The Sources of Carnitine in Human Semen

FERESHTEH FAHIMI,* LORAN BIEBER,† AND LAWRENCE M. LEWIN‡

*Department of Obstetrics and Gynecology and the †Department of Biochemistry, Michigan State University, East Lansing, Michigan, and the ‡Department of Chemical Pathology, Tel Aviv University, Sackler Medical School, Tel Hashomer, Israel

A study was initiated to determine the sources of carnitine in the male reproductive system. Free and total carnitine was measured in a group of healthy men before and after vasectomy. Each individual served as his own control. The results show that postvasectomy semen contains about 52% as much total carnitine and 40% as much free carnitine as prevasectomy samples. The data indicate that, in addition to the epididymis, the seminal vesicles and/or vas deferens are major contributors of carnitine in human semen.

Key words: carnitine, seminal vesicles, epididymis, semen, vasectomy.

Approximately 40% of infertility problems in humans can be attributed to a male factor. To date there are many unanswered questions concerning the role of carnitine in male reproductive physiology and pathophysiology.

There are indications of a role for carnitine in sperm metabolism (Casillas and Erickson, 1975), sperm maturation, fertilization capability (Casillas and Chaipayungpan, 1979), and motility (Tanphaichitr, 1977). Identification of the source of carnitine in human semen could help to define its physiologic role.

There are conflicting reports in the literature regarding the source of free carnitine in seminal plasma. Frankel et al (1974) compared levels of free carnitine in semen in normal donors with the levels in patients 30 days after vasectomy. They concluded that about 58% of free carnitine in human semen originates from the epididymis. Wetterauer and Heite (1978 and 1980) compared the mean free carnitine concentrations after vasectomy with those in normal ejaculates and reported that 94% of the free carnitine originates from the epididymis. Lewin et al (1979) compared the free carnitine levels in fertile semen (artificial insemination donors) with levels in postvasectomy semen and found one-third as much free carnitine in postvasectomy semen as in normal semen.

This study was initiated to: 1) determine the proportion of free carnitine originating from the epididymis; 2) determine whether free carnitine measurements can be used to evaluate epididymal function; and 3) to determine whether carnitine measurements can be helpful in detecting the location of obstruction in the male reproductive system in the treatment of obstructive azoospermia.

Materials and Methods

This study was conducted in a group of married men, between the ages of 27 and 37, who were determined to be healthy and were on no medication. Prior to elective vasectomy, ejaculated semen samples were collected and held frozen from the time they were received in the laboratory until used. Additional specimens were obtained five to six weeks after the vasectomies were performed, as suggested by Chun et al (1980). Each patient served as his own control.

The semen samples were deproteinized with perchloric acid (5%) and neutralized with potassium hydroxide. Aliquots of the supernatant fluid were analyzed for free carnitine by the radioenzyme method of Cederblad and Lindstedt (1972). Total carnitine was measured from an aliquot of the deproteinized supernatant fluid after adjusting the *p*H to >12 by the addition of potassium hydroxide (10 M) before incubation at 37 C for 1 hour. The resulting hydrolyzate was neutralized and assayed by the method of Cederblad and Lindstedt (1972).

Reprint requests: Fereshteh Fahimi, M.D., Department of Obstetrics and Gynecology, B316 Clinical Center, Michigan State University, East Lansing, Michigan 48824.

Submitted for publication August 18, 1980; revised version received April 21, 1980; accepted for publication April 22, 1981.

0196-3635/81/1100/0339/$00.70

TABLE I. Pre- and Postvasectomy Levels of Carnitine in Human Semen

Patient No. (Initials)	Total Carnitine (nmol/ml)		Free Carnitine (nmol/ml)	
	Prevasectomy	Postvasectomy	Prevasectomy	Postvasectomy
1. J.B.	916	372	842	269
2. D.B.	562	316	546	241
3. S.M.	533	211	464	87
4. H.R.	1348	786	1154	538
5. B.W.	712	266	529	214
6. J.W.	414	403	354	352
7. F.M.	816	170	699	79
8. E.R.	300	258	245	143
9. D.R.	452	214	408	159
10. R.R.	394	354	293	298
11. M.F.	410	221	325	59
Mean ± SEM	625 ± 92	324 ± 51*	532 ± 82	221 ± 42*

*$P < 0.005$.

The data were analyzed statistically using a t test for paired data to determine the significance of the differences between the means of pre- and postvasectomy concentrations of free and total carnitine. The 95% confidence interval of the mean was calculated for total and free carnitine in pre- and postvasectomy samples.

