

Variation in Tissue Carnitine Concentrations with Age and Sex in the Rat

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Diabetes, starvation and various hormonal treatments are known to alter drastically carnitine concentrations in the body. Before the mechanisms controlling carnitine metabolism could be determined, it was necessary to establish normal carnitine concentrations in both sexes at different ages. Carnitine was assayed in plasma, liver, heart and skeletal muscle of rats from birth to weaning. The plasma carnitine increased rapidly during the first 2 days after birth. Carnitine in both heart and skeletal muscle increased, whereas liver concentrations declined during the first week of life. A carnitine-free diet containing sufficient precursors for carnitine biosynthesis was fed to weaning rats. Groups of ten male and ten female rats were killed each week for 10 consecutive weeks. Carnitine was determined in plasma, liver, heart, skeletal muscle, urine and epididymis in the male. There was no difference in carnitine concentrations between the sexes at weaning. Plasma, heart and muscle concentrations were higher in adult male rats than in adult females. However, liver carnitine and urinary carnitine concentrations were higher in adult female than in adult male rats. The epididymal carnitine concentration increased very rapidly during 50 to 70 days of age and the differences in carnitine concentrations between the sexes also became apparent during this time. Thus both the age and the sex of the human subject or experimental animal must be considered when investigating carnitine metabolism.

Carnitine [(3-carboxy-2-hydroxypropyl)trimethylammonium hydroxide] is required for the transport of long-chain fatty acids from the cytosol to the site of β -oxidation in the mitochondrial matrix (Wolf, 1965). Thus, in a tissue that depends on fat as an important source of fuel, the concentration of carnitine would be expected to play an important role in the metabolism of that tissue. The results of Cederblad *et al.* (1976) indicate a relationship between the concentration of carnitine and the capacity for long-chain fatty acid oxidation in human skeletal muscle (Cederblad *et al.*, 1976).

The heart and epididymis of newborn rats are known to contain much less carnitine than the same organs of the adult (Wittels & Bressler, 1965; Marquis & Fritz, 1965). Infusion of anti-insulin serum or glucagon for 3 h, starvation for 24 h, and the induction of severe diabetic ketoacidosis with alloxan, all result in pronounced elevations in hepatic carnitine concentrations in the rat (McGarry *et al.*, 1975). Elevated concentrations of hepatic carnitine may indeed be required for the initiation of ketogenesis (McGarry & Foster, 1977). Preliminary experiments suggested there were significant differences in tissue carnitine concentrations due to age or sex. However, the factors controlling these concentrations are not well understood, and in many experiments on carnitine metabolism, transport or

function, the age, the sex and the diet of the experimental animals have not been stringently controlled. The purpose of this study was to investigate the role of age and sex in determining the concentrations of carnitine in plasma, liver, cardiac muscle, skeletal muscle and epididymis in normal rats. A brief account of these investigations has appeared (Borum & Broquist, 1977a).

Experimental

Animal care and diet

A group of pregnant Sprague-Dawley rats from Harlan Industries (Box 29176, Indianapolis, IN 46229, U.S.A.) were fed on laboratory chow during pregnancy and lactation. The pups were killed at various times from before birth to 24 days of age. A number of samples were obtained during the first 48 h after birth, and these time points were carefully determined.

For the experiments 100 male and 100 female 22-day-old Sprague-Dawley rats were purchased from Harlan Industries and maintained in suspended cages with screen bottoms. These rats were fed *ad libitum* with a defined diet that contained no detectable carnitine, but sufficient precursors for carnitine biosynthesis (Borum & Broquist, 1977b). Body weights determined twice each week indicated that

these animals maintained a normal growth rate on this diet. Ten female rats and ten male rats were killed on the day when the animals were received and each week thereafter for 10 weeks. Thus the nutritional state of the animals in the first time point of the following Figures is not as well controlled as in the remaining time points. Urine was collected by using suspended metabolic cages.

