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Plasma free fatty acids in fasting vitamin C-deprived guinea pigs

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SUMMARY

Plasma concentrations of free fatty acids (FFA) were measured in normal and scorbutic guinea pigs. In animals deprived of vitamin C for 15 or 25 days, FFA concentrations were higher than normal after a 5-hour fast. Conversely, they were lower than normal after a 29-hour fast. Plasma FFA concentrations were normal after a 29-hour fast in animals deprived of vitamin C for 25 days, but then given vitamin C at the beginning of the fasting period.

In the course of recent studies a possible effect of vitamin C on plasma free fatty acids (FFA) was noted.[1] Hypophysectomized rats receiving a chow diet had lower fasting FFA concentrations than did normal rats. Addition of oranges to their diet seemed to raise the FFA toward normal. It was decided to study this phenomenon in guinea pigs which, unlike normal rats, require dietary vitamin C.

Debons and associates (1) have shown that liver slices from fasting scorbutic guinea pigs liberate relatively few ketone bodies, despite decreased utilization of ketones, and despite the potential for excessive liberation of ketones if octanoic acid is added to the system. They suggest that this may be the result of a defect in the production of fatty acids from liver lipids. Their data are also consistent with a decreased transport of fatty acids to the liver. Since plasma FFA are a major means of fatty acid transport, it seemed probable to us that fasting scorbutic guinea pigs would have relatively low plasma FFA.

METHODS

Male NIH stock, mixed color, guinea pigs weighing from 221 to 329 g were given tap water and a commercial powdered ascorbic acid-free diet (Nutritional Biochemicals, Inc.) *ad libitum*. Every morning each animal was given a 0.08 ml intraperitoneal injection. "Normal" animals received 40 mg of ascorbic acid in solution (Lederle). "Scorbutic" animals received normal saline. Groups of normal or scorbutic animals were housed in separate cages, six per cage. The container from which they ate was six inches from the floor of the cage. Food intake was not measured, since it is well established that guinea pigs deprived of vitamin C eat much less than control animals (2).

Fifteen or 25 days after starting the experiments, the animals were sacrificed between 4 P.M. and 5 P.M. Food, but not water, had been removed from the cages at 11 A.M. the same day ("5-hour fast"), or the day before ("29-hour fast"). One group of 25-day scorbutic, 29-hour fasted animals received injections of 40 mg of ascorbic acid, instead of the usual saline, immediately after food was withdrawn ("25-day scorbutic plus vitamin C").

Animals were sacrificed and blood from the severed neck collected for 30 seconds through silicone-coated funnels into silicone-coated test tubes containing 3 drops of heparin. Blood samples were immediately placed in ice. When different experimental groups were sacrificed on the same day, the order of sacrifice alternated between groups.

Plasma FFA were determined by extraction of 1 ml of plasma for 24 hours in a mixture of 2,2,4-trimethylpentane (iso-octane), glacial acetic acid, and acetic anhydride, followed by titration against 0.02 N NaOH after two washings (3). The method is similar to the modification of Gordon's method described by Shafrir (4).

[1] P. S. Mueller, unpublished observations.

RESULTS AND DISCUSSIONS

The mean cumulative changes in body weights of normal and scorbutic animals are plotted against time in Figure 1. Average group weights in grams at day

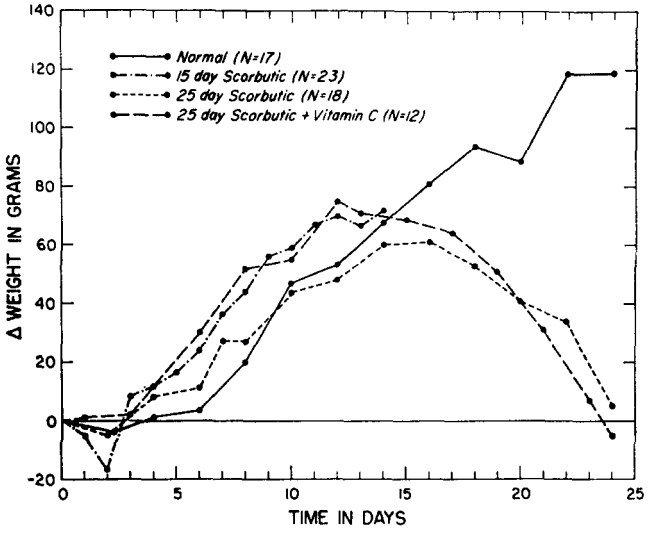

FIG. 1. Average cumulative change in body weight of control and scorbutic guinea pigs.

zero were: normal, 276; 15-day scorbutic, 240; 25-day scorbutic, 281; and 25-day scorbutic plus vitamin C, 249. Scorbutic animals gained normally for about 15 days, then lost weight. It was obvious that they began to eat less than the normal animals. They became weak and lethargic, often unable to raise themselves high enough to eat, but none died before sacrifice. No hemorrhagic phenomena were detected upon gross inspection of the skin and viscera. One normal animal died on the twenty-second day of intussusception caused by a small bowel tumor. The plasma of one scorbutic animal was lost through a laboratory error.

