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Dietary Carnitine Supplements Slow Disease Progression in a Putative Mouse Model for Hereditary Ceroid-Lipofuscinosis

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The childhood ceroid-lipofuscinoses are a group of autosomal recessively inherited disorders characterized by massive accumulation of autofluorescent lysosomal storage bodies in neurons as well as other cell types. The storage body accumulation is accompanied by severe degeneration of the central nervous system that results in blindness, cognitive and psychomotor degeneration, and premature death. On the basis of pathologic and biochemical criteria, a hereditary disease in the *mnd* mouse strain has been proposed as a model for certain types of human ceroid-lipofuscinosis. Experimental evidence suggests that the storage body accumulation in humans with juvenile and late-infantile ceroid-lipofuscinosis is linked to altered carnitine biosynthesis. On the basis of the latter observation, a study was performed to determine whether dietary carnitine supplements could slow the disease progression in the *mnd* mouse model. Carnitine supplementation begun at 4 weeks of age did not slow the retinal degeneration that is characteristic of this disease. It did, however, significantly elevate brain carnitine levels, slow the accumulation of autofluorescent storage bodies in brain neurons, and prolong the lifespans of the treated animals. These findings suggest that there is a link between carnitine biosynthesis and the disease pathology and indicate that carnitine supplementation may be beneficial in slowing the disease progression in humans with certain types of hereditary ceroid-lipofuscinosis. J. Neurosci. Res. 50: 123–132, 1997. © 1997 Wiley-Liss, Inc.

Key words: Batten disease; animal model; lysosomal storage; neuronal degeneration; carnitine; ceroid-lipofuscinosis

INTRODUCTION

A group of hereditary autosomal-recessive diseases in humans are characterized by massive accumulations of autofluorescent lysosomal storage bodies in neurons and numerous other cell types (Goebel, 1992). Based on the similarity in fluorescence properties of these storage bodies and of the normal age pigment lipofuscin, this group of disorders has been designated the ceroid-lipofuscinoses. Clinical symptoms in affected individuals include visual loss, psychomotor and cognitive deterioration, seizures, and premature death (Zeman, 1976; Boustany et al., 1988; Dyken, 1988). The childhood-onset ceroid-lipofuscinoses have long been classified into three major forms based on the age of clinical onset, the pattern with which symptoms appear, and the rate of progression of the disease (Boustany et al., 1988; Dyken, 1988). These major forms of ceroid-lipofuscinosis are commonly designated as the infantile, late-infantile, and juvenile types (Boustany et al., 1988; Dyken, 1988). Recent studies have established that each of these forms is genetically distinct. The infantile form maps to human chromosome 1p32 and results from a defect in the gene that codes for palmitoyl protein thioesterase (Vesa et al., 1995). The juvenile disease maps to human chromosome 16p12 and results from defects in a gene whose nucleotide sequence has been determined but whose function is as yet unknown (International Batten Disease Consortium, 1995). The classical late infantile disease gene was recently mapped to chromosome 11p15, but the specific gene that is defective is not yet known (Sharp et al., 1997).

Animals from a variety of species have been reported to have diseases that appear to be analogous to the human ceroid-lipofuscinoses (Jolly et al., 1980, 1988; Goebel et al., 1988; Kopang, 1988; Bronson et al., 1993; Jolly and Palmer, 1995). Among these is the *mnd* mouse, a spontaneous mutant first described by Messer and

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Flaherty (1986). The *mnd* mouse was proposed as a model for either juvenile or late-infantile ceroid-lipofuscinosis based on biochemical and pathologic similarities between the human and murine diseases (Bronson et al., 1993). Genetic linkage analysis appears to rule out a mutation in the juvenile ceroid-lipofuscinosis gene as the defect responsible for the disease in mice (Lee et al., 1996). On the basis of similarities in the composition of the lysosomal storage material, it is possible that the human late-infantile disease and the disease in the *mnd* mice are caused by mutations in homologous genes. A major constituent of the lysosomal storage bodies in affected mice and in humans with juvenile and late-infantile ceroid-lipofuscinosis is the subunit c protein of mitochondrial ATP synthase (Kominami et al., 1992; Faust et al., 1994). In both species, the stored protein contains a trimethyllysine (TML) residue (Katz et al., 1995; Katz et al., 1997). TML is a precursor in the biosynthesis of carnitine, a compound that plays an important role in mitochondrial fatty acid metabolism (Bremer, 1983; Rebouche, 1991). It was previously proposed that the accumulation of a TML-containing protein could result from a defect in carnitine biosynthesis (Katz and Siakotos, 1995; Katz, 1996). Indeed, many humans with juvenile and late-infantile ceroid-lipofuscinosis have depressed plasma carnitine concentrations (Katz, 1996). It is possible that elevating tissue carnitine levels through dietary supplementation would slow the synthesis of the methylated form of subunit c and thus slow its accumulation and the progression of disease symptoms. To evaluate this possibility, the effects of carnitine supplementation on disease progression in *mnd* mice was studied.

