

Seminal Carnitine Content in Obstructive Azoospermia

Correlation With the Anatomic Level of Obstruction

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Free carnitine in human semen originates predominantly in the epididymis. The role of carnitine in the evaluation of different forms of obstructive azoospermia was studied in 42 patients. In 14 of the men, a bilateral vasectomy had been performed. In the remaining 28 patients, the occlusion was located within the epididymis. In postvasectomy cases and where the occlusion was located in the cauda epididymidis, carnitine concentrations were low, with mean values of 115.57 $\mu\text{mol/l}$ and 121.28 $\mu\text{mol/l}$, respectively. When the occlusion was located in the corpus epididymidis, the mean value increased to 194.72 $\mu\text{mol/l}$. In patients having obstruction of the caput epididymidis or of the rete testis, the mean value of free carnitine was 416.0 $\mu\text{mol/l}$. After vasovasostomy, a return of free carnitine concentration to the normal range was observed in 10 of 12 cases. The results indicate that there is a significant correlation in patients with obstructive azoospermia between the concentration of free carnitine and the anatomic site of the obstruction. These findings may lead to important conclusions concerning therapy and prognosis for patients presenting with this condition.

Key words: carnitine, obstructive azoospermia, epididymal marker, vasovasostomy.

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Carnitine, a 3-hydroxy-4-trimethylamino-butyric acid, is a vitamin-like compound. Acetylation of carnitine plays an important role in normal sperm metabolism, and acetylcarnitine has been found in much higher concentrations in normal spermatozoa than carnitine itself (Casillas and Erickson, 1975; Brooks, 1979). Hutson et al (1977) have shown in bovine spermatozoa that carnitine increases pyr-

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uvate metabolism, which is known to be important for *in vitro* sperm capacitation.

In 1964, Marquis and Fritz were able to show for the first time that a high concentration of carnitine could be measured in the epididymis of rats. Their results have been confirmed by several other investigators in mammals as well as in humans, and it was demonstrated that free carnitine in human semen is predominantly of epididymal origin (Wetterauer and Heite, 1978; Brooks, 1979; Hinton and Setchell, 1980; Soufir et al, 1981). Casillas (1973) has shown in bulls that the carnitine concentration of spermatozoa increases progressively during their passage through the epididymal duct. Similar results have been obtained in other mammals and in humans (Bedford et al, 1973; Bedford, 1975; Wetterauer and Heite, 1978). Subsequently, several groups have studied the relevance of carnitine as a possible marker in different fertility problems, especially in azoospermia (Wetterauer and Heite, 1978; Sade et al, 1978; Soffer et al, 1981). Extremely low concentrations have been measured in semen samples of patients after vasectomy and in cases of obstructive azoospermia where the ejaculate consisted purely of prostatic secretions. Patients with testicular azoospermia, however, usually have carnitine levels within the normal range, except for patients with severely damaged testes, such as cases of bilateral cryptorchidism or Klinefelter's syndrome (Lewin et al, 1981).

We have determined free carnitine in the semen of patients with infertility problems starting in 1979.

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Our special interest was the correlation of seminal carnitine content and the anatomic level of epididymal obstruction in various forms of obstructive azoospermia.

Material and Methods

Free carnitine concentrations were measured in samples of fresh seminal plasma that were obtained by masturbation after 3 to 5 days of sexual abstinence. First, a normal range was defined by measuring carnitine levels in more than 100 normospermic patients. Normospermia was defined as: at least 20 million spermatozoa/ml with at least 60% normal progressive motility and normal morphology.

The determination of free carnitine was performed in the laboratory department of our hospital using an enzymatic-colorimetric method described by Pearson et al (1974). For measurement of carnitine, the carnitine acetyltransferase reaction was employed, in which L-carnitine is reacted with acetyl Co A, which reacts with 5,5-dithiobis-2-nitrobenzoate. The intensively colored 5-thio-2-nitrobenzoate-anion is measured photometrically at 412 nm. The ejaculate is first deproteinized with perchlorate, and the supernatant used for carnitine evaluation. The standard error is 3 to 5% using this technique, while the sensitivity of the assay is between 98 and 100%. Due to the exquisite specificity of the carnitine acetyltransferase reaction, there is no interference by other compounds.

Carnitine levels were measured in at least two semen samples from a total of 42 patients with proven obstructive azoospermia of different origins (Table 1).

