Concurrent occurrence of elevated ketone body and depressed carnitine level in underfed guinea-pigs

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Summary. Food ingestion of male guinea-pigs was restricted to 10 g/day. Total carnitine content of liver of the underfed animals fell to 3.75 μmoles per liver (46.1% of control). Serum level of total carnitine was 35.0 nmoles/ml (64.4%) in the underfed animals while they developed high ketonemia, 384.2 nmoles/ml (555.2%). In 'restricted' animals the carnitine levels also decreased in the muscles.

Carnitine (L-3-hydroxy-4-N-trimethylaminobutyrate) has a well-known role in intermediary metabolism; namely, it promotes fatty acid oxidation as acyl carrier. Being a substrate for carnitine palmitoyltransferase (CPT, EC 2.3.1.21) carnitine makes possible the transfer of activated fatty acids from cytoplasm into the mitochondrial matrix[1]. It seems that carnitine plays a very important role in the liver by promoting ketogenesis. The liver is enriched in carnitine in fasted and diabetic rats[2] and diabetic sheep[3], and the carnitine content is proportional to the ketogenic capacity[2]. These data are consistent with the present view about the role of carnitine in ketogenesis (for a recent review see McGarry and Forster[4]).

We have made studies of dietary factors affecting carnitine biosynthesis in guinea-pigs. In the course of these studies it was necessary to test how guinea-pigs responded to fasting and partial food deprivation. We found a severe depression of carnitine levels in tissues and sera of the underfed guinea-pigs. In comparison with the above[2,3] this finding was surprising, particularly because the animals were able to develop high ketonemia.

Methods. Young male guinea-pigs weighing 380–420 g were used. Since ascorbic acid (AA) has a defined role in carnitine biosynthesis[5] it was important to make sure that the animals took equal, known quantity of AA. So, the animals were fed AA-deficient diet and the both groups (see below) were given s.c. the recommended dose of AA, 10 mg on every 2nd day. The diet was prepared according to Woodruff et al.[6], but in order to reduce dietary carnitine, milk powder was replaced by an equivalent amount of vitamin-free casein and lactose. The resultant diet contained 56% carbohydrate, 18% protein, 8% fat, all the necessary vitamins (except AA) and minerals, 17.2 nmoles/g carnitine and 15.1 kJ/g (3.69 kcal/g). Animals in the 'restricted' group were given 10 g food per day at 12.00 h for 7 days. Control animals had free access to food and ingested daily 25–30 g. (In some experiments we used commercial guinea-pig chow containing 1–2 mg/g AA and 50.2 nmoles/g carnitine). On the 7th day the animals were sacrificed between 08.00 and 10.00 h under anesthesia. 100–200 mg specimens of organs were removed in the following order; extensor digitorum longus (EDL) muscle, superficial vastus lateralis muscle (SVL), deep vastus lateralis (DVL) muscle and liver. Lastly, blood was drawn from the abdominal aorta. The skeletal muscles chosen differ in composition of fiber type[7]. Total carnitine was measured after alkaline hydrolysis, using a spectrophotometric method[8] in the tissues and a radiochemical method[9] in the sera. Ketone bodies were determined in the sera according to Mellanby[10]. Activity of 4-N-trimethylaminobutyrate (butyrobetain) hydroxylase[11] (BBH, EC 1.14.1.) was also assayed in the liver.

Results and discussion. The table shows a marked decrease in carnitine levels in the tissues and serum of the underfed guinea-pigs, which is most pronounced in the liver, 61.0% of the control value when calculated on a weight basis. Because of shrinkage of the livers the real decrease is greater; the carnitine content of the whole liver fell to 46.1%. In other experiments (not shown) with commercial food the carnitine levels of control animals were similar to those seen in the table (380 ± 17.2 nmoles/g in liver) and underfeeding also decreased the carnitine levels.

The mechanism of development of carnitine depletion is not yet clear. A reduced intake of dietary carnitine can be excluded as cause; carnitine ingestion with the food used (17.2 nmoles/g) was negligible for the body pool. In fact, in control animals fed ad libitum variation in dietary carnitine (17.2 or 50.2 nmoles/g) did not affect the carnitine levels. The activity of BBH, the enzyme catalyzing the last step of carnitine biosynthesis in the liver, decreased nonsignificantly by 20% (not shown). Presumably changes in the endocrine background are responsible. It is worthy of note that we did not observe carnitine depletion in underfed rats (unpublished).