MATERIALS AND METHODS

Animals and Carnitine Supplements

Four-week-old male mice that were homozygous for the *mnd* mutation on a C57BL/6 background, as well as normal C57BL/6 mice, were obtained from Jackson Laboratories (Bar Harbor, ME). The mice were housed under 12 hr/12 hr cyclic light of 16 Lux average illuminance measured at the cage bottoms. The temperature in the animal facility was maintained at a relatively constant 21°C, and the relative humidity was maintained at 40–50%. All mice were provided with food and water ad libitum. Control *mnd* and C57BL/6 mice were provided with deionized water and Purina Rodent Chow 5001 mouse diet. This diet contains an average of 10.3 μg total carnitine/kg of chow (Berger and Sachan, 1991). One group of *mnd* mice, designated +Carn, were provided with the same diet supplemented with L-carnitine (8 g/kg diet) and deionized water supplemented with L-carnitine (4 g/L). Another group, designated HiCarn, were given Purina 5001 diet supplemented with L-carnitine (8 g/kg diet) and deionized water supplemented with L-carnitine (8 g/L). Average food consumption was 12–18 g/day/100 g body weight, and average water consumption was 15 ml/day/100 g body weight. The mice weighed an average of 26 g at 12 weeks of age. Thus, the daily carnitine intakes for the control, +Carn, and HiCarn groups were approximately 40 μg, 93 mg, and 155 mg/mouse/day, respectively. For the survival study, mice were maintained until they died without intervention. For other analyses, the mice were killed at designated times as described below.

Tissue Collection

After 4, 12, and 25 weeks of treatment (at 16 and 29 weeks of age), five mice from each treatment group were killed via carbon dioxide inhalation. Brains were obtained from the animals at the 4-week time point. In addition to brain, blood and eyes were obtained from mice at the other two time points. Blood was collected from the heart with a 1-ml syringe. After the blood clotted, the serum was separated by centrifugation. The serum was then stored at −70°C until used for carnitine analysis. The eyes were enucleated; the corneas, irises, and lenses were removed; and the remainders of the eyecups were fixed in a mixed aldehyde fixative (Katz and Robison, 1986). At the 4-week time point, the anterior half of the brain cerebrum was dissected and frozen at −70°C immediately after the animals were killed. At the other two time points, a region of the central-superior cerebral cortex was dissected from each animal and fixed in the same fixative used for the eyes. One portion of each fixed brain sample was prepared for fluorescence microscopic examination, and another portion was prepared for electron microscopic analysis.

Carnitine Analyses

Blood serum and brain carnitine levels were determined by using a radioenzymatic technique described by Parvin and Pande (1977). In this technique, free L-carnitine in the sample is reacted with ^{14}C-acetyl-CoA in the presence of the enzyme carnitine acetyl transferase to produce ^{14}C-acetyl-carnitine. After the reaction reaches completion, the residual labeled acetyl-CoA is separated from the ^{14}C-acetyl-carnitine with an ion exchange column, and the amount of labeled acetyl-carnitine is determined by liquid scintillation counting. Total carnitine (free carnitine plus carnitine esters) was determined in an aliquot of each sample by the same method after base hydrolysis of the carnitine esters. The concentration of carnitine esters in each sample was the difference between total and free carnitine concentrations. Blood serum carnitine concentrations were determined on five

mnd mice in each treatment group after 12 and 25 weeks and on age-matched C57BL/6 mice at the 12-week time point. After 4 weeks of treatment, brain carnitine levels were determined in five *mnd* mice in each dietary group and in five C57BL/6 mice in each of the control and +Carn treatment groups.

Photoreceptor Cell Density Determinations

A hallmark of the hereditary disease in *mnd* mice and in human ceroid-lipofuscinoses is progressive retinal degeneration (Messer et al., 1993; Chang et al., 1994). In young *mnd* mice, the retina appears normal, but as the animals age there is a dramatic loss of photoreceptor cells. Analyses were performed to determine whether carnitine supplementation would slow this photoreceptor cell loss. The fixed eyecups were bisected along a superior-inferior meridian approximately 0.5 mm to one side of the optic nerve head. The larger portion of each eyecup was postfixed in OsO_4 and embedded in epoxy plastic (Katz and Robison, 1986). Sections of the retina, 1 μm in thickness, were obtained along the superior-inferior meridian through the optic nerve head. The sections were mounted on glass microscope slides, stained with toluidine blue, and examined with a Zeiss Axiophot microscope with a 40× Plan Neofluor objective. Four regions of each retina were analyzed for photoreceptor density determinations: two peripheral regions extending from 230- to 580-μm distance from the superior and inferior ora serratas and two central regions extending from 350- to 700-μm distance from the two edges of the optic nerve head. The numbers of photoreceptor cell nuclei present in a measured length of retina in each region were counted. Photoreceptor density determinations were performed on three sections from each eye and the data averaged.

