Effect of Ascorbic Acid on Lipoprotein Lipase Activity

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SUMMARY

Baboons kept on hypovitaminotic C diets, but without clinical signs of scurvy, had significantly higher heart muscle lipoprotein lipase activity than baboons on vitamin C 34 mg/kg body mass/day. When the serum vitamin C levels were above 0,35 mg/100 ml the heart muscle lipoprotein lipase was repressed. Serum vitamin C levels below 0,35 mg/100 ml stimulated lipoprotein lipase to between 2 and 3 times the repressed value.

Heart muscle lipoprotein lipase from baboons receiving dietary vitamin C was inhibited by 0,34 mM vitamin C *in vitro*, **whereas heart muscle lipoprotein lipase from baboons on scorbutogenic diets were stimulated by addition of vitamin C** *in vitro*.

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The uptake of triglyceride fatty acids from the plasma by extrahepatic tissues is facilitated through hydrolysis of the triglycerides by the enzyme, clearing factor lipase or lipoprotein lipase. Various authors have given evidence that the enzyme acts at the surface of the capillary endothelial cells where the triglycerides, carried in the plasma very-low-density lipoproteins or chylomicrons, are sequestered.[1,2]

Robinson[3,4] also maintained that lipoprotein lipase plays a directive role in determining the pattern of triglyceride fatty acid uptake by extrahepatic tissues.

Recently, various workers have presented data indicating that the adipose tissue and heart muscle contain lipoprotein lipases having different characteristics.[1,5-9]

The activities of the enzyme in specific tissues have been shown to change in particular physiological situations and such changes can be correlated with alterations in the uptake of triglyceride fatty acids by these tissues. In adipose tissue, for example, the activity of the enzyme is high in the fed state, when triglyceride fatty acids are taken up, and low in the fasted state, when uptake of triglyceride fatty acids is low. In the heart muscle, on the other hand, the activity of lipoprotein lipase is low during feeding and is elevated during fasting, when the flow of triglyceride fatty acids is from the depot fat region towards utilisation of the heart muscle.

Ascorbic acid has been implicated in the control of serum cholesterol levels by a number of investigations,[10-12] although Anderson *et al.*[13] have questioned the serum lipid-lowering effect of ascorbic acid.

Sokoloff *et al.*[11] also discussed the decrease of serum lipoprotein lipase activity during severe atherosclerosis, and serum lipid values and the improvement of lesions during lipid intake restriction and high ascorbic acid intakes.

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Andrews *et al.*[14] found that in the elderly human, vitamin C deficiencies often occurred and that higher intakes than the recommended 30 mg/day were necessary to bring leucocyte values back to those of young people. Similarly, postheparin lipase activity was often found to be deficient in old age.[15] We therefore set out to determine the relationship between serum ascorbic acid levels and heart lipoprotein lipase. A preliminary report of our findings has been published.[16]

MATERIALS AND METHODS

Baboons were kept on diets as described by Kotzé and Kempff,[17] and De Klerk *et al.*[18]

The diets were supplemented with various quantities of ascorbic acid to obtain different serum levels. Blood samples were obtained by venepuncture under phencyclidine hydrochloride anaesthesia (1 mg/kg body mass) (Sernylan; Parke-Davis). Blood samples were allowed to clot and the serum was separated by centrifugation. Serum ascorbic acid was determined according to the method of Roe and Kuether[19] as modified by Schaffert and Kingsley.[20]

Heart muscle samples were obtained after phenobarbitone euthanasia and immediate autopsy. The apex of the heart muscle was excised and rinsed in Krebs-Ringer bicarbonate buffer, blotted on filter paper, weighed, and three times its mass of Krebs-Ringer bicarbonate buffer added. It was then minced with scissors and homogenised for 1 minute with Ultra Turrax homogeniser in the cold. Heparin was added to the homogenate to yield a final concentration of 250 units/ml, and this was subsequently incubated for 30 minutes at 25°C under a constant flow of a gas mixture containing 95% O_2 and 5% CO_2.

Lipoprotein lipase was determined according to the method of Huttunen and Steinberg,[21] with the modification that the liberated free fatty acids were determined according to Novak's[22] method. Dry mass of the homogenate was determined by mass difference after drying an aliquot for 24 hours at 105°C.

Lipoprotein lipase results are always the average of at least 6 different determinations. The effect of ascorbic acid on lipoprotein lipase activity was done by addition of ascorbic acid to the assay medium to a final concentration of 0,34 mM (6 mg/100 ml).