Azoospermia was documented in all of these patients by two or more spermograms. In 14 of the 42 men, a bilateral vasectomy had been performed no less than 3 months previously. Microscopic examination revealed no spermatozoa.

In the remaining 28 patients, the origin of the azoospermia was not known. Hormone assays had shown normal levels of FSH, LH and prolactin. Serum testosterone (T) also was normal. In all 28 cases, a bilateral testicular biopsy was done to exclude any testicular cause of the disturbance and normal spermatogenesis was demonstrated in all histologic specimens. Surgical exploration of both testes and the epididymis was performed on these 28 men with proven obstructive azoospermia by means of a scrotal incision. After exploration of the scrotal contents, the exact anatomic site of the obstruction was localized. The seminal duct was opened close to the cauda epididymidis and the liquid was examined microscopically. In no case were spermatozoa seen. The epididymal duct was then exposed further, incised in the region of the cauda epididymidis and its contents examined microscopically again. When no spermatozoa were found, exposure proceeded toward the corpus and finally the caput epididymidis. After exact localization of the obstruction on both sides, an epididymovasostomy was performed using a single-layer suture technique. Usually when the duct was obstructed in the region of the head of the epididymis or even at the rete testis, no attempt was made to perform reconstructive surgery. In another group of patients (n = 12) who had been vasectomized earlier for family-planning

TABLE 1. Causes of Azoospermia in Patients Evaluated for Seminal Carnitine

Diagnosis	n
Vasectomy	14
Occlusion of rete testis or caput epididymidis	14
Occlusion of corpus	8
Occlusion of cauda	6
Total	42

reasons, and who presented at our institution wishing to have their fertility restored, carnitine content also was measured in a preoperative ejaculate. A vasovasostomy was performed on both sides using magnifying glasses. Carnitine concentration was measured again in the postoperative spermogram 2 to 3 months later.

Results

As shown in Table 2, carnitine concentration in normospermic males ranged between 440 and 990 $\mu\text{mol/l}$. After vasectomy, carnitine values in the 14 ejaculates ranged from 51.49 to 134.0 $\mu\text{mol/l}$ with a mean of 115.57 $\mu\text{mol/l}$. In the group of men with bilateral obstruction of the epididymis (n = 28), the following results were obtained: in cases where the occlusion was located on both sides in the cauda epididymidis, carnitine concentrations were very low, and were similar to those found after vasectomy (mean = 121.28 $\mu\text{mol/l}$). Carnitine levels increased when the obstruction was closer to the testis. In patients with occlusions in the region of the corpus epididymidis, the mean value was 194.72 $\mu\text{mol/l}$, and in those with obstruction of the caput or the rete testis the mean was 416.0 $\mu\text{mol/l}$, which is almost within the normal range.

After successful vasovasostomy, carnitine concentrations returned to the normal range in 10 cases

TABLE 2. Correlation of Carnitine Content and Anatomic Site of Obstruction*

	Mean†	Standard Deviation	SEM
Vasectomy	115.57	19.42	5.19
Occlusion of rete testis or caput epididymidis	416.00	34.55	9.24
Occlusion of corpus	194.72	39.47	13.95
Occlusion of cauda	121.28	13.74	5.61
Successful vasovasotomy	509.93	104.82	33.15

*Normal range of free carnitine: 440 $\mu\text{mol/l}$ -990 $\mu\text{mol/l}$.
† $\mu\text{mol/l}$. Significance of the differences checked by the Wilcoxon-Mann-Whitney-test (nonparametric on the $\alpha = 0.05$ level.

Significantly different: 2 vs. 3, 2 vs. 4, 3 vs. 4.

Not significantly different: 1 vs 4.

after 3 to 6 months. The two patients who showed persistent azoospermia after reconstructive surgery had relatively low carnitine values (173.70 and 223.33 $\mu\text{mol/l}$).

Discussion

The results of our investigation indicate that free carnitine concentrations in human semen correlate well with the level of epididymal obstruction. This is clinically important for handling refertilization procedures because spermatozoa from different regions of the epididymis show marked differences in fertilizing capacity (Casillas, 1973).

