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Characterization of organic cation/carnitine transporter family in human sperm

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Abstract

Spermatozoan maturation, motility, and fertility are, in part, dependent upon the progressive increase in epididymal and spermatozoal carnitine, critical for mitochondrial fatty acid oxidation, as sperm pass from the caput to the cauda of the epididymis. We demonstrate that the organic cation/carnitine transporters, OCTN1, OCTN2, and OCTN3, are expressed in sperm as three distinct proteins with an expected molecular mass of 63 kDa, using Western blot analysis and our transporter-specific antibodies. Carnitine uptake studies in normal control human sperm samples further support the presence of high-affinity (OCTN2) carnitine uptake (K_m of $3.39 \pm 1.16\,\mu M$; V_{max} of 0.23 ± 0.14 pmol/min/mg sperm protein; and mean ± SD; $n = 12$), intermediate-affinity (OCTN3) carnitine uptake (K_m of $25.9 \pm 14.7\,\mu M$; V_{max} of 1.49 ± 1.03 pmol/min/mg protein; $n = 26$), and low-affinity (OCTN1) carnitine uptake (K_m of $412.6 \pm 191\,\mu M$; V_{max} of 32.7 ± 20.5 pmol/min/mg protein; $n = 18$). Identification of individuals with defective sperm carnitine transport may provide potentially treatable etiologies of male infertility, responsive to L-carnitine supplementation.

Keywords: Carnitine/organic cation transporters; OCTN1; OCTN2; OCTN3; Sperm motility and maturation; Epididymis; Fatty acid oxidation

Carnitine (β-hydroxy-γ-trimethylaminobutyric acid) is a small water-soluble quaternary amine that, in conjunction with carnitine acyltransferases, plays an essential role in the transport of long-chain fatty acids as acylcarnitine esters into mitochondria for β-oxidation and ATP generation, as well as into peroxisomes and endoplasmic reticulum [1,2]. In non-vegetarians, approximately 75% of body L-carnitine is derived from the diet and 25% from de novo biosynthesis from lysine and methionine [3]. Most tissues are capable of the synthesis of γ-butyrobetaine, the immediate precursor of carnitine, which must then be exported to the liver, kidney, or brain for final hydroxylation to carnitine [4]. The carnitine concentration is normally 20–50-fold higher in most tissues (e.g., muscle, heart, and liver) than in serum [5], with the highest concentration being found in the mammalian epididymis (up to 2000-fold) [6]. Uptake into tissues therefore occurs across a large concentration gradient [5,7]. Transport of carnitine into the cell is mediated by a high-affinity, sodium-dependent plasmalemmal carnitine transporter, OCTN2, which has recently been cloned as a member of the carnitine/organic cation transporter family and has a predicted molecular mass of ~63 kDa [8,9]. This high-affinity carnitine transporter is expressed in muscle, heart, kidney, testis, epididymis, etc. and has a K_m of 2–6 μM for carnitine [10–14]. In addition, a low-affinity carnitine transporter with a $K_m \geqslant 500\,\mu M$ is expressed in liver, brain, small intestine, epididymis, testis etc. [15–18] and an intermediate-affinity carnitine transporter has been suggested by kinetic studies in testis, muscle culture, renal tubular cells, and intestinal epithelial cells [12,18–21].

Organic cation transporters function primarily in the elimination of cationic drugs and other xenobiotics in tissues such as kidney, intestine, liver, and placenta by distinct mechanisms that are either dependent on or independent of a sodium-gradient, membrane potential

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or pH [22,23]. The subfamily of carnitine/organic cation transporters, Octn1, Octn2, and Octn3, has been recently isolated and characterized in mice, each having a molecular mass of ~63 kDa [18]. When murine Octn1, Octn2, and Octn3 cDNAs were transfected into HEK293 cells, their relative uptake activity ratios of carnitine to tetraethyl-ammonium (TEA) were 1.78, 11.3, and 746, respectively, suggesting high specificity of Octn3 for carnitine and significantly lower carnitine transport activity of Octn1 [18]. All three were expressed in kidney and testis, and Octn1 and Octn2 were expressed in various other tissues, whereas Octn3 was primarily expressed in testis, suggesting it plays a significant role in testis.

