

Characterization of guinea pigs adapted to differently high vitamin C supplies

1. Blood-levels of cholesterol, glucose, triacylglycerides and hemoglobin

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Summary: Guinea pigs adapted (6–10 weeks) to low supply with vitamin C in the food show the tendency to increased levels of cholesterol and triacylglycerides in the blood and to decreased levels of hemoglobin and of glucose in comparison to guinea pigs adapted to medium and high supply.

Zusammenfassung: Meerschweinchen, die an geringe Vitamin-C-Gehalte im Futter adaptiert worden sind (6–10 Wochen), zeigen im Vergleich zu Tieren, die an mittlere und hohe Gehalte adaptiert sind, Tendenzen zu erhöhten Blutspiegeln an Cholesterin und Triacylglyceriden und zu erniedrigten Gehalten an Hämoglobin und an Glucose.

Key words: guinea pigs, blood levels of cholesterol, glucose, triacylglycerides and hemoglobin, adaptation to different vitamin C supplies

Schlüsselwörter: Meerschweinchen, Blutspiegel an Cholesterin, Glucose, Triacylglyceride und Hämoglobin, Adaptation an unterschiedliche Vitamin-C-Gehalte

Introduction

In another series of papers (1–3) we present indications that guinea pigs are able to adapt their ascorbic acid metabolism in certain limits to the extent of their vitamin C supply and that during this adapting time the amounts of microsomal and mitochondrial cytochromes as well seem to pass through transient values. Since the adapting needs the long period of six-eight weeks and longlasting adapting times usually include an involvement of slowly acting hormones and more over since there is no clear metabolic connexion between ascorbic acid and cytochromes it had to be taken into account that all these symptoms are due to certain hormonal constellations reigning the actual metabolism.

For the above reasons we examined in this series to begin with common clinical-biochemical parameters in order to characterize guinea pigs adapted to differently high vitamin C supplies and simultaneously to get indications for the states of the respective metabolism.

The guinea pig groups were adapted to defined supplies in a suit between 5 mg/100 g and 680 mg/100 g vitamin C in the food. A supply with

5 mg/100 g seems to be marginal for survival (1) and a supply with 680 mg/100 g seems to be at the limit of the absorptive capacity of the intestine. We could not increase the ascorbic acid levels in the blood or in the liver by still higher substitution.

Materials and Methods

The guinea pigs were yellow short- and smooth-haired and of own rearing. All animals were kept at 20°C and at about 60 % humidity of the atmosphere in air-conditioned rooms and were ad libitum provided with water. The guinea pigs breeding and their youngsters were kept in large cages and fed ad libitum the standard diet C 3012 from "Altromin", and supplementary with hay a grass (summertime) or pieces of apples powdered with vitamin C (wintertime).

The experimental guinea pigs had an initial body weight of about 400 g (i.e. an age of about six weeks) and were kept in macrolon cages M4 with insert-floors out of stainless steel. In order to avoid diarrhoea and other symptoms of irritability, they were accustomed to the semisynthetic diet C 3015 (without vitamin C) from "Altromin" for two weeks by a supply with a mixture (1:1) of the standard diet C 3011 and of the semisynthetic diet supplemented to that amount of vitamin C to which animals were supposed to adapt to. Thereafter the guinea pigs were fed with the sole supplemented semisynthetic diet for at least six weeks.

Blood for estimation of glucose and hemoglobin was obtained between 8 and 9 a.m. by vein puncture. In order to obtain serum for estimation of cholesterol and triacylglycerides the guinea pigs were killed between 8 and 9 a.m. by a blow on the neck and bled.

With regard to the habit of guinea pigs to reduce food intake in between for several days and the resulting consequences (1) the animals were weighed two times a week. Guinea pigs which had no gained weight continuously during the last three weeks were excluded from investigation.

Guinea pigs completely deprived of vitamin C supply were substituted with 680 mg vitamin C in 100 g food for eight weeks and thereafter fed ad libitum the unsupplemented semisynthetic diet C 3015 for 14 days. The fasting guinea pigs were deprived of food for 16 hours (from 4 p.m. to 8 a.m. the next day).