Results

The amounts of total carnitine and free carnitine in seminal plasma from pre- and postvasectomy specimens are shown in Table 1. The range of total carnitine in the prevasectomy specimens was between 300 and 1348 nmol/ml. The range in postvasectomy specimens was between 170 and 786 nmol/ml. The mean ($\bar{x}$) and standard error of the mean (SEM) for total carnitine were 625 ± 92 nmol/ml for prevasectomy specimens and 324 ± 51 nmol/ml for postvasectomy specimens (Fig. 1). The difference between pre- and postvasectomy production of total carnitine is statistically significant ($P = 0.005$). The 95% confidence interval of the mean for total carnitine in the prevasectomy group was between 419 and 831 nmol/ml and for the postvasectomy group was between 210 and 439 nmol/ml (Fig. 1).

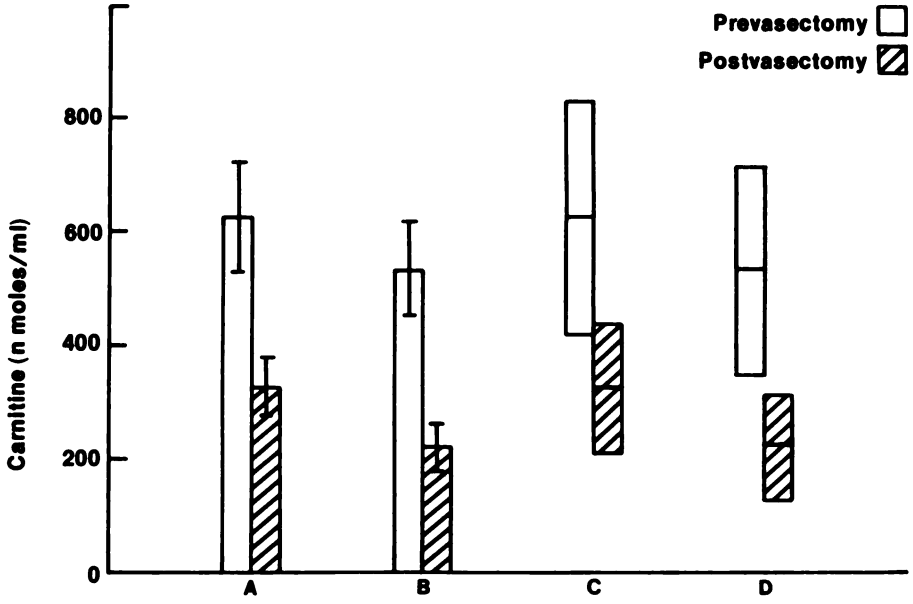

Fig. 1. Comparison of total and free carnitine levels in pre- and postvasectomy semen samples. A. Mean of total carnitine in pre- and postvasectomy human semen. B. Mean of free carnitine in pre- and postvasectomy human semen. (The standard errors of the mean are indicated by the vertical lines.) C. Ninety-five percent confidence interval of the mean for total carnitine in human semen. D. Ninety-five percent confidence interval of the mean for free carnitine in human semen. (The horizontal line in the middle of each bar represents the mean.)

The range of free carnitine for prevasectomy specimens was between 245 and 1154 nmol/ml and for postvasectomy specimens, between 59 and 538 nmol/ml. The mean and standard error of the mean for free carnitine were 532 ± 82 nmol/ml in the prevasectomy group and 221 ± 42 nmol/ml in the postvasectomy group (Fig. 1). This difference between pre- and postvasectomy levels of free carnitine is statistically significant ($P < 0.005$). The 95% confidence interval of the mean for free carnitine in the prevasectomy group was between 349 and 716 nmol/ml and for the postvasectomy group was between 126 and 316 nmol/ml (Fig. 1). There was no overlap of the free carnitine level in the pre- and postvasectomy samples. The total carnitine level, however, shows a slight overlap between pre- and postvasectomy specimens.

The results indicate that postvasectomy semen contains about 40% as much free carnitine and 52% as much total carnitine as prevasectomy semen (Fig. 1). These data show that, in addition to the epididymis, the accessory sex organs contribute free carnitine to seminal plasma.

Discussion

The semen ejaculated after vasectomy does not contain secretions from the testis or the epididymis but consists mainly of secretions from the seminal vesicles and the prostate gland and a small contribution from the vasa deferentia. Since bioautography of prostatic fluid (obtained by prostatic massage) shows only traces of carnitine and its acyl derivates, some of the carnitine in postvasectomy semen must be contributed by the seminal vesicles (Lewin et al, 1979). The results in Table 1 show that free carnitine in seminal plasma is derived from both the epididymis and accessory reproductive organs. This is in contrast to the lack of carnitine in semen of vasectomized rams (Brooks, 1979) and to the results obtained in the rat. In the rat, the epididymis appears to be the major source of carnitine (Brooks et al, 1974).