Human OCTN1 and OCTN2 have been shown to share >75% homology [9] and to transport TEA as well as acylcarnitines [8,24–26]. Using a proteomics approach, we recently confirmed the existence of the hOCTN3 protein which is expressed in liver peroxisomes and has an intermediate-affinity K_m of 20 μM for carnitine [27]. OCTN2 has been demonstrated in the basolateral membrane of epithelial cells in the distal caput, corpus, and proximal cauda epididymides of the adult rat [28]. OCTN1 has low affinity for carnitine and appears to be a multispecific organic cation transporter that is ubiquitously expressed [26,29]. There is thus a family of transporters with different affinities for carnitine and differential expression profiles.

Male infertility is a significant problem affecting 7.5% of the male population [30]. Approximately 60% of these cases are idiopathic and related to problems with sperm motility. An important source of aerobic energy production which appears to be necessary for sperm maturation and the acquisition and maintenance of motility is derived from mitochondrial fatty acid oxidation and the generation of acetyl-CoA for which carnitine is an essential cofactor. The concentrations of free L-carnitine in epididymal plasma and spermatozoa are the highest recorded in the mammalian organism (2–100 mM) [17]. Carnitine is progressively concentrated from the bloodstream by the epididymal epithelium of the caput and corpus and secreted into the epididymal lumen [31–35]. Several groups have therefore suggested that carnitine may play an important role in sperm maturation and the development of sperm motility [36–38].

Although carnitine transport has been characterized in many different tissues, the carnitine transport system in human sperm has not been characterized. The juvenile visceral steatosis (*jvs*) Octn2-deficient mouse, which is a murine model for the human, high-affinity carnitine transporter (OCTN2) defect, presents with cardiac hypertrophy, microvesicular fatty infiltration of the viscera as well as male infertility due to obstructive azoospermia which is reversible with L-carnitine supplementation [39,40]. It has been suggested that the carnitine deficiency in the spermatozoa leads to dysmotility and poor survival, resulting in the observed obstruction. For an increased understanding of infertility related to carnitine deficiency, it is therefore important to identify and functionally characterize the relevant carnitine transporters in sperm. We have raised three anti-murine transporter-specific antibodies to Octn1, Octn2, and Octn3 which specifically cross-react with and exquisitely differentiate between their human orthologues [27]. The present study was undertaken to determine the expression of OCTN1, OCTN2, and OCTN3 in human sperm and to characterize their kinetic features.

Materials and methods

Sperm preparation and analysis. All studies were performed with the approval of the Institutional Review Board of the Hospital for Sick Children, Toronto. Residual unused portions of semen samples were obtained, with informed consent, from men, with normal semen parameters, who were donating samples for artificial insemination at the Sunnybrook and Women's College Hospital Fertility Centre (Toronto, Canada). Samples were examined and selected according to World Health Organization [41] laboratory guidelines. The ejaculates were collected by masturbation, following 72 h of sexual abstinence, in sterile containers and allowed to liquefy for at least 30 min at room temperature. Semen suspensions were examined in a Makler chamber and characterized with respect to their concentration, linear progressive movement (motility), and morphology. After liquefaction, the semen samples were processed by adding an equal volume of ENHANCE-S *Plus* gradient system (Conception Technologies, San Diego, CA) in a conical falcon tube. The semen samples were then centrifuged (450*g*) for 20 min. The supernatant was discarded and the pellet was suspended in 2 ml of Sperm Washing Medium (Conception Technologies, San Diego, CA) and centrifuged once again (450*g*, 10 min) to wash the seminal plasma and to remove any exogenous L-carnitine loosely bound to the plasma membrane. Sperm Washing Medium (1 ml) was added to the pellet which was resuspended. Sperm viability was determined by trypan blue dye exclusion and samples with a viability above 80% were used in the study. The sperm pellet was diluted into aliquots containing adequate sperm counts or protein as required for the carnitine uptake studies and Western blot analysis.