Fluorescence and Electron Microscopic Analysis of Brain Tissue

Analyses were also performed to determine whether carnitine supplementation would slow the accumulation of lysosomal storage bodies in brain neurons. A portion of each fixed brain sample was embedded in HistoPrep (Fisher Scientific, Pittsburgh, PA) and frozen for cryostat sectioning. Cryostat sections of each specimen were cut at a thickness of 5 μm and mounted on glass slides in 0.17 M sodium cacodylate, pH 7.4. The sections were examined with laser confocal fluorescence microscopy using a Bio-Rad confocal system mounted on a Nikon inverted microscope with a 60× objective. The fluorescence intensities of the storage bodies in each section were measured from the digitized confocal images after standardization of the system with a uranyl glass fluorescence intensity standard. Between a 0.175- and 0.350-mm^2 cross-sectional area of tissue was analyzed from each specimen. Quantitative image analysis was performed using Image 1 software (Universal Imaging Corp., West Chester, PA).

Some of the brain specimens were examined with electron microscopy to determine whether the carnitine treatments had any effect on the ultrastructural appearances of the neurons and the lysosomal storage bodies. Portions of the cerebral cortex gray matter were postfixed in OsO_4 and embedded in epoxy plastic (Katz and Robison, 1986). Ultrathin sections of the specimens were cut with a Sorvall MT5000 ultramicrotome. The sections were stained with uranyl acetate and lead citrate and were examined and photographed with a JEOL 1200 EX electron microscope.

Statistical Analyses

All data were subjected to analysis of variance (ANOVA) to assess differences among the treatment groups in the various parameters that were measured. Comparisons between the individual treatment groups (control, +Carn, and HiCarn) were performed by using the Student-Newman-Keuls test (Winer, 1971).

RESULTS

Effect of Carnitine Treatment on Longevity

Both levels of dietary carnitine supplementation significantly increased the longevity of the *mnd* mice (Fig. 1). The mean lifespan of mice fed the basal diet without carnitine supplements was 302 days. The mean longevity of the mice in the +Carn group was 342 days, an increase of 13% over that of the untreated animals ($P < .01$). Mice in the HiCarn treatment group had an slightly greater increase in mean lifespan (Fig. 1). The mean longevity of mice in the latter group was 351 days. This was an increase of 16% over the mean lifespan of mice fed the basal diet ($P < .001$). The mean longevity of the HiCarn mice was not significantly different from that of the +Carn animals. The maximum lifespans of the *mnd* mice were also increased as a result of dietary carnitine supplementation. The longest lived of the mice fed the basal diet was 341 days. The animals in the +Carn and HiCarn treatment groups survived maxima of 420 and 380 days, respectively.

Blood Serum and Brain Carnitine Levels

The *mnd* mice fed the control diet had significantly higher blood serum carnitine concentrations than did the normal congenic C57BL/6 animals fed the same diet (Table I). The mean total serum carnitine concentrations in the *mnd* mice fed the control diet for 12 and 25 weeks were 33% and 39% higher, respectively, than in the

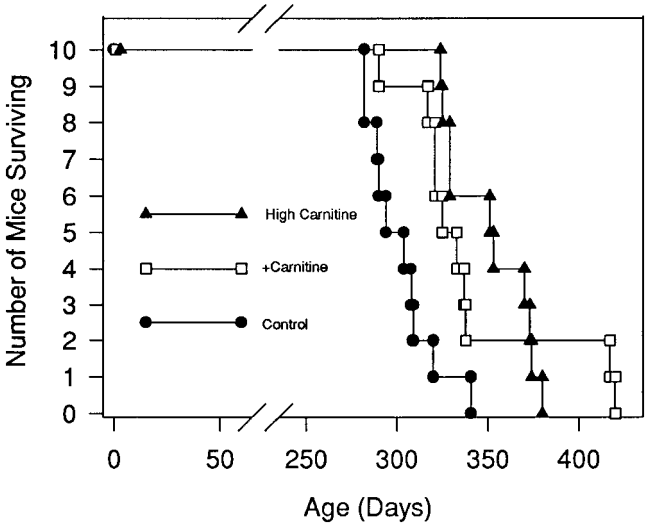

Fig. 1. Effect of carnitine treatment on longevity in *mnd* mice.

TABLE I. Effect of Dietary Carnitine Supplements on Serum Carnitine Concentrations

	Serum carnitine* (nmol/ml)		
Treatment group	Free	Esters	Total
mnd Control—12 weeks	44.3 ± 1.9	58.4 ± 3.0	101.7 ± 3.1
mnd +Carn—12 weeks	46.4 ± 2.7	61.6 ± 2.1	108.0 ± 3.4
mnd HiCarn—12 weeks	53.2 ± 12.5	62.3 ± 10.9	115.5 ± 22.5
mnd Control—25 weeks	48.4 ± 2.9	58.1 ± 5.4	106.5 ± 7.2
mnd +Carn—25 weeks	44.2 ± 2.0	54.9 ± 4.9	99.1 ± 6.7
mnd HiCarn—25 weeks	52.2 ± 6.7	66.2 ± 5.4	118.4 ± 12.0
C57BL/6—12 weeks	34.4 ± 2.2	42.1 ± 2.8	76.5 ± 3.3

*All values are mean ± SE of five animals.