Assay procedures

Tissue carnitine concentrations were measured by a modification of the method of Cederblad & Lindstedt (1972) as described by Borum *et al.* (1977). Plasma protein concentration was determined by the micro biuret method (Itzhaki & Gill, 1964). Non-collagen protein was solubilized by the method of Lilienthal *et al.* (1950), and the concentration assayed by the micro biuret method. Urinary creatinine was measured by a modification of the Folin and Wu procedure as described by Technicon Bulletin N-11b (1969).

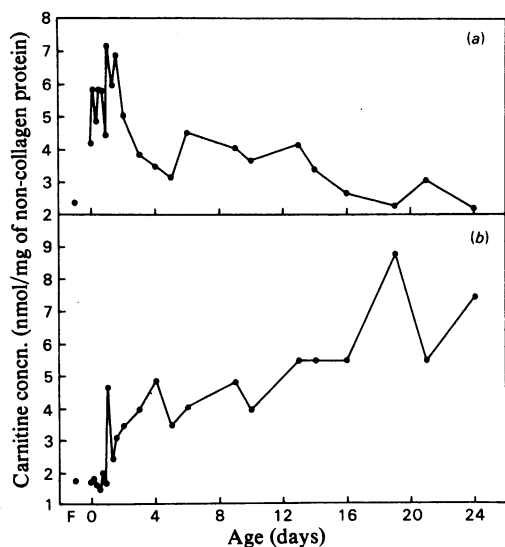


Fig. 1. Carnitine concentrations in the liver and heart of preweaned rats

The assay methods and animal treatments are described in the Experimental section. Points for animals up to 6 days of age are the means for triplicate measurements of a pooled sample from 4–11 animals. (a) Liver carnitine concentrations. The S.E.M. are: 9 days, 0.3 ($n=5$); 10 days, 0.3 ($n=7$); 13 days, 0.2 ($n=11$); 14 days, 0.2 ($n=4$); 16 days, 0.2 ($n=4$); 19 days, 0.1 ($n=6$); 21 days, 0.3 ($n=4$); 24 days, 0.1 ($n=6$). (b) Heart carnitine concentrations. The S.E.M. are: 9 days, 0.3; 10 days, 0.3; 13 days, 0.3; 14 days, 0.3; 16 days, 0.5; 19 days, 0.7; 21 days, 0.2; 24 days, 0.7.

Statistical analysis

The *t*-test program in the 2200 General Library Statistics/Engineering of Wang Laboratories Inc., Tewksbury, MA 01874, U.S.A., was used to analyse the data on a 2200 series calculator.

Results

Carnitine concentrations in perinatal rats

Since there are no differences in carnitine concentrations between male and female rats before weaning, so the data for both sexes are presented together. Data presented in the following Figures are based on non-collagen protein. The same conclusions are reached when the carnitine concentrations are expressed per g wet wt. of tissue. As shown in Fig. 1(a), there are low carnitine concentrations in foetal liver, with a high concentration at birth, which decreases throughout the suckling period. These values agree well with those of Robles-Valdes *et al.* (1976).

Carnitine concentrations are low in the heart of the foetus at birth, but increase during the suckling period. The reported large increase in heart carnitine concentrations between 5 and 15 days of age (Robles-Valdes *et al.*, 1976) was not observed in these studies. The most rapid increase in heart carnitine appeared to occur during the first 4 days after birth (see Fig. 1b). The increase in carnitine concentrations after