The cholesterol levels were estimated by the CHOD-PAP method employing an automated analysis in the BM/Hitachi system 737 (7-9).

The triacylglycerides levels were estimated by the GPO-PAP method employing an automated analysis in the BM/Hitachi system 737 (9+10).

The hemoglobin concentrations were estimated by the cyanomethhemoglobin method employing an automated analysis in the Sysmex CC-700 TOA System (11+12).

The blood glucose levels were estimated by means of the oxygen rate method (5+6) employing a Beckman oxygen electrode.

All the reagents were procured from Boehringer Mannheim.

Statistical analysis

On the assumption that the data observed were normally or lognormally distributed, analysis of variance was used for testing the hypothesis H_0 : there is no influence of the suit of vitamin C supplies on the blood levels of cholesterol, hemoglobin and triacylglycerides. If H_0 was rejected groups of interest were compared by Scheffe's procedure. The influence of fasting and of normal providing with food on the levels of glucose was tested by two-factorial analysis of variance.

Results

a) Serum levels of cholesterol (Table 1)

Since there was a tendency to a dependence of the cholesterol levels from the body weights after very long feeding times (more than 15 weeks) we restricted the feeding period to six to ten weeks of providing with the defined vitamin C supply.

It may be assumed that the cholesterol levels are not equal ($p < 0.0001$) in the suit of guinea pig groups with different vitamin C supplies. The cholesterol levels show the tendency to be increased in the guinea pigs adapted to 5, 10 and to 20 mg/100 g vitamin C in the food in comparison with the groups supplied with higher amounts than 20 mg/100 g. In comparison to the latter the additional Scheffe-tests yield: p at least < 0.02 in the 5 mg/100 g group; p at least < 0.015 in the 10 mg/100 g animals and in the

20 mg/100 g group $p < 0.002$ with exception of $p = 0.05$ for comparison to the 680 mg/100 g group.

Guinea pigs at first adapted to 680 mg/100 g vitamin C in the food and completely deprived of a supply thereafter for fourteen days had mean levels of 65.85 ± 19.676 [mg Cholesterol/dl serum] ($n = 13$) and hence tend to a high increase of the cholesterol levels ($p < 0.0001$) in comparison to the initial values (= 680 mg/100 g group in Table 1).

b) Serum levels of triacylglycerides (Table 2)

Since there was a tendency to a dependence of the triacylglycerides levels from the body weights after very long feeding times (more than 15 weeks), we restricted the feeding period to six to ten weeks of providing with the defined vitamin C supply.

It may be assumed that the triacylglycerides levels are not equal ($p < 0.001$) in the suit of guinea pig groups with different vitamin C supplies. The triacylglycerides levels show the tendency to be increased in the guinea pigs adapted to 5, 10 and to 20 mg/100 g vitamin C in the food in comparison to the groups supplied with higher amounts than 20 mg/100 g. In comparison to the latter animals the additional Scheffe-tests yield: p at least < 0.002 in the 5 mg/100 g guinea pigs; p at least < 0.001 in the 10 mg/100 g group and $p < 0.0001$ in the 20 mg/100 g animals.

Table 1. Serum levels of cholesterol in guinea pigs adapted to different supplies with vitamin C.

[mg vit. C 100 g food]	n	[mg cholesterol/dl serum] mean	standard deviation
5	14	56.35	11.862
10	14	56.71	9.902
20	9	56.33	8.185
90	9	32.33	9.178
330	8	35.75	5.496
680	10	41.80	6.373

Table 2. Serum levels of triacylglycerides in guinea pigs adapted to different supplies with vitamin C.

$\left[\frac{\text{mg vit. C}}{100 \text{ g food}} \right]$	n	mean	[mg triacylglycerides/dl serum] standard deviation
5	14	78.07	15.168
10	11	74.09	17.846
20	10	90.00	10.954
90	9	49.66	9.420
330	8	50.12	13.820
680	10	52.70	9.821

Guinea pigs at first adapted to 680 mg/100 g vitamin C in the food and completely deprived of a supply thereafter for fourteen days had mean values of 199.85 ± 81.212 [mg triacylglycerides/dl serum] ($n = 13$) and hence tend to a sharp increase of the triacylglycerides levels ($p < 0.0001$), in comparison to the initial values (= 680 mg/100 g group in Table 2).

c) Hemoglobin levels (Table 3)

It may be assumed that the hemoglobin levels are not equal in the suit of guinea pig groups with different vitamin C supplies ($p < 0.0001$). This is mainly due to a decrease in the guinea pig group adapted to 5 mg/100 g vitamin C in the food.