C57BL/6 animals ($P < .01$). Dietary carnitine supplementation did not result in significant elevations of serum carnitine concentrations after either 12 or 25 weeks of supplementation. The fraction of the serum carnitine that was in the free form did not vary significantly among the treatment groups in the *mnd* mice, nor between the *mnd* and the parent C57BL/6 strains. In all cases, the fraction of the serum that was in the free form was 43–45%. This is significantly lower than in humans, in which an average of 82–89% of blood carnitine is in the free form.

In contrast to the failure of dietary supplementation to elevate blood carnitine levels, brain carnitine levels were substantially increased in mice fed diets supplemented with this compound (Table II). Mean brain carnitine concentrations in *mnd* mice fed the +Carn and HiCarn diets were 77% and 65% higher, respectively, than in *mnd* mice fed the control diet ($P < .001$ in both cases). The elevation in brain carnitine levels in *mnd* mice fed the +Carn diet was similar to that in the C57BL/6 mice fed the same diet. In the latter animals, mean brain carnitine concentrations were 75% higher than control

TABLE II. Effect of Dietary Carnitine Supplements on Brain Carnitine Concentrations

	Brain carnitine* (nmol/g wet weight)		
Treatment group	Free	Esters	Total
mnd Control	107.4 ± 8.5	24.2 ± 4.9	131.7 ± 3.7
mnd +Carn	151.4 ± 4.3	81.4 ± 3.4	232.8 ± 4.9
mnd HiCarn	153.4 ± 4.3	63.8 ± 8.2	217.1 ± 9.6
C57BL/6 control	94.5 ± 2.4	45.5 ± 3.3	140.0 ± 3.0
C57BL/6 +Carn	164.8 ± 4.8	79.8 ± 7.3	244.6 ± 10.5

*All values are mean ± SE of five animals.

levels ($P < .001$) (Table II). There was no significant difference in mean brain carnitine concentration between the *mnd* and C57BL/6 mice fed the control diet (Table II). In the *mnd* mice fed the control diet, the mean fraction of the brain carnitine that was in the free form (82%) was significantly higher than in the C57BL/6 animals fed the same diet (68%) ($P < .02$). In the *mnd* mice fed either the +Carn or HiCarn diets, the fraction of brain carnitine in the free form was reduced to that seen in normal C57BL/6 mice (Table II). Dietary carnitine supplementation did not affect the free to esterified carnitine ratio in the C57BL/6 mice.

Retinal Degeneration

One of the pathologic changes that occurs in mice with the *mnd* mutation is a progressive retinal degeneration characterized primarily by a loss of photoreceptor cells (Chang et al., 1994). To determine whether carnitine supplementation slowed the retinal degeneration, photoreceptor cell densities were determined in the retinas of mice in each of the groups after 12 weeks of treatment. Both central and peripheral regions of the retinas were examined. No significant differences in photoreceptor cell densities were observed among the three treatment groups (Fig. 2; Table III).

Autofluorescent Storage Body Content in Brain

The hereditary disease in *mnd* mice is characterized by a massive accumulation of autofluorescent lysosomal storage bodies in cerebral cortex neurons (Bronson et al., 1993). Analyses were performed to determine whether the increased longevity in mice treated with carnitine was accompanied by a reduced rate of accumulation of these storage bodies. After 12 weeks of treatment, there were no significant differences between the control and carnitine-supplemented mice in the amounts of autofluorescent storage material in the cerebral cortex neurons (Figs. 3 and 4). However, after 25 weeks of treatment, both the +Carn and HiCarn animals had significantly less of this storage material in cortical neurons than did the control mice not given carnitine supplements (Figs. 3–5). The

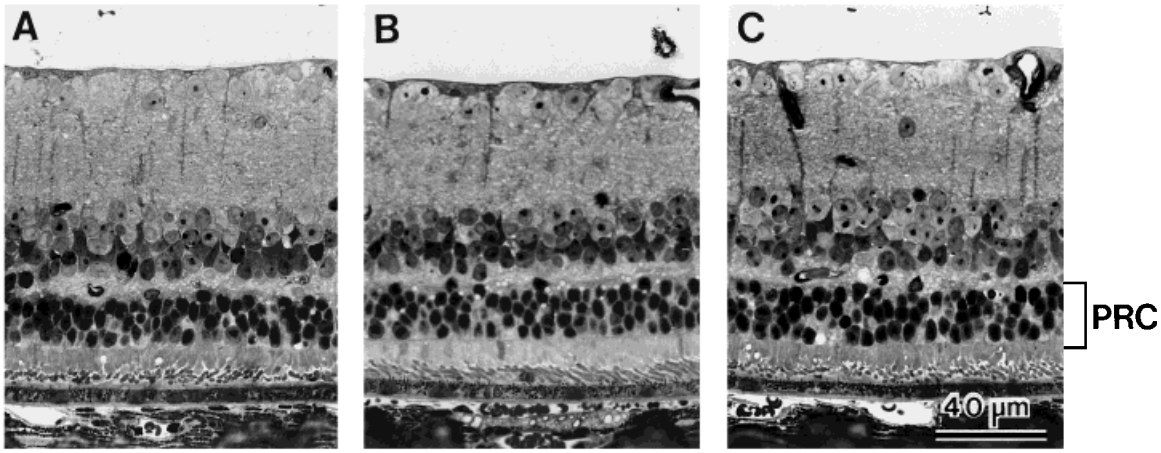

Fig. 2. Light micrographs of the posterior retinas from *mnd* mice after 12 weeks of treatment. **(A)** Control; **(B)** +Carn; **(C)** HiCarn. Photoreceptor cell densities were determined by counting photoreceptor cell nuclei (PRC) per unit length of retina measured along the base of the retina. Bar in C indicates the magnification for all micrographs.