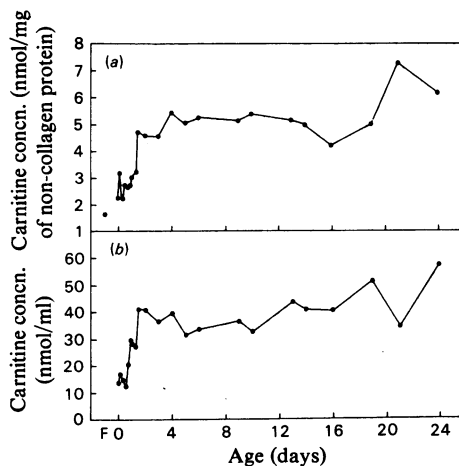


Fig. 2. Carnitine concentrations in the muscle and plasma of preweaned rats

The animals are described in the legend to Fig. 1. (a) Muscle carnitine concentrations. The S.E.M. are: 9 days, 0.2; 10 days, 0.3; 13 days, 0.3; 14 days, 0.5; 16 days, 0.2; 19 days, 0.5; 21 days, 2.0; 24 days, 0.5. (b) Plasma carnitine concentrations. The S.E.M. are: 9 days, 3.0; 10 days, 3.0; 13 days, 1.8; 14 days, 1.1; 16 days, 1.1; 19 days, 2.0; 21 days, 1.5; 24 days, 2.8.

birth observed in cardiac-muscle and skeletal-muscle tissue are very similar (Fig. 2a). Plasma carnitine concentrations are presented in Fig. 2(b). There is a very rapid increase in plasma carnitine concentrations during the first 48h after birth.

Plasma carnitine concentrations

As observed in Fig. 3, the plasma carnitine concentrations do not change appreciably in female rats from 22 to 85 days of age. However, they increase dramatically in male rats, such that the adult male rat has approximately twice that found in the plasma of the female rat.

Liver carnitine concentrations

The adult male rat does not have a higher concentration of carnitine in the liver than the female, as was observed in the plasma. Rather, carnitine concentrations appear to be somewhat higher in female liver (see Fig. 4).

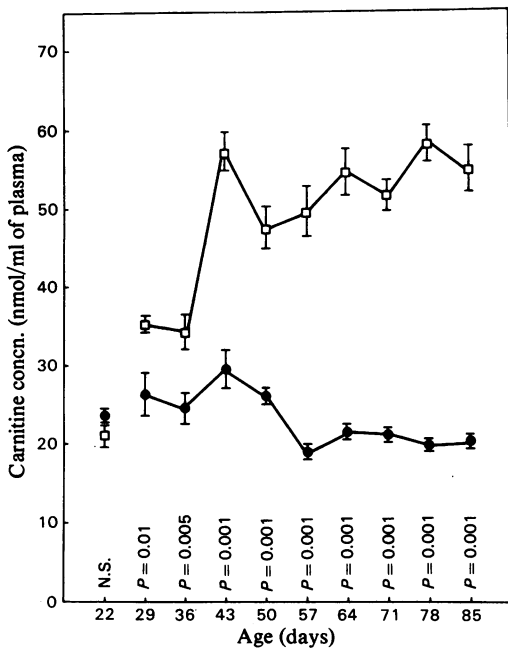


Fig. 3. Carnitine concentrations in the plasma of female and male rats from age 22 days to 85 days

The assay methods and the animal treatments are described in the Experimental section. Each point is the mean for plasma carnitine determinations on ten animals, with the s.e.m. indicated by the bars. The P values for the difference between carnitine concentrations for female and male rats were determined as described in the Experimental section. Symbols: ●, females; □, males. N.S., P > 0.05.