Acetone extracts of heart muscle homogenates were prepared as described by Garfinkel and Schotz.[23]

RESULTS

Determinations of serum ascorbic acid levels and the lipoprotein lipase activities of baboon heart muscle homogenates indicate a definite inverse relationship between these entities. The serum ascorbic acid level varied 12,7-fold from a low value of 0,11 mg/dl to 1,41 mg/dl. It is of interest because after 7 young baboons had been kept on ascorbic acid 34 mg/kg body mass/day for at least 3 months, 1,41 mg/dl was the highest serum level obtained, which was still lower than the average value of 1,56 mg/dl found in more than 200 free-living baboons in the Kruger National Park.[24] The level of ascorbic acid of 34 mg/kg/day is also much higher than the requirements of 2 mg/kg/day[25] and 7,5 mg/kg/day reported by Shaw *et al.*[26] for primates.

TABLE I. RELATIONSHIP BETWEEN SERUM ASCORBIC ACID LEVELS AND BABOON HEART MUSCLE LIPOPROTEIN LIPASE

Serum ascorbic acid (mg/100 ml)	Lipoprotein lipase activity n.e.q FFA released per mg dry mass per minute
0,11	384
0,12	549
0,16	495
0,16	492
0,18	444
0,21	320
0,24	249
0,25	345
0,29	271
0,30	142
0,34	269
0,45	201
1,09	186
1,25	76
1,26	209
1,26	231
1,26	217
1,36	222
1,41	125

The heart lipoprotein lipase activity decreased by a factor of 7,2, from a high value of 549 to a low value of 76 (Table I).

Examination of the data indicated that apparently a minimal serum ascorbic acid level of about 0,35 mg/dl existed, below which the lipoprotein lipase actively increased steeply (Fig. 1). At serum ascorbic acid values above 0,35 mg/dl, however, heart lipoprotein lipase appeared to be repressed. This is of interest, because Taylor[27] reported that petechial haemorrhages around hair follicles appeared at serum ascorbic acid levels of about 0,2 mg/dl, which corresponds to 15 μg ascorbic acid per 10^8 leucocytes.

When heart muscle homogenates of baboons with different serum ascorbic acid levels were incubated *in vitro* with 0,34 mM ascorbic acid during the assay, it was found that at low serum ascorbic acid levels the addition of ascorbic acid stimulated the enzyme activity (103,7%), and at high serum ascorbic acid levels inhibited it (61,3%) (Table II).

The acetone powder of baboon heart muscle was depleted of ascorbic acid. Addition of ascorbic acid to the assay medium also effected a stimulation of the lipoprotein lipase activity to 122,2% of the original value. Although stimulation of lipoprotein lipase by ascorbic acid occurred, the effect was always small.

Heart muscle vitamin C levels paralleled those of the serum.

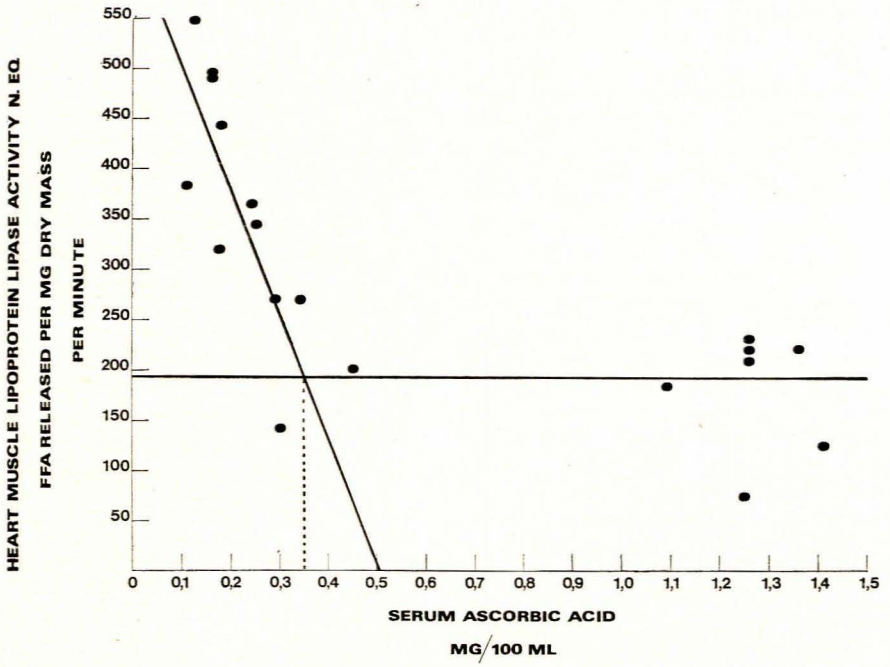

Fig. 1. The relationship between serum ascorbic acid levels and baboon heart muscle lipoprotein lipase.