Plasma FFA values of various experimental groups appear in Table 1. After a 5-hour fast, normal animals had lower FFA values than did 15-day ($p < .01$) or 25-day ($p < .05$) scorbutic animals. Conversely, after a 29-hour fast, normal values were higher than in either 15-day or 25-day ($p < .05$) scorbutic animals.

Thus, prolonging the fast by 24 hours resulted in a marked increase of FFA in normal animals, but did not cause a significant increase of FFA in either group of scorbutic animals. Fifteen-day and 25-day scorbutic animals did not differ significantly from each other after comparable fasts, although values for the latter group were slightly lower.

When 25-day scorbutic animals had received an injection of vitamin C immediately after food had been withdrawn, 29-hour fasting FFA values were normal. There is no overlap between this group and the other 25-day scorbutic groups, in which comparable weight loss occurred ($p < 0.001$). After the results reported had been obtained, it was decided to check the possibility that the injection of excessive quantities of vitamin C might have altered the normal response of FFA to fasting. Groups of six animals, maintained since weaning on a chow and kale diet without injections, and weighing the same as the original vitamin C-injected normals, were sacrificed after 5- and 29-hour fasts. FFA concentrations were $0.44 \pm .07$ meq/l and $0.93 \pm .12$meq/l, respectively; values similar to those observed in the "normal" animals of the original study.

The findings in the five groups of animals subjected to a 29-hour fast are in agreement with the initial hypothesis. Vitamin C deficiency impairs the process by which high plasma FFA concentrations occur during a prolonged fast. In the light of the finding that scorbutic guinea pigs have more body fat than do pair-fed control animals (2), and the previously cited work of Debons *et al.* (1), one may assume that there is impairment of mobilization of FFA from adipose tissue. The impairment appears to be abolished within 29 hours when vitamin C is again provided.

The relatively high plasma FFA in scorbutic 5-hour fast animals was unexpected. It might be argued that if scorbutic impairment of FFA mobilization was incomplete, the relatively high FFA is due to starvation in the 25-day group. However, this would not account for the 15-day group which was, as a group, gaining weight at the time of sacrifice. It is true that the rate of gain had slowed considerably in the four days before sacrifice, but if 5-hour FFA concentrations were

TABLE 1. PLASMA FREE FATTY ACID CONCENTRATION IN VARIOUS EXPERIMENTAL GROUPS

	5-Hour Fast			29-Hour Fast		
	N	FFA Mean ± S.D.	FFA Range	N	FFA Mean ± S.D.	FFA Range
		meq/liter	*meq/liter*		*meq/liter*	*meq/liter*
25-day Normal	11	0.41 ± 0.12	0.23 – 0.55	6	0.93 ± 0.27	0.67 – 1.30
15-day Scorbutic	11	0.60 ± 0.15	0.40 – 0.91	12	0.71 ± 0.15	0.52 – 1.00
25-day Scorbutic	12	0.55 ± 0.14	0.38 – 0.84	6	0.63 ± 0.08	0.52 – 0.76
25-day Scorbutic plus vitamin C	—	—	—	12	1.04 ± 0.15	0.86 – 1.37

so responsive to relative caloric deficit, one would expect to find an intraindividual correlation in this group of 11 animals, between weight change and FFA concentration. In fact, although individual changes in weight from the thirteenth to the fourteenth days ranged from +13 g to −20 g, the coefficient of correlation with FFA was only −0.123. Finally, it is difficult to see how a single fat-mobilizing process could be very responsive to a short fast, yet show very little further response to a much longer fast.

Previous investigations have described impaired glucose utilization (decreased glucose tolerance) after as few as 10 days of vitamin C deprivation (5, 6, 7). Furthermore, high plasma FFA concentrations have been observed in another condition in which glucose utilization is impaired, namely, diabetes mellitus (8). It is possible that the relatively high FFA concentrations within 5 hours of withdrawing food resulted from impaired glucose utilization. Such an explanation requires that the mechanism of FFA mobilization which is related to prolonged fasting be different from that related to decreased glucose utilization, because only the former mechanism appears to be impaired in the scorbutic state. This postulate must be tested by additional experiments.

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