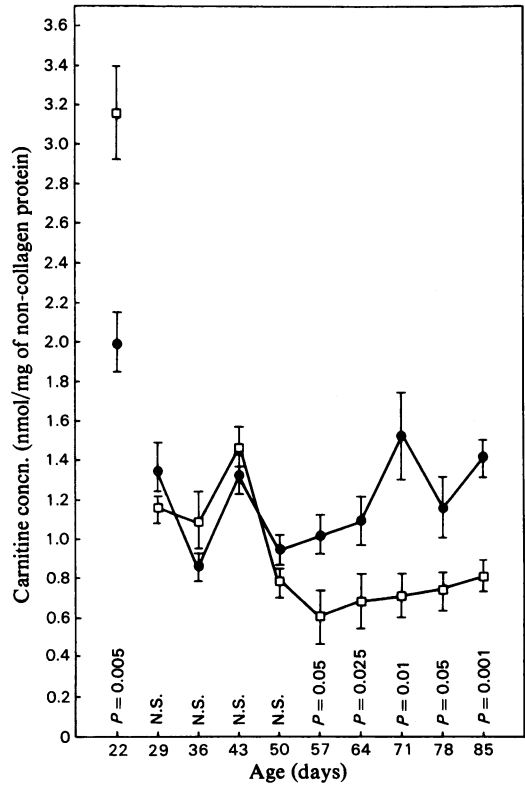


Fig. 4. Carnitine concentrations in the liver of female and male rats from age 22 days to 85 days

Liver carnitine concentrations were determined in the same animals described in the legend to Fig. 3. Symbols: ●, females; □, males. N.S., P > 0.05.

Cardiac-muscle and skeletal-muscle carnitine concentrations

Cardiac-muscle carnitine values are presented in Fig. 5(a). The carnitine concentrations in the cardiac muscle of the adult male rat are significantly higher than the carnitine concentrations of the adult female heart. Fig. 5(b) shows the same trend in skeletal muscle. The concentration of skeletal-muscle carnitine is higher in males than in females after about 50 days of age.

Epididymal carnitine concentrations

Epididymal carnitine concentrations in the male rats used in this experiment are presented in Table 1. There is a dramatic increase in the epididymal carnitine concentrations at the same time that significantly higher concentrations of carnitine are found in the plasma, heart and muscle of the male rat compared with that of the female rat.

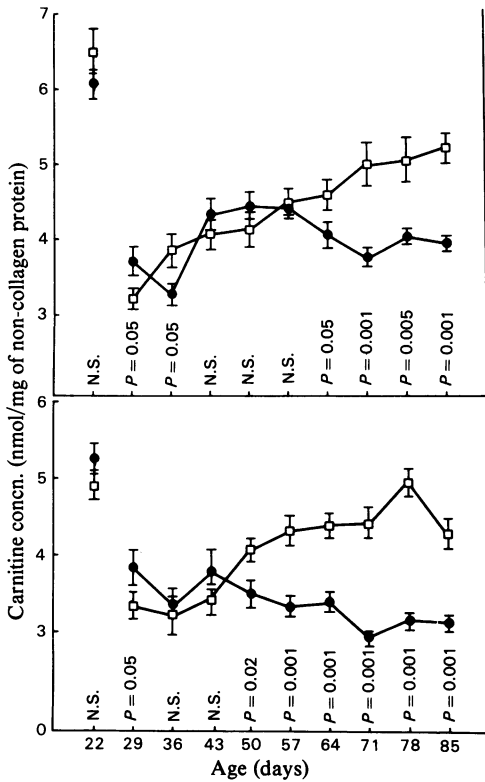


Fig. 5. Carnitine concentrations in the heart and muscle of female and male rats from 22 days to 85 days (a) Heart carnitine concentrations were determined in the same animals described in the legend to Fig. 3. (b) Skeletal-muscle carnitine concentrations. Symbols: ●, females; □, males. N.S., $P > 0.05$.

Urinary carnitine concentrations

The urinary carnitine values in Table 2 are based on mg of creatinine in the urine. The same trend emerges when the carnitine values are expressed on the basis of a 24h collection period. At the same time points at which earlier data showed higher concentrations of carnitine in most tissues of the male rat than in the female rat, the female is excreting more carnitine in her urine than is the male rat.

Discussion

Nutritionally the laboratory rat must quickly switch from the predominantly carbohydrate trans-placental alimentation to a high-fat diet of milk. During the suckling period, the rat obtains most of its energy from fatty acid oxidation, so the presence of an adequate amount of carnitine in the newborn may be of the utmost importance (Bailey & Lockwood, 1973). The primary source of carnitine in neonatal tissues, at least 2-3 days *post partum*, is the mother rat, whose liver and milk carnitine concentrations are very high (Robles-Valdes *et al.*, 1976). It is not known if the newborn liver is capable of synthesizing adequate amounts of carnitine when the newborn is deprived of dietary carnitine.