TABLE III. Effect of Carnitine Supplementation on Photoreceptor Cell Densities

	Photoreceptor cell density* (nuclei/100 μm retina)		
Treatment group	*n*	Central	Peripheral
Control	5	59 ± 5	22 ± 3
+Carnitine	5	60 ± 6	20 ± 3
HiCarnitine	7	58 ± 2	23 ± 1

*All values are mean ± SE.

mean brain storage body content in the +Carn group was only 48% of that in the control mice ($P < .03$). In the HiCarn animals, the mean brain storage body content was 60% of that in the control mice after 25 weeks of treatment ($P < .05$). In the control animals, brain storage body content increased an average of 2.7-fold between the 12- and 25-week time points ($P < .01$). In the +Carn and HiCarn groups, however, mean brain storage body content only increased by 63% and 59%, respectively, between 12 and 25 weeks (Fig. 4). There were no significant differences in storage body content between the groups given the two different levels of carnitine supplementation at either time point.

Ultrastructural examination of cerebral cortical neurons confirmed the finding that carnitine supplementation decreased the accumulation of disease-specific storage bodies in the brain (Fig. 5). The storage bodies were present primarily in the perinuclear regions of the neurons in all three treatment groups. After 25 weeks, carnitine treatments resulted in decreases in the numbers of storage bodies in most neurons examined but did not alter the sizes or morphologic appearances of these inclusions (Fig. 5). In the carnitine-treated animals, many cortical neurons appeared to be almost devoid of storage bodies, whereas most neurons examined in the control animals had large numbers of these inclusions. The fine structure of the storage bodies was similar to that reported for storage bodies from brains of humans with juvenile ceroid-lipofuscinosis (Katz et al., 1995) (Fig. 6).

DISCUSSION

Massive lysosomal storage of the subunit c protein of mitochondrial ATP synthase occurs in neurons of humans with late-infantile and juvenile ceroid-lipofuscinosis and of animals with apparently analogous diseases (Palmer et al., 1989, 1990; Kominami et al., 1992; Faust et al., 1994; Katz et al., 1994). Among the animal models that display lysosomal subunit c accumulation is the *mnd* mouse (Faust et al., 1994). The mechanisms responsible for the specific lysosomal storage of this one particular protein have not been identified. It has been determined, however, that in both forms of the human disease and in all the animal models examined, including the *mnd* mouse, one of the lysine residues in the stored form of subunit c is trimethylated (Katz and Gerhardt, 1992; Katz, 1993; Katz et al., 1994, 1995; Katz et al., 1997). Thus, certain types of ceroid-lipofuscinosis appear to entail abnormal processing of the methylated form of subunit c. Trimethylation of protein lysine residues is the first step in the biosynthesis of carnitine, an essential cofactor in mitochondrial fatty acid metabolism (Rebouche, 1991). The presence of TML in the stored form of the protein suggests that subunit c may normally act as a major source of TML for carnitine biosynthesis. This possibility is supported by the finding that in both humans and dogs with ceroid-lipofuscinosis, accumulation of TML-containing subunit c is accompanied by significant

