# SOME BIOCHEMICAL EFFECTS OF MEGADOSES OF ASCORBIC ACID DURING FASTING

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## ABSTRACT

Some biochemical effects of megadoses of ascorbic acid (3 gm/day) during a four-day fast were determined by measuring selected plasma and urine constituents of twelve male volunteers. Plasma glucose level was markedly higher two hours after a glucose challenge during the early part of the ascorbic acid supplemented fasting but was slightly lower towards the end of the four-day fast. In contrast, free fatty acids and ketone bodies were lower two hours after the glucose challenge during the early phase of ascorbic acid supplemented fasting, but reached higher levels during the later phase. Levels of plasma lactic acid, alanine, total amino acids and urea, as well as levels of urea nitrogen excretion were consistently lower during the entire duration of ascorbic acid supplemented fasting. These were compared with corresponding values of unsupplemented fasting.

These observations can be explained on the effects of ascorbate in promoting cAMP accumulation which can consequently alter the rate of cAMP-dependent processes such as glycogenolysis, glycogenesis, gluconeogenesis and lipolysis.

## INTRODUCTION

Ascorbic acid has been proposed by Lewin (1) to promote cAMP accumulation as a result of competition with ascorbate at the active site of phosphodiesterase. This has been experimentally confirmed by a number of investigators (2, 3, 4).

There has been no complete agreement concerning the effects of ascorbic acid on carbohydrate metabolism in man. Secher (5) and Sylvest (6) have shown that intravenous infusion of ascorbic acid (0.3 to 1.2 grams) resulted in significant lowering of blood sugar in normal and diabetic patients. Pleger and Scholl (7) noted that higher intake of ascorbic acid resulted in the decrease of the required insulin dose in several diabetic patients. However, Scarlett, et al. (8) observed no change in plasma glucose concentration during ascorbic acid infusion (1 to 2 gm every 3 hours). Mehnert (9) reported that the oral administration of 1.5 gm of ascorbic acid per day for 6 weeks

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to normal subjects did not alter glucose tolerance.

So far, there seems to have been no study undertaken concerning the effects of megadoses of ascorbic acid on carbohydrate, lipid, and amino acid metabolism during fasting. And if ascorbic acid promotes cAMP accumulation in tissues, it may be interesting to study the effect of megadoses of ascorbic acid on cAMP-dependent processes such as glycogenolysis, glycogenesis, gluconeogenesis, and lipolysis during ascorbic acid supplemented fasting.

## MATERIALS AND METHODS

Human subjects were used. Twelve healthy male volunteers, whose ages ranged from 19 to 27 years, participated in this study. All were within 10% of their ideal body weight computed according to the Tamhauser method. None has history of diabetes, gout, cardiac, renal and hepatic diseases.

The chemicals used were obtained from J.T. Baker Chemicals, Ltd., Merck, Darmstadt, and Sigma Chemical Company.

The subjects were divided into two groups. Each group underwent a four-day fast twice, with a 30-day interval between each fasting period. In Group 1, the first fasting period (designated as unsupplemented fasting) was preceded by the daily intake of 2 placebo capsules containing cellulose for 14 days. The second fasting (the ascorbic acid supplemented fasting period) was preceded by the daily intake of 2 ascorbic acid capsules (each of which contained 500 mg ascorbic acid in cellulose) for 14 days. In Group 11, the order of two fasting periods was reversed.

After 12 hours of overnight fasting, glucose (1 gm/kg body wt.) as 50% solution in water, was ingested by each subject at 6 A.M. This was followed by first blood extraction and complete emptying of urinary bladder at 8 A.M., which was considered the start of fasting (zero hour). Further blood extractions and complete voiding of urine took place regularly at 8 A.M. during the succeeding days of fasting. Subjects remained in supine position for at least an hour before each blood extraction which closely preceded complete voiding of urine. Subjects were obliged to take 2 liters of water (*ad libitum*) daily and to maintain minimal activity during the entire duration of fasting. They were allowed to walk within the room, watch television, listen to music, read newspapers or magazines, play cards or sleep whenever they like. Daily bath and physical exertions were strictly prohibited. Subjects in group 11 were subjected to the same conditions as those in group 1, except that blood extractions were totally omitted during the two fasting periods.

Ten ml of venous blood was drawn by the use of plastic syringe and were transferred to a test tube containing a sodium fluoride and potassium oxalate mixture. Urine samples were collected in brown bottles with a capacity of 2 liters. Aliquots of the 24-hr urine were transferred into individual test tubes which were frozen immediately and stored. Using methods based

on published procedures, the following constituents were analyzed: plasma glucose (10), plasma free fatty acids (11), plasma and urinary ketone bodies (12), plasma alanine (13), plasma lactic acid (14), total amino acids in plasma (14) and plasma and urinary urea (14).

## RESULTS AND DISCUSSION

Table 1 shows that at the start of fasting, ascorbic acid supplementation resulted in hyperglycemic response which lasted for two hours. This could be a consequence of impaired utilization of glucose at the start of fasting. In the fasted state, carbohydrate utilization was low.

