.0±1.0	65.0±6.1*	21.2±2.3*	25.6±3.7
Sub	39.2±3.8	56.4±2.5*	25.0±3.4*	27.6±0.8
Mod	36.4±3.3	46.3±7.0	34.6±3.2	35.0±3.2
Plasma total protein concentration (g/l)				
Max	66.9±1.1	73.0±1.5*	68.0±0.6	67.5±0.7
Sub	67.1±1.1	71.3±0.8*	67.5±0.9	67.7±0.9
Mod	66.9±0.9	70.3±1.3*	68.2±0.7	67.8±0.4
Hemoglobin(g/dl)				
Max	15.6±0.4	16.8±0.5*	15.5±0.3	15.4±0.4*
Sub	14.6±0.4	15.6±0.4*	14.3±0.4	14.4±0.3
Mod	15.0±0.4	15.4±0.4*	14.8±0.4	15.6±0.5
Hematocrit (Vol %)				
Max	46.4±0.8	50.4±0.8*	45.9±0.6	45.7±0.6*
Sub	44.6±1.2	47.3±0.8*	43.6±0.8	43.7±0.8
Mod	45.2±0.9	46.7±0.8*	44.7±0.9	45.9±0.8

**P<0.05 compared to their respective resting control level.*

Discussion

In adults aged 20-60 years, pHA normally ranges from 10 to 100 µg/l with a mean between 30-40 µg/l (Eriksson *et al.* 1991, Fraser *et al.* 1981) as seen in our subjects. The average molecular weight in human plasma is in the range of 140 kD, making it all but impermeable to most capillary walls (Tengblad *et al.* 1986). Factors like the diet, posture, activity, or clinical deviations influence pHA to a different extent (Engström-Laurent and Hällgren 1987, Gegout *et al.* 1991, Onarheim *et al.* 1991, Rössler *et al.* 1998). Animal experiments suggested a salient role of physical exercise for promoting hyaluronan turnover between tissue matrix and the circulatory system (Schad and Berchtelsbauer 1977, Tulamo *et al.* 1990).

Hyaluronan influx to the circulatory system.

Interstitial hyaluronan concentration is usually one to two orders of magnitude higher than pHA, with considerable interindividual differences (Laurent and Laurent 1981, Laurent and Fraser 1992, Laurent *et al.* 1996). Therefore, increased lymphatic propulsion can be expected to increase pHA. Hyaluronan output from the tissue spaces *via* the lymphatics is assisted by muscular activity, which also elevates interstitial pressure (Guyton *et al.* 1976, Laurent and Laurent 1981, Fraser and Laurent 1989, Rowell 1993. Lymph flow is particularly enhanced when large muscle groups exercise >65% of their maximal capacity, e.g. a 300W work load was found to double pHA (Engström-Laurent and Hällgren 1987).

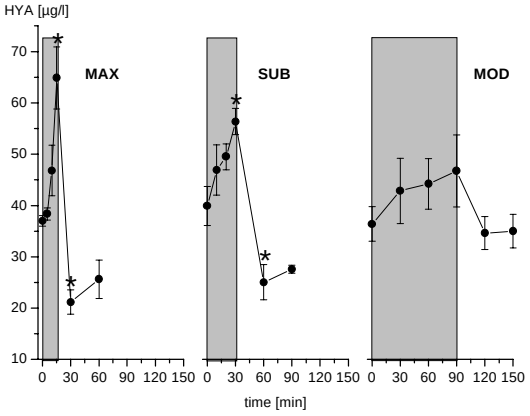

Fig. 1. *Plasma hyaluronan concentration (HYA mean ± S.E.M.) in 6 subjects at the beginning (time=0), during and after exercise of maximal (MAX; 15 min), submaximal (SUB; 30 min) and moderate (MOD; 90 min) intensity. Asterisks indicate significant differences to compared pre-exercise values.*

Since exercise promotes fluid loss in the microcirculation, the concentration of macromolecules left behind in the bloodstream increases. From the changes in hematocrit and hemoglobin (3) and plasma protein concentration (van Beaumont *et al.* 1973), an average plasma volume decrease of 12 and 10 % can be estimated for the end of the MAX and SUB experiments of this study. Consequently, only a small increase of pHA would be expected; indeed, plasma protein concentration only rose by 9 and 6 %, respectively, during MAX and SUB. The much larger rise of pHA (76 and 43 %) must therefore have been mainly due to an increased distribution into the bloodstream; capillary fluid loss contributed much less to this effect, perhaps by 10-15 %.

Besides increased lymphatic output, there is another possible reason for the rise of pHA during exercise. Pulmonary microvessels contain transitorily immobilized hyaluronan (Lebel *et al.* 1988); with increased cardiac output, vascular recruitment within previously underperfused areas ensues (Coates *et al.* 1993), and more hyaluronan could thus enter the macrocirculation. Despite probably unaltered pulmonary lymph flow during exercise-induced hyperventilation or tachypnea (Bland *et al.* 1977, Martin *et al.* 1983), this mechanism could increase pHA during exercise.

Hyaluronan clearance from the circulation.