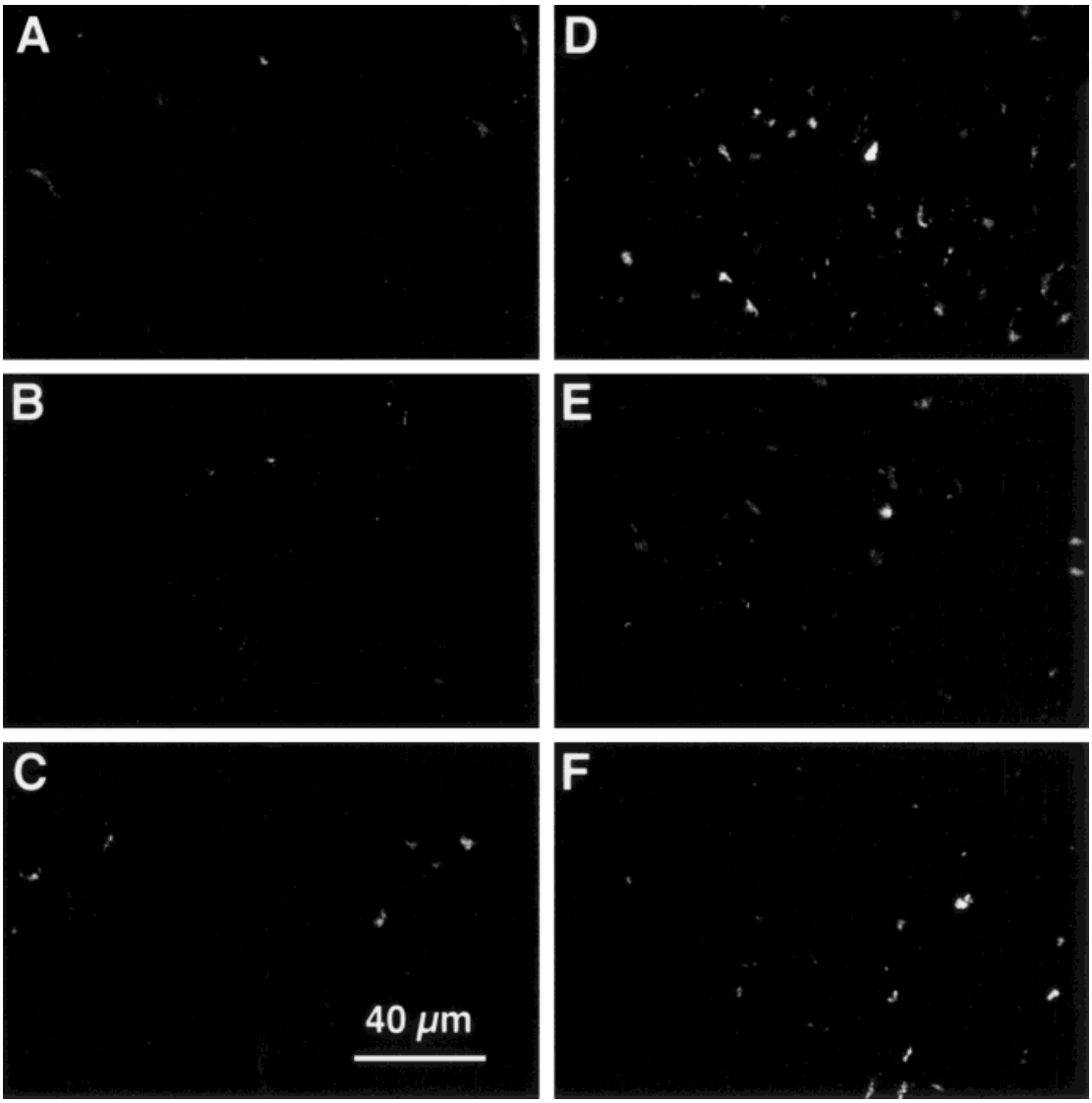

Fig. 3. Representative confocal fluorescence microscope images of cryostat sections of cerebral cortex gray matter from **(A)** control, **(B)** +Carn, and **(C)** HiCarn animals after 12 weeks of treatment, and from **(D)** control, **(E)** +Carn, and **(F)** HiCarn mice after 25 weeks of treatment. Images such as these were used to quantify the fluorescent storage body content of the tissue. Bar in C indicates the magnification for all micrographs.

depression in plasma carnitine levels (Katz and Siakotos, 1995; Katz, 1996). In humans with the juvenile form of Batten disease, plasma TML levels are also significantly depressed (Katz, 1996). This indicates that the disease involves a failure to degrade TML-containing subunit c, resulting in a reduced availability of TML for carnitine biosynthesis.

Unlike affected humans and dogs, the lysosomal accumulation of TML-containing subunit c in the *mnd* mice is not accompanied by depressed blood carnitine levels. On the contrary, blood carnitine levels are significantly higher than normal in these animals. In brain, however, carnitine concentrations in the *mnd* mice are almost the same as those in the control C57BL/6 strain. Carnitine supplementation resulted in elevated brain but not blood carnitine concentrations in the *mnd* mice, and the elevated brain carnitine concentrations were accompanied by decreased rates of storage body accumulation.

The latter finding indicates that the accumulation of TML-containing subunit c can be regulated by tissue carnitine levels. Tissue carnitine normally originates from a combination of two sources: endogenous biosynthesis from TML and dietary intake (Heinonen and Takala, 1991; Rebouche, 1991). A number of tissues, including

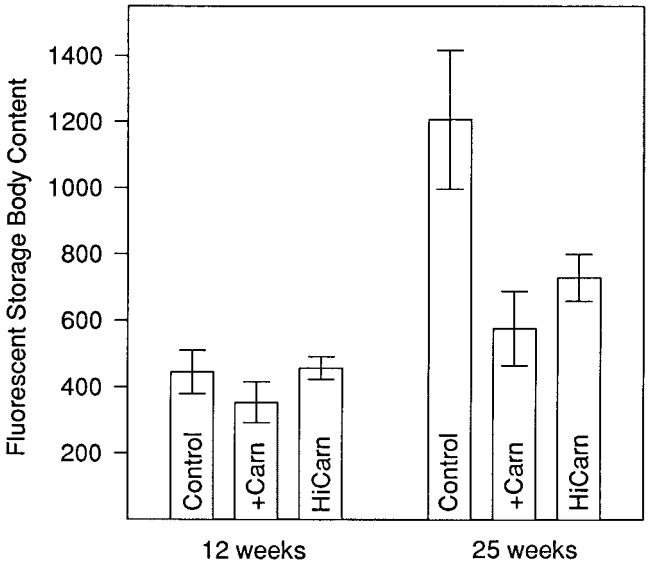

Fig. 4. Effect of carnitine treatment on brain storage body content as determined by quantitative confocal fluorescence microscopic analysis. Fluorescent storage body content is expressed as fluorescence emission intensity relative to a uranyl glass standard. All values are mean ± SEM for five animals per treatment group.