Measurements of carnitine levels in human semen have been suggested by Wetterauer and Heite (1978) to be useful as a "representative biochemical parameter" of epididymal function. In a subsequent publication, these authors (Wetterauer and Heite, 1980) have suggested that the estimation of carnitine plus fructose and citrate is useful for locating the site of obstructive azoospermia. The fructose level would be an indicator of seminal vesicle function; citrate or acid phosphatase and, to a lesser degree, gamma-GT would be indicators of prostate function; and the carnitine level would be an indicator of epididymal function. However, our study strongly indicates that carnitine in seminal plasma is derived from both the epididymis and the seminal vesicles and/or vas deferens and thus supports the work of Lewin et al (1976) on the usefulness of measuring carnitine and fructose levels for assessment of both epididymal and seminal vesicle functions. The data reported here indicate that there is no overlap in the 95% confidence interval of the mean for free carnitine levels in pre- and postvasectomy specimens and that the overlap is minimal when total carnitine levels are considered (Fig. 1).

There were no differences in the levels of free carnitine before and after vasectomy in two of the cases studied (#6 and #10) (Table I). Since the samples were collected, stored, and measured under similar conditions, it seems likely that free carnitine in the prevasectomy samples was contributed mainly by the seminal vesicle and/or vas deferens, probably due to epididymal dysfunction. The amounts of total and free carnitine in these two samples were lower than the average, also indicating an impaired output of carnitine and a possibility of epididymal dysfunction.

In summary, carnitine levels have been measured in human semen before and after vasectomy. The results indicate that postvasectomy specimens contain 40% as much free carnitine and 52% as much total carnitine as prevasectomy semen. Therefore, in addition to the epididymis, the seminal vesicle and/or vas deferens contribute to the carnitine level in human semen.

Acknowledgment

The authors express their appreciation to Dr. Burton Fink for his assistance in obtaining samples of pre- and postvasectomy semen.

References

Brooks DE. Carnitine, acetylcarnitine and the activity of carnitine acyltransferases in seminal plasma and spermatozoa of men, rams and rats. J Reprod Fertil 1979; 56:667–673.

Brooks DE, Hamilton DW, Mallek AH. Carnitine and glycerylphosphorylcholine in the reproductive tract of the male rat. J Reprod Fertil 1974; 36:141–160.

Casillas ER, Chaipayungpan S. The distribution of carnitine and acetylcarnitine in the rabbit epididymis and the carnitine content of rabbit spermatozoa during maturation. J Reprod Fertil 1979; 56:439–444.

Casillas Er, Erickson BJ. The role of carnitine in spermatozoan metabolism: substrate-induced elevations in the acetyla-

tion state of carnitine and coenzyme A in bovine and monkey spermatozoa. Biol Reprod 1975; 12:275–283.

Cederblad G, Lindstedt S. A method for the determination of carnitine in the picomole range. Clin Chim Acta 1972; 37:235–243.

Chun NL, Mumford SD, Atwood RJ. Postvasectomy residual sperm pregnancy. Fertil Steril 1980; 33:668–669.

Frankel G, Peterson RN, David JE, Freund M. Glycerylphosphorylcholine and carnitine in normal human semen and in postvasectomy semen: differences in concentration. Fertil Steril 1974; 24:84–87.

Lewin LM, Beer T, Lunenfeld B. Epididymis and seminal vesicle as sources of carnitine in human seminal fluid: the clinical significance of the carnitine concentration in human seminal fluid. Fertil Steril 1976; 27:9–13.

Lewin LM, Holzman BG, Fahimi F, Choi Y, Bieber LL. Carnitine acyl esters of human semen. Int J Androl 1979; 2:542–548.

Tanphaichitr N. *In vitro* stimulation of human sperm motility by acetylcarnitine. Int J Fertil 1977; 22:85–91.

Wetterauer U, Heite HJ. Carnitine in seminal fluid as parameter for the epididymal function. Andrologia 1978; 10: 203–210.

Wetterauer U, Heite JJ. Carnitine in seminal plasma: its significance in diagnostic andrology. Arch Androl 1980; 4: 137–143.

U.S. POSTAL SERVICE

STATEMENT OF OWNERSHIP, MANAGEMENT AND CIRCULATION

(Required by 39 U.S.C. 3685)

1. TITLE OF PUBLICATION	A. PUBLICATION NO.	2. DATE OF FILING
JOURNAL OF ANDROLOGY	5 3 5 - 6 9 0	October 1, 1981
3. FREQUENCY OF ISSUE	**A. NO. OF ISSUES PUBLISHED ANNUALLY**	**B. ANNUAL SUBSCRIPTION PRICE**
BI-MONTHLY	6	$49 Pers. $53 Inst.