Intravenously administered labeled hyaluronan is rapidly cleared from the bloodstream (t/2=2-6 min; Reed *et al.* 1990, Reed and Laurent 1992). The increase of pHA seems to boost its elimination by hepatic, kidney and splenic endothelial hyaluronidase (Tengblad *et al.* 1986, Fraser and Laurent 1989, Kreil 1995, Laurent *et al.* 1996, Natowicz and Wang 1996). Accelerated clearance of hyaluronan from a tissue might serve an important function as an edema safety factor (Lebel *et al.* 1988). Uptake in the reticuloendothelial system is carried out by a receptor mechanism and cellular metabolization; an increase of this degradation may be involved in the observed post exercise fall of pHA.

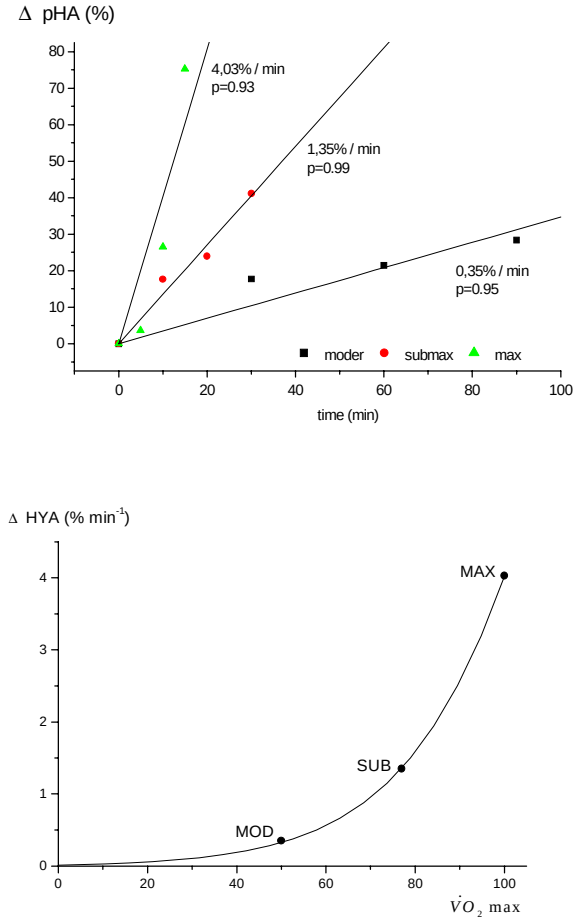

Fig. 2. *(a) Linear best fit of plasma hyaluronan concentration with exercise duration (in % of pre-exercise control values) in MAX, SUB and MOD. The pHA increase is probably a mixed effect of the incremental work load and test duration. See text for details. (b) Slope of linear pHA (=HYA) increase (from Fig. 2a) as a function of work intensity.*

Our results support the view that elevated pHA as induced by physical exercise increases the elimination of plasma hyaluronan from the (macro)circulation. Although lymph protein concentration decreases during

exercise by up to 80 % (Schad and Berchtelsbauer 1977), a mere 'washout' effect cannot explain the magnitude of the observed pHA decrease after exercise. Only greater hyaluronan degradation and/or removal, proportional to the amount that reaches the circulation, seems to explain our observations: intensified hyaluronan input is followed by increased clearance. The hepatic saturation limit for hyaluronan degradation was not reached in this study since this would require about a 10-fold value of the steady state level (Fraser and Laurent 1989). This would be way beyond the levels seen in our test subjects. Enhanced hepatic clearance may occur because of preferential uptake of high molecular weight hyaluronan (Laurent *et al.* 1986) that might escape degradation in the lymph nodes due to increased lymph flow. If exercise-induced tissue heating is able to fasten receptor-mediated endocytosis in hepatic endothelial cells is unknown.

Although blood contains hyaluronidase (as well as hyaluronidase inhibitors), since the enzyme is not active except at very low pH (Roden *et al.* 1989), hyaluronan is not degraded in the plasma (Tengblad *et al.* 1986). According to the findings of molecular weight limitations in renal hyaluronan clearance (Fraser *et al.* 1981), variations of renal perfusion are also unlikely to contribute to the observed effects in pHA.

The lungs could cause, or at least contribute to, the post-exercise fall of pHA below pre-exercise control levels. If pulmonary microvessels discharge an extra amount of 'trapped' hyaluronan with increased cardiac output (Lebel *et al.* 1988, Coates *et al.* 1993), the reverse could occur after exercise: hyaluronan redistribution back to an immobilized pool. With promoted degradation at the same time, this could well lower pHA below pre-exercise levels.

This study has limitations because the urinary output of hyaluronan is unknown, the group of subjects was small, and no women were included. However, our results not only indicate increased hyaluronan turnover during exercise – confirming previous findings – but also after exercise had been discontinued. We could show that the amount of hyaluronan turnover increases with exercise intensity in a non-linear fashion. It remains to be investigated if these observations have clinical implications, particularly in cases of compromised hyaluronan regulation. Further studies need to be conducted to clarify the relative contribution of lymphatic output, mobilization/redistribution within stationary microvascular hyaluronan pools, and breakdown kinetics, to rapid changes in plasma hyaluronan concentration during and after muscular exercise as observed in this study.

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