Regardless of the mechanism, the underfed guinea-pigs demonstrate that a ketotic state may come into existence without a concomitant increase of liver carnitine. Moreover, these animals were able to develop high ketonemia (555%, table) even with a depressed liver carnitine level. The change in level of one substrate of CPT, i.e. the change of carnitine, is only one possibility for altering the flux through the enzyme. Recently, other factors (glycogen, malonyl-coenzyme-A) have been reported to affect CPT activity[4,12]. This work shows that liver carnitine is not an obligatory rate limiting factor for ketogenesis and calls attention to other factors activating the ketogenic machinery.

Carnitine levels and serum ketone body concentration in guinea-pigs subjected to restricted feeding

Group (No. of animals)	Liver Carnitine (μmoles per liver)	Liver (nmoles/g tissue ± SEM)	EDL	SVL	DVL	Serum Carnitine (nmoles/ml ± SEM)	Ketone bodies (30HB/AcAc ratio)
Control (6)	8.19 ± 0.70	334 ± 16.2	353 ± 19.1	237 ± 17.2	807 ± 40.1	52.7 ± 2.1	69.2 ± 5.1 (1.02)
'Restricted' (6)	3.78 ± 0.27	204 ± 15.1	216 ± 18.0	168 ± 16.1	603 ± 32.2	35.0 ± 1.8	384.2 ± 10.1 (1.22)
Significance	$p < 0.001$	$p < 0.001$	$p < 0.001$	$p < 0.02$	$p < 0.01$	$p < 0.001$	$p < 0.001$

All animals were fed an ascorbic acid (AA) deficient diet containing 17.2 nmoles/g carnitine and were given 5 mg/day AA s.c. Controls were fed ad libitum and the 'restricted' animals were given 10 g/food per day for 7 days. The animals initially weighed 398 ± 11.0 g. At termination of the experiment the controls and 'restricted' weighed 453 ± 28.2 g and 396 ± 21.0 g, respectively. Carnitine refers to the sum of the free and esterified carnitine. Ketone bodies refers to the sum of the acetoacetate (AcAc) and 3-OH-butyrate (3OHB). EDL, extensor digitorum longus muscle; SVL, superficial vastus lateralis muscle; DVL, deep vastus lateralis muscle.

The inverse relation between liver carnitine and serum ketone bodies, presented here, is not a unique precedent. In a human disease termed 'systemic carnitine deficiency' the liver carnitine level is also depressed[13]. In such patients ketoacidosis has occasionally been observed[13-15].

1 G. Delisle and I.B. Fritz, Proc. natl Acad. Sci., USA *58*, 790 (1967).
2 J.D. McGarry, C. Robles-Valdes and D.W. Foster, Proc. natl Acad. Sci., USA *72*, 4385 (1975).
3 A.M. Snoswell and G.D. Henderson, Biochem. J. *119*, 59 (1970).
4 J.D. McGarry and D.W. Foster, A. Rev. Biochem. *49*, 395 (1980).
5 J.D. Hulse, S.R. Ellis and L.M. Henderson, J. biol. Chem. *253*, 1654 (1978).
6 C.W. Woodruff, M.E. Cherrington, A.K. Stockell and W.J. Darby, J. biol. Chem. *178*, 861 (1949).
7 J.B. Peter, R.J. Barnard, V.R. Edgerton, C.A. Gillespie and K.E. Stempel, Biochemistry *11*, 2627 (1972).
8 D.J. Pearson, P.K. Tubbs and J.F.A. Chase, in: Methods of Enzymatic Analysis, 2nd edn, p.1758. Ed. H.U. Bergmayer. Verlag Chemie, Weinheim 1974.
9 J.D. McGarry and D.W. Foster, J. Lipid Res. *17*, 277 (1976).
10 J. Mellanby and D.H. Williamson, in: Methods of Enzymatic Analysis, 2nd edn, p.1740. Ed. H.U. Bergmayer. Verlag Chemie, Weinheim 1974.
11 G. Lindstedt and S. Lindstedt, J. biol. Chem. *245*, 4178 (1970).
12 J.D. McGarry, G.F. Leatherman and D.W. Foster, J. biol. Chem. *253*, 4128 (1978).
13 G. Karpati, S. Carpenter, A.G. Engel, G. Watters, J. Allen. S. Rothman, G. Klassen and O.A. Mamer, Neurology, Minneap. *25*, 16 (1975).
14 F. Cornellio, S. Didonato, D. Peluchetti, A. Bizzi, B. Bertagnolio, A. D'Angelo and U. Wiesemann, J. Neurol. Neurosurg. Psychiat. *40*, 1970 (1977).
15 G. Bouding, J. Mikol, A. Gillard and A.G. Engel, J. neurol. Sci. *30*, 313 (1976).