TABLE II. EFFECT OF ADDED ASCORBIC ACID TO IN VITRO LIPOPROTEIN LIPASE ACTIVITIES OF BABOON HEART MUSCLE HOMOGENATES (ASCORBIC ACID IN ASSAY WAS 0,34 mM)

	Lipoprotein lipase activity as % (control = 100%)		Serum AA level (mg/100 g wet mass)	Heart muscle AA level (mg/100 g wet mass
	− Asc. acid	+ Asc. acid		
	100	35,0	0,60	9,1
	100	56,8	1,27	14,8
	100	84,2	0,76	9,3
	100	39,5	0,41	2,2
	100	91,0	1,29	15,6
Average		61,3	0,87	10,2
SD		±25,46	±0,40	± 5,39
	100	105,6	0,31	5,2
	100	106,0	0,29	2,7
	100	107,0	0,30	5,7
	100	103,7	0,09	1,4
	100	107,0	0,22	4,5
	100	93,1	0,18	4,6
Average		103,7	0,27	4,0
SD		± 2546	±0,10	± 1,14
Acetone powder	100	122,2	—	—

DISCUSSION

There is experimental evidence that during fasting lipoprotein lipase from heart and skeletal muscle increases.[6-7,9] Borenstajn *et al.*[7] found that insulin could not prevent the increase of heart lipoprotein lipase elicited by starvation. It thus appears that there are other factors besides insulin that control heart lipoprotein lipase activity.

It is not clear how the feeding of ascorbic acid can have a repressive effect on lipoprotein lipase synthesis; because such a mechanism, if any, is unknown.

According to recent reports the lipoprotein lipase from heart muscle is not a single entity. Schotz and Garfinkel[8] described two molecular species of rat heart lipoprotein lipase, one, LPL_a, was stimulated by heparin, whereas the other, LPL_b, was inhibited by heparin, and concluded that both species were increased during fasting.

Sokoloff *et al.*[11] suggested that ascorbic acid at doses of 150 mg/kg body mass/day could counteract the decrease of lipoprotein lipase of rabbit and rat serums caused by cholesterol feeding. This finding is, however, not directly relevant, since these animals synthesise their daily ascorbic acid requirements from glucose, whereas primates, like the baboon and man, do not synthesise their own ascorbic acid but must receive it from external sources.

The fact that adipose tissue and heart muscle lipoprotein lipase react differently to physiological stimuli like feeding and fasting, suggests that whereas an ascorbic acid deficiency caused a rise in heart lipoprotein lipase activity, a concomitant decrease of adipose tissue lipoprotein lipase should occur. However, high serum ascorbic acid levels should increase adipose tissue lipoprotein lipase and, therefore, tend to lower serum triglyceride fatty acid levels. Sokoloff *et al.*[11] maintained that large doses of ascorbic acid (2 - 3 g/day) and restriction of lipid intake lowered the triglyceride and cholesterol levels of hyperlipidaemic patients.

It is also of interest that a serum ascorbic acid level of

about 0,35 mg/dl was found to be sufficient to result in a low heart muscle lipoprotein lipase activity and probably to ensure an efficient level of adipose tissue lipoprotein lipase activity. This serum ascorbic acid level is just above the 0,2 mg/dl serum ascorbic acid level reported to cause petechial haemorrhage of hair follicles.[27]

In vitro incubation of heart lipoprotein lipase with additional 0,34 mM ascorbic acid caused a stimulation of the enzyme activity if the homogenate originated from a baboon with a low serum ascorbic acid level, and inhibited the lipoprotein lipase when the homogenate was from a baboon with a high serum ascorbic acid level. This finding suggests that we may be dealing with three phenomena. Firstly, the feeding of ascorbic acid controls the level of lipoprotein lipase synthesis, or only one of the LPL_a or LPL_h species as reported by Garfinkel and Schotz.[23] Secondly, that in addition to this effect, which probably involves protein synthesis, we have a more direct effect of allosteric enzyme activity modulation by different ascorbic acid concentrations *in vitro*. A third possibility exists in that the two species LPL_a and LPL_b react differently to ascorbic acid, as was reported for the reaction with heparin.[5]

It would seem, therefore, that ascorbic acid definitely exerts a controlling effect on lipoprotein lipase activity and consequently on serum lipid levels.

At present our knowledge about the intricacies of the process is still lacking and further investigation is warranted.

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