Measurement of L-[^{3}H]carnitine uptake. Sperm L-carnitine uptake was measured by a modification of our technique established for cultured skin fibroblasts and lymphoblasts [10,42]. L-[^{3}H]carnitine was purchased from Amersham and L-carnitine was a gift from Sigma-Tau Pharmaceuticals (Pomezia, Italy). The sperm pellet was suspended in 1 ml RPMI 1640 at a concentration of 20×10^6 cells/ml and divided into eight aliquots. An aliquot of 50 μl L-[^{3}H]carnitine label was added to a series of concentrations of unlabeled L-carnitine to make final concentrations of 0.1, 0.2, 0.5, 1, 2, 5, and 10 μM L-carnitine for the measurement of high-affinity carnitine uptake; 1, 2, 5, 10, 20, 50, and 100 μM L-carnitine for the measurement of intermediate-affinity carnitine uptake; and 10, 20, 50, 100, 200, 500, and 1000 μM L-carnitine for the measurement of low-affinity carnitine uptake in a total volume of 1 ml. Non-specific uptake was measured at 10 mM L-carnitine. The sperm was incubated in 95% humidified air and 5% CO_2 at 37 °C for precisely 4 h. The tubes were then placed on ice for 1 min. The sperm were centrifuged at 1400*g* at 4 °C for 5 min. The supernatant was carefully aspirated with transfer pipettes. The pellet was washed twice with 1 ml of ice-cold phosphate buffered saline containing 10 mM unlabelled L-carnitine and centrifuged to remove any exogenous L-carnitine loosely bound to the plasma membrane. The final pellet was resuspended in 1 ml of ice-cold 0.5 M NaOH to lyse the cells. Eight hundred microliters of the sperm lysate was transferred to a

scintillation vial and 10 ml of scintillation liquid cocktail (Beckman Counter) was added to each vial. Samples were counted after overnight incubation in the cold room. Specific uptake of radioactivity was used to calculate the rates of total carnitine uptake in picomole per minute per milligram sperm protein. Protein concentration of the sperm solubilized in 0.5 M NaOH was determined by the Bradford method [43] using the Bio-Rad Protein Assay Kit (Bio-Rad Laboratories, CA) with bovine serum albumin as a standard.

Generation of polyclonal antibodies to mouse Octn1, Octn2, and Octn3. Synthetic peptides to the deduced amino acids at the differentiating carboxy-termini of each of the three carnitine transporters were prepared with an additional cysteine residue which could be used in the MAP (multiple antigen peptide system). The sequences of the synthetic peptides for mOctn1, mOctn2, and mOctn3 were NH_2-CGKKSTV SVDREESPKVLIT-COOH; NH_2-CTRMQKDGEESPTVLKSTAF-COOH; and NH_2-CKESKGNVSRTSRTSEPKGF-COOH, respectively. The polyclonal antibodies were raised in New Zealand white (NZW) male rabbits. We have shown that these antibodies are transporter-specific and cross-react specifically with and differentiate between their human orthologues [27].

Western blot analysis. For Western blot analysis, sperm pellets were added to a lysis buffer after centrifugation and then dispersed ultrasonically. Samples were centrifuged (3000*g*, 15 min, 4 °C) and the supernatant was transferred to a fresh tube. A total of 40 μg protein was electrophoresed on a 10% SDS–PAGE and transferred onto a polyvinylidene fluoride transfer membrane. Non-specific binding sites were blocked by incubating the membranes for 1 h with 10% non-fat milk and Tris-buffered saline containing 0.1% Tween 20. Primary antibody was diluted (1:500) in 1× TBS/10% milk/0.1% Tween 20 and incubated with the blots overnight at 4 °C. Blots were washed in TBST three times for 10 min at room temperature and incubated with HRP-protein A, diluted 1:4000 in 1× TBS/10% milk/0.1% Tween 20 for 1 h at room temperature. The membranes were washed three times with Tris-buffered saline containing 0.5% Tween 20. The ECL Kit (enhanced chemiluminescence kit) (Amersham Pharmacia) was used for the detection system. The blot was then exposed to BioMax X-ray film (Kodak).