kidney, liver, and brain, contain all the enzymes necessary for carnitine biosynthesis (Rebouche and Engel, 1980). Most tissues, including brain, also demonstrate active transport of carnitine to and from the blood (Bremer, 1983; Bieber, 1988). Thus, at least for kidney, liver, and brain, tissue carnitine levels would be determined by a combination of rates of endogenous synthesis and exchange with the blood pool. Carnitine biosynthesis requires the formation of TML. It appears that in mammals, TML can only be synthesized by methylation of protein lysine residues; free lysine is not a substrate for enzymatic methylation (LaBadie et al., 1976; Bieber, 1988). In humans, the finding that lysosomal accumulation of TML-containing subunit c is accompanied by depressed plasma carnitine and TML levels indicates that subunit c is a major source of TML for carnitine biosynthesis (Katz, 1996). This is likely to be the case in mice as well. It is therefore possible that tissue carnitine levels regulate subunit c synthesis and turnover via a feedback mechanism.

In the light of these considerations, the observations reported in this study may be explained as follows. The *mnd* mice have a genetic defect that results in an impaired degradation of the TML-containing subunit c protein. Animals fed a normal diet compensate for the resulting reduction in carnitine biosynthesis from subunit c by increasing the turnover in other TML-containing proteins. An overall overcompensation would account for the somewhat elevated serum carnitine concentrations relative to those in a control strain of mice. In brain, the compensation from degradation of alternate TML-containing proteins and increased uptake from the blood are apparently sufficient to maintain almost normal brain carnitine levels. When dietary carnitine intake is increased to a degree sufficient to elevate brain carnitine concentrations, the carnitine apparently acts via a feedback mechanism to slow the synthesis of TML-containing subunit c. This would explain the decreased accumulation of subunit c containing storage bodies in the *mnd* mice fed supplemental carnitine. Consistent with the conclusion that dietary supplementation alters carnitine metabolism in the brains of the *mnd* mice is the observation that the elevation in the ratio of free to esterified carnitine was reversed by the dietary supplements.

It is not clear why dietary carnitine supplementation did not result in further elevation of blood levels of this compound in the *mnd* mice, whereas blood carnitine levels do increase in genetically normal mice given dietary carnitine supplements (Costell and Grisolia, 1993). The failure of large dietary supplements of carnitine to significantly increase the already elevated blood concentrations of this compound in the *mnd* mice is probably a reflection of the rapid urinary excretion of carnitine (Costell and Grisolia, 1993; Brass et al., 1994).

Amounts of storage material in brain tissue were assessed using laser-induced fluorescence emission measurements. Autofluorescence is a characteristic feature of the storage bodies that accumulate in the ceroid-lipofuscinoses (Katz et al., 1988; Palmer et al., 1993; Goebel et al., 1996). The chemical basis for the fluorescence has not been definitively determined, but some evidence suggests that the fluorescence properties are due solely to the aggregation of the subunit c protein (Palmer et al., 1993). Regardless of its chemical basis, fluorescence intensity appears to be a good measure of the amounts of lysosomal storage material in tissues. Because subunit c is such a major constituent of the storage material, the decrease in accumulation of autofluorescent storage bodies in animals treated with carnitine probably indicates that subunit c storage has been reduced.

It is not clear why carnitine supplementation was effective in slowing the accumulation of lysosomal storage bodies in the brain but did not delay the loss of photoreceptors from the retina. The mechanisms underlying photoreceptor cell death in the *mnd* mice or in humans with ceroid-lipofuscinosis are not known, nor is it known how the loss of photoreceptor cells is related to the accumulation of the lysosomal storage bodies that occurs in tissues such as brain but apparently does not occur in the photoreceptor cells (Messer et al., 1993). Thus, one can only speculate as to why the carnitine supplements were ineffective in delaying photoreceptor cell loss. One possibility is that the processes responsible for photorecep-