Spermatozoa from the caput epididymidis are more or less immobile or have irregular vibrations. Only after passage through the body, and especially the tail, of the epididymis are they able to exhibit regular propulsive movements. This difference in motility is one reason for the very poor results of operative procedures that seek to reestablish fertility in cases of occlusions at the level of the caput and even the corpus epididymidis. As our results show, the lower the carnitine level, the more distal the occlusion is likely to be located and the better the prognosis after surgery. Vasectomized patients and those with an obstruction in the tail of the epididymis will show mean carnitine values under 130 $\mu\text{mol/l}$ and can be expected to have the best results after refertilization procedures. On the other hand, patients with occlusion of the epididymal duct close to the testis or even at the level of the rete testis will show carnitine values close to normal. Their postoperative prognosis as regards fertility probably will be rather poor.

The finding that very low carnitine concentrations can be measured after vasectomy (Sade et al, 1978; Soffer et al, 1981; Lewin et al, 1981) also was confirmed in our investigation. Extremely low carnitine levels have been found in semen consisting purely of prostatic fluids (Lewin et al, 1981). This seems to prove that the prostate does not play an important role in carnitine production. As soon as the patency of the vas has been restored, carnitine levels return to normal.

Invasive diagnostic measures such as deferentography, which in itself may cause an inflammatory reaction, should be considered superfluous if not obsolete today. Evaluation of free carnitine is simple and will hint at the site of obstruction. Our investigation, like those of others (Wetterauer and Heite,

1978; Lewin et al, 1981), clearly indicates a role for carnitine as a useful marker of epididymal function. The routine examination of this substance in the ejaculates of patients with azoospermia represents an important step in patient evaluation prior to surgical procedures that attempt to restore fertility. Because carnitine concentrations are also low in some cases of testicular azoospermia where the testis is severely damaged and Leydig cell function is altered, the differentiation of a testicular or obstructive origin of azoospermia cannot be made based on carnitine measurements alone. In these cases, histologic examination of bilateral testicular biopsies remains a prerequisite.

References

- Bedford JM. Maturation, transport and fate of spermatozoa in the epididymis. In: Hamilton DW, Greep RO, eds. Handbook of physiology. Sect 7: Endocrinology; Vol 5: Male reproductive system. Washington, D.C.: American Physiology Society, 1975; 304-388.
- Bedford JM, Calvin HI, Cooper GW. The maturation of sperm in the human epididymis. *J Reprod Fertil* 1973; 18 (Suppl): 199-202.
- Brooks DE. Carnitine, acylcarnitine and the activity of carnitine acyltransferases in seminal plasma and spermatozoa of men, rams and rats. *J Reprod Fertil* 1979; 56:667-673.
- Casillas EP. Uptake of carnitine by bovine spermatozoa during maturation in the epididymis. *J Biol Chem* 1973; 248:8227-8232.
- Casillas ER, Erickson BJ. The role of carnitine in spermatozoan metabolism: Substrate-induced elevations in acetylation state of carnitine and coenzyme A in bovine and monkey spermatozoa. *Biol Reprod* 1975; 12:275-283.
- Hinton BT, Setchell BP. Concentration of some organic compounds in the luminal fluid of the testis and epididymis of the rat and some other mammals. *J Androl* 1980; 1:83-87.
- Hutson SM, Van Dop C, Lardy HA. Mitochondrial metabolism of pyruvate in bovine spermatozoa. *J Biol Chem* 1977; 252:1309-1315.
- Lewin LM, Shalev DP, Weissenberg R, Soffer Y. Carnitine and acylcarnitines in semen from azoospermic patients. *Fertil Steril* 1981; 36:214-218.
- Marquis R, Fritz IB. Enzymological determination of free carnitine concentrations in rat tissues. *J Lipid Res* 1964; 5:184-187.
- Pearson DJ, Tubbs PHK, Chase JFA. Carnitine and acylcarnitine. In: Bergmeyer HU, ed. *Methoden der enzymatischen analyse*. Weinheim, FRG: Verlag Chemie, 1974; 1806-1808.
- Sade M, Saverymuth I, Dinsdale OA, Gow JE. The management of azoospermia. *Br J Urol* 1978; 50:595-597.
- Soffer Y, Shalev DP, Weissenberg R, Orenstein H, Nebel L, Lewin LM. Survey of carnitine content of human semen using a semiquantitative auxanographic method: decreased semen total carnitine concentration in patients with azoospermia or severe oligozoospermia. *Andrologia* 1981; 13: 440-444.
- Soufir JC, Marson J, Jouannet P. Free L-Carnitine in human seminal plasma. *Int J Androl* 1981; 4:388-390.
- Wetterauer U, Heite H-J. Carnitine in seminal fluid as parameter for epididymal function. *Andrologia* 1978; 10:203-210.