Results and discussion

Western blot of OCTN1, OCTN2, and OCTN3

To date, three proteins have been shown to transport L-carnitine, namely OCTN1, OCTN2, and OCTN3. Importantly, we have demonstrated the expression of all three, each with an expected molecular weight of ~63 kDa, in human sperm by Western blot analysis using our anti-murine transporter-specific antibodies (Fig. 1).

Carnitine uptake in sperm

Carnitine uptake studies in human sperm from normal control samples support the presence of a high-affinity transport process with an apparent K_m of 3.39 ± 1.16 μM and a maximal velocity of uptake (V_{max}) of 0.23 ± 0.14 pmol/min/mg of sperm protein (mean ± SD) (Table 1). We also demonstrate an intermediate-affinity carnitine uptake system with a K_m of 25.9 ± 14.7 μM and V_{max} of 1.49 ± 1.03 pmol/min/mg of protein. We further demonstrated low-affinity carnitine uptake system with a K_m of 412.6 ± 191 μM and a V_{max} of 32.7 ± 20.5 pmol/min/mg of protein.

Carnitine is an essential cofactor for long-chain fatty acid oxidation through its transfer of long-chain fatty acids as acylcarnitine esters across the inner mitochondrial membrane [1]. In mammalian metabolism, a major role of free L-carnitine involves the conjugation of acyl residues to the β-hydroxyl group of the carnitine molecule, with subsequent translocation from one cellular compartment to another. Carnitine also modulates the intramitochondrial acyl-CoA/CoA sulfhydryl ratio, providing cells with a critical source of free CoA, and has a detoxifying role of trapping potentially toxic acyl-CoA metabolites that may increase during acute metabolic crises [1]. The role of the carnitine system is therefore to maintain homeostasis in the acyl-CoA pools of the cell, keeping the acyl-CoA/CoA pool constant even under conditions of very high acyl-CoA turnover. Carnitine derivatives can be moved across intracellular barriers thereby providing a shuttle mechanism between

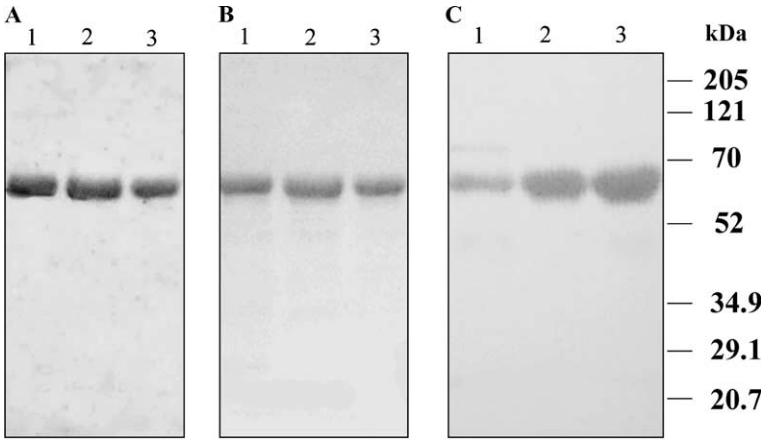

Fig. 1. Western blot analysis of cell lysates isolated from normal control human sperm demonstrating specific expression of hOCTN1, hOCTN2, and hOCTN3, respectively. (A) Blot incubated with rabbit anti-mOctn1 antibody (40 μg sperm protein per lane). (B) Blot incubated with rabbit anti-mOctn2 antibody (40 μg sperm protein per lane). (C) Blot incubated with rabbit anti-mOctn3 antibody (4, 12, and 36 μg sperm proteins loaded in lanes 1, 2, and 3, respectively). Each protein has a molecular mass of ~63 kDa as predicted.