The complete series of experiments with male and female rats from 22 to 85 days of age has been carried out twice with identical results. An important aspect of this work is that since all rats were fed on a diet containing no carnitine, but sufficient lysine and methionine for carnitine biosynthesis, the plasma carnitine concentrations are not simply a reflection of recent oral carnitine intake. The high concentration of carnitine found in heart, muscle, and liver on the day of shipment (Figs. 4, 5a and 5b) may indeed

Table 1. Age-dependence of carnitine concentrations in the epididymis of rats

The mean epididymal carnitine concentrations of male rats at various ages are presented \pm s.e.m. for ten animals. The increase in carnitine concentrations over that of the weanling is also given.

Age (days)	Carnitine (nmol/mg of non-collagen protein)	Increase over weanling concentration (-fold)
22	8 \pm 1	1.0
29	16 \pm 1	2.0
36	25 \pm 1	3.2
43	29 \pm 2	3.7
50	48 \pm 4	6.0
57	79 \pm 7	9.8
64	129 \pm 17	16.1
71	154 \pm 10	19.2
78	161 \pm 10	20.2
85	205 \pm 22	25.6

Table 2. Age-dependence of urinary carnitine excretion in female and male rats

The mean urinary carnitine concentrations of male and female rats are presented \pm s.e.m. for 16 animals. The P value is for female urinary carnitine values against male urinary carnitine values. N.S., $P > 0.05$.

Age (days)	Carnitine (nmol/mg of creatinine)		
	Females	Males	P
22	397 \pm 56	543 \pm 54	N.S.
29	338 \pm 33	180 \pm 25	0.001
36	233 \pm 36	92 \pm 15	0.001
43	207 \pm 23	57 \pm 13	0.001
50	162 \pm 26	41 \pm 8	0.001
57	247 \pm 27	59 \pm 19	0.001
64	217 \pm 18	55 \pm 10	0.001
71	136 \pm 18	61 \pm 9	0.001
78	215 \pm 12	106 \pm 15	0.001
85	211 \pm 13	165 \pm 16	0.05

reflect the response of tissue carnitine concentrations to starvation (McGarry *et al.*, 1975) and the normal changes brought on by the stress of shipment. Although the mechanism resulting in the altered tissue concentrations after shipment are not well understood, the data emphasize the need to control stringently both the diet and the environment of animals that are used in tissue carnitine studies.

The results of these experiments clearly show that tissue carnitine concentrations in the rat are influenced both by the age and sex of the animal. Present work indicates that the same situation may apply to humans. It has been reported that in humans plasma carnitine concentrations are lower in the female than in the male (Cederblad, 1976). We have confirmed these results, and have also found that histologically normal male muscle has 27.4 ± 1.6 (\pm S.E.M.; $n=23$) nmol of carnitine/mg of non-collagen protein, whereas normal female muscle has 20.3 ± 1.5 (\pm S.E.M.; $n=16$) nmol of carnitine/mg of non-collagen protein. The difference between carnitine concentrations in normal male and normal female muscle is significantly different ($P=0.005$). Thus both the age and the sex of each subject must be considered, whether an investigation involves the effect of some hormonal or environmental factor on carnitine metabolism in experimental animals, or the study of a patient with a disease characterized by abnormal tissue carnitine concentrations.

The main site of carnitine biosynthesis is the liver, with some carnitine being synthesized in the testes (Haigler & Broquist, 1974; Cox & Hoppel, 1974). Thus the carnitine concentration in any other tissue is the summation of the transport of carnitine into and out of that tissue. The carnitine concentration of a tissue would therefore be regulated by factors controlling the rate of carnitine biosynthesis and carnitine transport. Preliminary studies indicate that a product of the ovaries and a product of the testes as well as a product of the pituitary have a role in

the control of tissue carnitine concentrations (P. R. Borum, unpublished work).

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