4. COMPLETE MAILING ADDRESS OF KNOWN OFFICE OF PUBLICATION *(Street, City, County, State and ZIP Code) (Not printers)*

2350 VIRGINIA AVENUE, HAGERSTOWN, MD 21740

5. COMPLETE MAILING ADDRESS OF THE HEADQUARTERS OR GENERAL BUSINESS OFFICES OF THE PUBLISHERS *(Not printers)*

EAST WASHINGTON SQUARE, PHILADELPHIA, PA 19105

6. FULL NAMES AND COMPLETE MAILING ADDRESS OF PUBLISHER, EDITOR, AND MANAGING EDITOR *(This item MUST NOT be blank)*

PUBLISHER *(Name and Complete Mailing Address)*

J. B. LIPPINCOTT COMPANY, EAST WASHINGTON SQUARE, PHILADELPHIA, PA 19105

EDITOR *(Name and Complete Mailing Address)*

ANDRZEJ BARTKE, PH.D., DEPT. OF OBSTETRICS AND GYNECOLOGY, UNIV. OF TEXAS HEALTH SCI. CTR., 7703 FLOYD CURL DR., SAN ANTONIO, TX 78284

MANAGING EDITOR *(Name and Complete Mailing Address)*

DEBORAH A. FINCH, EAST WASHINGTON SQUARE, PHILADELPHIA, PA 19105

7. OWNER *(If owned by a corporation, its name and address must be stated and also immediately thereunder the names and addresses of stockholders owning or holding 1 percent or more of total amount of stock. If not owned by a corporation, the names and addresses of the individual owners must be given. If owned by a partnership or other unincorporated firm, its name and address, as well as that of each individual must be given. If the publication is published by a nonprofit organization, its name and address must be stated.)* (Item must be completed)

FULL NAME	COMPLETE MAILING ADDRESS
THE AMERICAN SOCIETY OF ANDROLOGY	c/o OREGON REGIONAL PRIMATE CENTER 505 N.W. 185TH STREET BEAVERTON, OR 97005

8. KNOWN BONDHOLDERS, MORTGAGEES, AND OTHER SECURITY HOLDERS OWNING OR HOLDING 1 PERCENT OR MORE OF TOTAL AMOUNT OF BONDS, MORTGAGES OR OTHER SECURITIES *(If there are none, so state)*

FULL NAME	COMPLETE MAILING ADDRESS
NONE	

9. FOR COMPLETION BY NONPROFIT ORGANIZATIONS AUTHORIZED TO MAIL AT SPECIAL RATES *(Section 411.3, DMM only)*

The purpose, function, and nonprofit status of this organization and the exempt status for Federal income tax purposes (Check one)

☐ (1) HAS NOT CHANGED DURING PRECEDING 12 MONTHS ☐ (2) HAS CHANGED DURING PRECEDING 12 MONTHS *(If changed, publisher must submit explanation of change with this statement.)*

10. EXTENT AND NATURE OF CIRCULATION	AVERAGE NO. COPIES EACH ISSUE DURING PRECEDING 12 MONTHS	ACTUAL NO. COPIES OF SINGLE ISSUE PUBLISHED NEAREST TO FILING DATE
A. TOTAL NO. COPIES *(Net Press Run)*	1,893	1,077
B. PAID CIRCULATION 1. SALES THROUGH DEALERS AND CARRIERS, STREET VENDORS AND COUNTER SALES	51	118
2. MAIL SUBSCRIPTION	556	483
C. TOTAL PAID CIRCULATION *(Sum of 10B1 and 10B2)*	607	601
D. FREE DISTRIBUTION BY MAIL, CARRIER OR OTHER MEANS SAMPLES, COMPLIMENTARY, AND OTHER FREE COPIES	941	29
E. TOTAL DISTRIBUTION *(Sum of C and D)*	1,548	630
F. COPIES NOT DISTRIBUTED 1. OFFICE USE, LEFT OVER, UNACCOUNTED, SPOILED AFTER PRINTING	345	447
2. RETURN FROM NEWS AGENTS	-	-
G. TOTAL *(Sum of E, F1 and 2 - should equal net press run shown in A)*	1,893	1,077

11. I certify that the statements made by me above are correct and complete

SIGNATURE AND TITLE OF EDITOR, PUBLISHER, BUSINESS MANAGER, OR OWNER Joseph W. Lippincott, III, Circulation Manager

PS Form July 1981 3526 *(See instruction on reverse)* *(Page 1)*