Table 1
Kinetic studies of carnitine uptake by OCTN1, OCTN2, and OCTN3 in normal control human sperm

High-affinity carnitine uptake by OCTN2			Intermediate-affinity carnitine uptake by OCTN3			Low-affinity carnitine uptake by OCTN1		
Sample #	K_m (μM)	V_{max}*	Sample #	K_m (μM)	V_{max}*	Sample #	K_m (μM)	V_{max}*
W024	2.94	0.407	W112	51.9	2.63	W006	361.7	46.2
W027	5.03	0.191	W114	23.8	4.21	W007	402.8	20.1
W029	2.25	0.369	W119	54.2	3.66	W012	434.6	16.8
W030	2.08	0.066	W120	11.6	0.93	W015	249.9	10.3
W034	3.72	0.149	W121	44.2	0.84	W106	186.5	46.8
W038	4.32	0.452	W122	59.8	2.86	W017	558.8	63.2
W039	2.07	0.165	W132	28.6	1.23	W023	309.9	13.8
W040	4.33	0.357	W133	25.0	1.51	W068	401.9	10.8
W041	3.61	0.099	W134	20.2	1.84	W078	706.9	26.9
W042	3.82	0.091	W141	18.3	1.78	W079	764.6	39.2
W044	4.85	0.314	W142	16.9	3.04	W080	570.7	68.9
W181	1.68	0.100	W146	25.9	2.44	W081	660.1	66.4
			W150	21.1	1.09	W082	405.8	26.4
			W156	18.7	1.11	W083	144.1	18.4
			W157	13.8	0.36	W088	424.7	37.4
			W160	18.4	0.66	W089	520.2	53.3
			W161	10.5	0.56	W108	126.3	9.0
			W162	10.5	1.21	W117	197.5	14.9
			W165	13.3	0.62			
			W168	11.7	0.30			
			W170	9.46	0.62			
			W171	26.4	1.19			
			W173	46.9	1.58			
			W174	39.4	1.05			
			W176	19.2	0.70			
			W178	34	0.90			
Mean	3.39	0.23		25.9	1.49		412.6	32.7
SD	1.16	0.14		14.7	1.03		191	20.5

Key: *, pmol/min/mg sperm protein; SD, standard deviation.

mitochondria, peroxisomes, and microsomes for complex lipid-synthetic and -breakdown pathways [2]. The end-product of β-oxidation, acetyl-CoA, may be completely oxidized in tissues such as muscle and heart by the tricarboxylic acid cycle for ATP production or converted into ketones by liver.

Carnitine is thus of key importance in heart, muscle, kidney, liver, epididymis, and sperm which rely heavily on fatty acid oxidation for energy production. Spermatozoan maturation, motility, and fertility are acquired during their journey down the epididymal duct [44] and are, in part, dependent upon the progressive increase in epididymal and spermatozoal carnitine. The epididymis is dependent to a considerable degree on lipids for its energy supply [45]. The mammalian genital tract contains several compartments which maintain the highest free L-carnitine concentrations in the body (1–80 mM in epididymal tissue [46], seminal plasma [47], and spermatozoa [48]). The initiation of sperm motility occurs in parallel with the increase in concentration of free L-carnitine in the epididymal lumen. Free L-carnitine is taken up from blood plasma into the epididymal cell and transferred into the lumen of the rat epididymis, being more rapid in the distal caput and corpus epididymis [31,49], presumably by a double transport mechanism similar to kidney [1]. L-carnitine must then be taken up by sperm. The free L-carnitine concentration in the cauda epididymal lumen of rat and boar is 2000-fold greater than blood plasma concentrations [48,50,51]. The major epididymal acylcarnitine ester is acetylcarnitine. Spermatozoa from the caput are relatively immobile and have undetectable or very low free L-carnitine content. During transit from the caput to the cauda, the spermatozoa accumulate free L-carnitine which is immediately acetylated. The free L-carnitine and acetylcarnitine concentrations in cauda epididymal spermatozoa have been estimated to be 20–100 mmol/L [52,53]. The precise mechanism of carnitine uptake in spermatozoa during maturation has been hitherto unknown but evidence suggests that the sperm membrane is not freely permeable to carnitine [53,54]. Once sperm reach the cauda, they contain high concentrations of carnitine and acetylcarnitine and have high carnitine acetyltransferase (CAT) activity [46,50]. Addition of acetyl-L-carnitine in vitro to ejaculated human sperm has been shown to significantly increase the percentage of motile sperm [55,56]. It has been suggested that free L-carnitine acts on the tricarboxylic acid cycle by buffering CoA concentrations in the mitochondrial matrix of the sperm and that, in the form of acetyl-L-carnitine,