**Table 1. Different plasma and urinary constituents at the start of both unsupplemented and ascorbic acid supplemented fasting.**

| Constituents | Unsupplemented | Supplemented |
|---|---|---|
| Plasma | | |
| Glucose (mmole/L) | 5.56 ± 0.57 | 7.08 ± 0.73 (a) |
| FFA (mmole/L) | 0.95 ± 0.15 | 0.63 ± 0.09 (a) |
| Ketone bodies (mmole/L) (b) | 93.34 ± 32.27 | 75.68 ± 23.14 |
| Lactin acid (mmole/L) | 0.59 ± 0.06 | 0.50 ± 0.04 |
| Alanine (mmole/L) | 0.28 ± 0.06 | 0.25 ± 0.04 |
| Urea (mmole/L) | 4.66 ± 0.75 | 3.95 ± 0.81 |
| Total amino acid (mmole/L) | 4.73 ± 0.33 | 4.55 ± 0.25 |
| Urine | | |
| Ketone bodies (mmole/24 hrs) (b) | 7.92 ± 4.30 | 5.85 ± 3.27 |
| Urea nitrogen (gm/24 hrs) | 2.16 ± 0.32 | 1.44 ± 0.12 (a) |

*(a) Significantly different from unsupplemented value ($p < 0.05$).*
*(b) Expressed in acetone equivalence.*

At the start ascorbic acid supplementation depicted lower values of plasma free fatty acids, ketone bodies, lactic acid, alanine, urea, and total amino acids. This was also true with urinary ketone bodies and urea nitrogen. Towards the fourth day of fasting, urinary ketone bodies increased while urea nitrogen remained low (Table 2). The plasma free fatty acids also increased while those of glucose, lactic acid, alanine, urea, and total amino acids in the plasma remained low as compared to the unsupplemented values (Table 3).

**Table 2. Urinary profile during the start and fourth day of both unsupplemented and ascorbic acid supplemented fasting in two groups of subject.**

| Urinary Constituents | Unsupplemented | Supplemented |
|---|---|---|
| Start of fasting | | |
| Group I | | |
| Ketone bodies (mmole/24 hrs) (a) | 7.92 ± 4.30 | 5.85 ± 3.27 |
| Urea nitrogen (gm/24 hrs) | 2.16 ± 0.32 | 1.44 ± 0.12 (b) |
| Group II | | |
| Ketone bodies (mmole/24 hrs) (a) | 8.95 ± 4.99 | 5.34 ± 2.07 (b) |
| Urea nitrogen (gm/24 hrs) | 1.67 ± 0.19 | 1.33 ± 0.12 (b) |
| End of fasting | | |
| Group I | | |
| Ketone bodies (mmole/24 hrs) (a) | 45.80 ± 7.40 | 64.04 ± 7.74 (b) |
| Urea nitrogen (gm/24 hrs) | 3.32 ± 0.46 | 1.78 ± 0.30 (b) |
| Group II | | |
| Ketone bodies (mmole/24 hrs) (a) | 57.51 ± 8.44 | 82.47 ± 11.36 (b) |
| Urea nitrogen (gm/24 hrs) | 8.01 ± 0.55 | 1.71 ± 0.20 (b) |

*(a) Expressed in acetone equivalence.*

*(b) Significantly different from unsupplemented value. ($p < 0.05$).*

If ascorbic acid competitively inhibits phosphodiesterase (1) the concentration of cAMP would increase during ascorbic acid supplementation. This will favor lipolysis hence the increase in plasma free fatty acids which can account for the increase in ketone bodies. The increased concentration of free fatty acids cannot be due to increased synthesis of free fatty acids since these observations are of the fasted state. In the fasted state, there is no malonyl coenzyme A that will stimulate fatty acid synthesis. Thus, ascorbic acid stimulates lipolysis. The increase in ketone bodies is not an adverse effect of ascorbic acid supplementation, because ketone bodies provide alternative fuel to body tissues when carbohydrates are in short supply. This is especially true in the nervous system which for practical purposes cannot utilize fatty acids as an energy source (15).

Ascorbic acid supplementation in the fasted state slows down glycogenesis and glycolysis. This is indicated by low concentration of lactic acid. Low

**Table 3. Different plasma and urinary constituents at the fourth day of both unsupplemented and ascorbic acid supplemented fasting.**

| Constituents | Unsupplemented | Supplemented |
|---|---|---|
| Plasma | | |
| Glucose (mmole/L) | 3.99 ± 0.38 | 3.72 ± 0.39 |
| FFA (mmole/L) | 2.91 ± 0.21 | 3.41 ± 0.15 (a) |
| Ketone bodies (μmole/L) (b) | 234.86 ± 48.41 | 329.43 ± 46.77 (a) |
| Lactic acid (mmole/L) | 0.63 ± 0.06 | 0.38 ± 0.04 (a) |
| Alanine (mmole/L) | 0.79 ± 0.07 | 0.60 ± 0.08 (a) |
| Urea (mmole/L) | 7.01 ± 0.71 | 4.99 ± 0.42 (a) |
| Total amino acid (mmole/L) | 5.66 ± 0.20 | 5.32 ± 0.15 (a) |
| Urine | | |
| Ketone bodies (mmole/24 hrs) (b) | 45.80 ± 7.40 | 64.04 ± 7.74 (a) |
| Urea nitrogen (gm/24 hrs) | 3.32 ± 0.46 | 1.78 ± 0.30 (a) |

*(a) Significantly different from unsupplemented value ($p < 0.05$)*

*(b) Expressed in acetone equivalence.*

concentration of lactic acid may come from low concentration of pyruvate which explains the low concentration of alanine.

Protein sparing effect of ascorbic acid is shown by the low total amino acids in the plasma during fasting and the low urea and urea nitrogen. Ascorbic acid supplementation can therefore reduce gluconeogenesis. This is evidenced by the low alanine concentration.

There are two significant effects of ascorbic acid supplementation in mega doses: lipid mobilization and protein sparing effect.

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