High glucose-induced enzyme activity changes in cultured cell lines established from the kidneys of Chinese hamsters with aglycosuria or spontaneous glycosuria

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Summary. Chinese hamster kidney epithelial-like cells derived from highly inbred nondiabetic *(AV)* and diabetic *(XA)* genetic sublines were passaged in medium containing 100 or 400 mg/dl glucose. The effect of high medium glucose on the activities of 5 enzymes involved in glucose metabolism was followed and significant glucose-dependent difference was observed. The effects, however, were opposite in cells derived from *AV* and *XA* sublines.

Diabetes-related changes in the activities of many enzymes involved in carbohydrate metabolism have been documented in the highly inbred Upjohn Chinese hamster colony[1,2]. These changes could arise from hormonal imbalances or, alternatively, a direct effect of hyperglycemia per se. In view of the fact that excessive nonenzymatic glycosylations of proteins have been increasingly found in diabetic human patients and animal models[3-10], an attempt to ascertain the effect of high glucose in the medium on the activities of enzymes involved in carbohydrate metabolism in kidney cell cultures was thus made. Pyruvate kinase (PK, EC 2.7.1.40), malate dehydrogenase (MDH, EC 1.1.1.37), isocitrate dehydrogenase (IDH, EC 1.1.1.42) and phosphoenolpyruvate carboxykinase (PEPCK, EC 4.1.1.32) were studied because of their pivotal roles in the glycolytic-gluconeogenic and citric acid cycles which are the major pathways in carbohydrate metabolism. UDP-Glucose: galactosylhydroxylysine-collagen glucosyltransferase (CGT, EC 2.4.1.66) was also included due to its implicated role in the development of diabetic microangiopathy[11].

Materials and methods. 2 lines of epithelial-like cells, designated CHK-XA_E and CHK-AV_E, were established from the kidneys of 2 8-week-old male Chinese hamsters derived from the diabetic *XA* and the nondiabetic *AV* genetic sublines respectively. The method used and the lineage of the animals are described elsewhere[12,13]. The CHK-XA and CHK-AV cells were further divided, at the 5th and the 3rd passages, into E-100 and E-400 sublines by culturing in medium containing 100 or 400 mg/dl glucose respectively in successive passages. The cells were grown until confluent in 25 cm^2 tissue culture flasks. The medium was removed and the attached cells were rinsed 5 times with ice-cold saline. 2 ml saline was added to each flask and the cells were removed with a rubber policeman. The cells were transferred to 12×75 glass tubes and then combined with 2 ml saline used to wash the flask. The cells were centrifuged at $180 \times g$ for 10 min, the supernatant was discarded and the cells were suspended in 0.25 ml saline. The cells were frozen-thawed rapidly 5 times and sonicated for 15 sec at 30 W using a Sonifier Cell Disruptor Model W185 (Heat Systems-Ultrasonics, Inc., Plainview, Long Island, NY). The samples were then centrifuged at $260 \times g$ for 10 min, the supernatant collected and stored on ice until use. All enzyme assays were done on the day the samples were prepared. Aliquots of 0.01-ml samples were assayed for PK, MDH and IDH; aliquots of 0.05 ml were assayed for PEPCK and CGT. The enzymes were assayed by methods previously described[1,14] with the exception that the precipitates formed in the CGT assay were washed 4 times in 1% (w/v) phosphotungstic acid, 0.5 N HCl instead of 16% (w/v) trichloroacetic acid[14]. 1 unit (U) of enzyme was defined as substrate turnover at a rate of 1 μmole/min. Proteins were determined by a modified Lowry's procedure[15]. The experiments were carried out in cells harvested on 4 successive passages with 4 sister flasks per passage resulting in a total of 16 observations for each enzyme in each subline. PK and PEPCK were studied in one series of experiments and MDH, IDH and CGT in another. All E-400 cells had been grown in 400 mg/dl glucose for at least 7 passages and they were matched with E-100 cells cultured pairwise in 100 mg/dl glucose under identical conditions. Studies on CHK-AC_E and CHK-XA_E were performed separately on different days whereas E-100 and E-400 cells in each cell line were always studied in pairs.

Results and discussion. Our data are expressed in terms of U per 1×10^6 cells or U per mg proteins in cell extracts and summarized in the figure. In CHK-AV_E cells, successive passages in 400 mg/dl glucose led to significant increase in PK activity (1.05 ± 0.27 U/mg and 0.081 ± 0.024 U/10^6 cells in E-100 vs 1.94 ± 0.21 U/mg and 0.157 ± 0.023 U/10^6 cells in E-400) and decrease in MDH (0.721 ± 0.035 U/mg protein and 0.080 ± 0.005 U/10^6 cells in E-100 vs