it serves as a store of readily available acetyl groups which may be used to replace the energy storage function of high-energy phosphate compounds [57,58]. Acetyl-L-carnitine may also influence sperm motility through a direct cholinergic effect [45]. Hinton and Setchell [49] suggest that high cauda epididymal carnitine may repress motility once it is developed, following which sperm can be activated once the carnitine is diluted by accessory gland secretions during ejaculation. Another novel action of carnitine is its inhibition in vitro of clusterin-induced aggregation of spermatozoa [59]. The binding of cationic carnitine to cell surface proteins may result in augmented repulsion between sperm, leading to increased dispersion in seminal fluid. Thus, L-carnitine likely has multifunctional roles including spermatozoal maturation, motility acquisition, metabolism of lipids and carbohydrates, membrane stabilization, and maintenance of mature spermatozoa in a quiescent state prior to ejaculation [36–38].

The progressive accumulation of carnitine and acetylcarnitine in epididymal fluid from the caput to the cauda and in maturing sperm is likely facilitated by a coupled series of different affinity carnitine transporters. Defects in any one of these carnitine transporters could lead to insufficient carnitine accumulation in spermatozoa. This would result in diminished fatty acid oxidation which would likely result in delayed spermatic maturation and decreased motility which may in turn predispose to obstructive azoospermia and infertility as seen in the juvenile visceral steatosis Octn2-deficient mouse [40]. Histologically, the duct of the proximal epididymis of the *jvs* mouse is dilated due to the accumulation of unusually high concentrations of spermatozoa which are extravasated into the stroma. In contrast, the duct of the distal epididymis is constricted and contains no spermatozoa. It has been suggested that carnitine deficiency in the spermatozoa leads to dysmotility and poor survival, resulting in the observed obstruction. Alternatively, the stromal components, including the carnitine-dependent fibroblasts and smooth muscle cells, may also participate in the obstruction due to frailty of the sustentacular tissues in supporting the transport of large amounts of spermatozoa.

OCTN2 is a high affinity, sodium ion-dependent carnitine transporter expressed in multiple tissues, including kidney, skeletal muscle, heart, placenta, testis, and epididymis [10,18,28]. The human *OCTN2* gene has been sequenced as part of Human Genome Project and maps to human Chr 5q31. It consists of 10 exons and 9 introns [8]. The hOCTN2 cDNA codes for a protein of 557 amino acids with twelve putative transmembrane domains and a predicted molecular mass of 63 kDa [8]. We and others have described mutations in the *SLC22A5* gene encoding OCTN2 in patients with primary systemic carnitine deficiency (SCD) [60–63]. SCD presents with progressive, infantile-onset hypertrophic cardiomyopathy, recurrent hypoglycemic hypoketotic encephalopathy, weakness, failure to thrive, and microvesicular steatosis in muscle, heart, and liver, and impaired renal reabsorption of carnitine [10,64]. This formerly lethal autosomal recessive disorder is now highly treatable with reversal of the cardiomyopathy, provided there is early intervention with high dose oral carnitine therapy [10,64,65]. Moreover, we had shown previously that a GFP-human OCTN2 protein localized to the plasma membrane and that this is a high-affinity carnitine transporter with a K_m of 5 μM [10,66]. In the present study, using our specific anti-mOctn2 antibody, we obtained a clear band on Western blot confirming the expression of the 63 kDa OCTN2 protein in sperm. We further demonstrated that sperm expressed a high-affinity carnitine transporter with a K_m of 3.39 μM. This is very similar to the K_m values of 2–6 μM observed for high affinity carnitine transport in kidney, skeletal muscle, heart, placenta, and cultured skin fibroblasts [9–14]. We therefore suggest that OCTN2 is likely responsible for carnitine transport across the spermatozoan plasma membrane.