Guinea pigs at first adapted to 680 mg/100 g vitamin C in the food and completely deprived of a supply thereafter show no decrease of the hemoglobin levels after 14 days ($p > 0.05$, $n = 10$) [nor after 21 days ($p > 0.05$, $n = 7$)].

d) Serum levels of glucose (Table 4)

A comparison of the blood glucose levels in guinea pigs fasting ($n = 59$) and those provided with food ($n = 59$) shows no tendency to interaction ($p = 0.55$) in the two suits of groups with different vitamin C supplies. But they are at different range ($p < 0.001$), the fasting guinea pigs have lower

Table 3. Hemoglobin levels in guinea pigs adapted to different supplies with vitamin C.

$\left[\frac{\text{mg vit. C}}{100 \text{ g food}} \right]$	n	mean	[gHb/dl blood] standard deviation
5	15	12.00	1.328
10	19	13.01	1.318
20	17	13.67	0.984
90	12	14.30	0.939
210	21	14.22	0.678
680	10	14.16	0.572

Table 4. Serum levels of glucose in guinea pigs adapted to different supplies with vitamin C. Combination: A :[mg vitamin C/100 g food]. B:0 = fasting guinea pigs; 1 = guinea pigs provided with food.

Combination A	B	n	[mg glucose/dl Serum] mean standard	standard deviation
5	1	10	117.9	8.23
5	0	10	98.0	8.16
10	1	10	121.5	10.33
10	0	10	93.8	4.21
90	1	13	122.8	6.59
90	0	13	101.4	8.40
167	1	6	122.2	6.67
167	0	6	100.7	5.12
550	1	6	125.0	3.52
550	0	6	106.0	8.02
680	1	14	128.7	7.53
680	0	14	103.0	6.65

levels. In the suit of guinea pigs provided with food as well as in the suit of fasting animals there is a tendency to reduced levels of blood glucose after adaptation to low supply with vitamin C in the food ($p < 0.002$). An additional Scheffe-test yields $p = 0.025$ for the difference between guinea pigs with 5 mg/100 g and 680 mg/100 g vitamin C in the food (mean values 108.0 and 115.9 mg glucose/dl serum).

In guinea pigs provided with food the blood levels of glucose are not dependent on the time point of estimation between 8 and 12 a.m. (estimations at 8, 9, 10, 11 and 12 a.m. with 8 samples for each time point yield as result $p = 0.6$).

Discussion

The tendencies to an increase of the cholesterol and triacylglycerides levels in the blood and to a decrease of the hemoglobin and the glucose levels in guinea pigs adapted to low supply with vitamin C seem hardly explainable by lack of vitamin C for one of the classical metabolic steps known to be dependent on ascorbic acid. The results seem to point to a generally slowed down metabolism, with less need for substances yielding energy or building materials. It seems to fit that guinea pigs completely deprived of the vitamin show a tendency to even pronounced increases of the cholesterol and triacylglycerides blood levels. Because the condition of guinea pigs completely deprived of the vitamin for fourteen days is shortly prior to the critical position leading to an impetuous derangement of the metabolism (4) on behalf of an intensified catabolism and diminished anabolic intensity. Since hemoglobin has a rather long half-life time it is comprehensible that guinea pigs deprived of the vitamin for two or three weeks maintain normal hemoglobin levels.

The British Medical Association recommended for man a daily intake of 30 mg vitamin C. This recommendation is based on the conception that the vitamin C supply is sufficient if it avoids the classical scurvy symptoms.

Our results point to the fact that there are far earlier symptoms of insufficient vitamin C supply still compatible with longlasting survival but as well to be avoided.

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