OCTN1 has been shown to be a low-affinity, pH-dependent, sodium-independent, L-carnitine transporter with a K_m for TEA of 436 μM [23,26]. OCTN1 encodes a 551-amino acid protein with 11 transmembrane domains. OCTN1 has been shown to have wide substrate specificity and is suggested to function as a proton/organic cation antiporter [26]. The amino acid sequence of hOCTN1 is very similar to that of hOCTN2, although OCTN1 has low affinity for carnitine [9,18]. In the present study, using anti-mOctn1 antibody, we confirmed the expression of OCTN1 in sperm. We also demonstrated low-affinity carnitine uptake in sperm with a K_m of 412 μM, which is consistent with the previously demonstrated K_m values for OCTN1 in different tissues and species. As OCTN3 is expressed in peroxisomes, we speculate that OCTN1 may serve an important role in the intracellular shuttling of acylcarnitines in sperm mitochondria.

Octn3 is strongly expressed in murine testis and encodes a protein of 564 amino acids with 78% similarity to hOCTN2 and 67% similarity to hOCTN1 [9,18,23]. We recently confirmed expression of a human OCTN3 protein which is localized to liver peroxisomes and has a K_m of 20 μM for carnitine, indicating that it belongs to the intermediate-affinity carnitine transporter class [27]. In the present study, we confirmed the expression of OCTN3 in human sperm using our anti-mOctn3 antibody. Kinetic studies demonstrated a K_m of 26 μM for L-carnitine which is also consistent with an intermediate-affinity carnitine transporter. Peroxisomes have been demonstrated in the epithelial cells of the rat epididymis and in the Leydig cells of the testis [67]. Plasmalogens, for which the initial biosynthetic steps are catalyzed by

two peroxisomal enzymes, namely dihydroxyacetonephosphate acyltransferase and alkyl-dihydroxyacetonephosphate synthase, are a major component of the spermatozoan membrane, and play a crucial role in sperm maturation [67]. The plasmalogen content in the sperm plasma membrane increases during sperm passage through the epididymis [67,68]. Localization of both enzymes in peroxisomal membranes implies that cells producing plasmalogens should contain peroxisomes. Furthermore, significant catalase activity, a marker enzyme for peroxisomes, has been demonstrated in migrated, motile spermatozoa [69].

In conclusion, expression of OCTN1, OCTN2, and OCTN3 in sperm suggests that all three transporters are important for the accumulation and intracellular homeostasis of carnitine in sperm and likely play a significant role in sperm maturation and motility and thereby fertility. Carnitine uptake studies in sperm may allow precise identification of individuals with infertility due to a defect in one of these transporters, within the large population of idiopathic spermatozoal dysmotility disorders. There may also be phenotypic variations in the expression of these defects depending upon the severity of the underlying mutation. A precedent for this has has been established in another autosomal recessive disease, namely cystic fibrosis, in which men with mild mutations may present with infertility, primarily due to congenital absence of the vas deferens, in the absence of the other clinical manifestations of cystic fibrosis [70]. Furthermore, carriers of a carnitine transporter defect may also be at risk for infertility in the context of additive risk factors causing secondary carnitine deficiency (e.g., dialysis, renal disease, medications, etc.). Finally, diagnosis of a specific carnitine transporter defect in sperm will be important for the identification of males whose infertility may be responsive to L-carnitine supplementation.

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