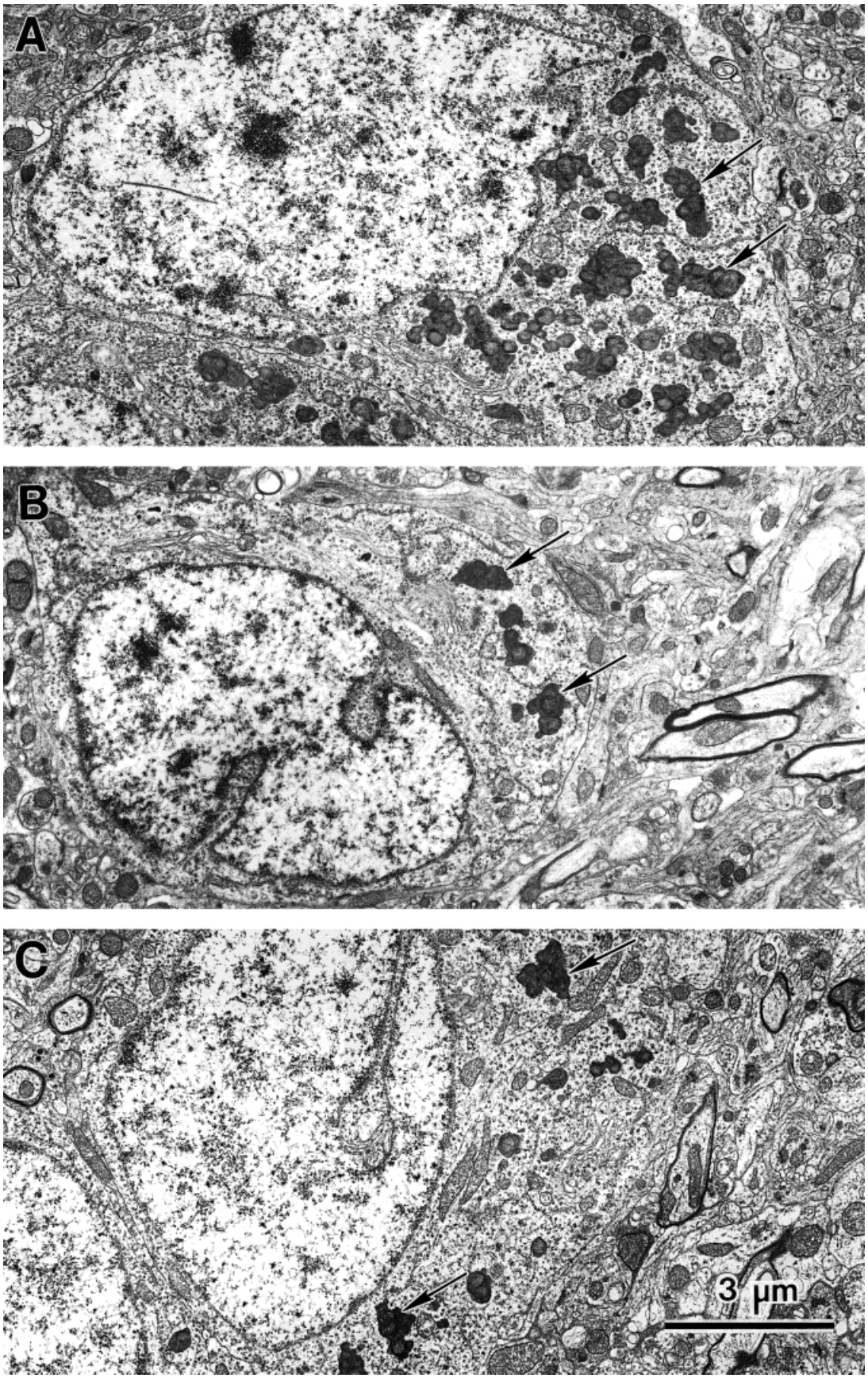

Fig. 5. Electron micrographs of cerebral cortical neurons from **(A)** control, **(B)** +Carn, and **(C)** HiCarn mice after 25 weeks of treatment, illustrating the relative amounts and ultrastructural appearances of the storage bodies (arrows). Bar in C indicates the magnification for all three micrographs.

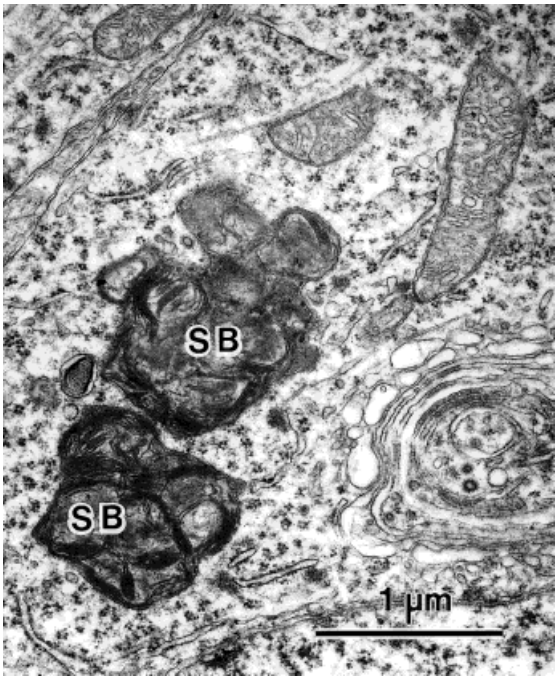

Fig. 6. Electron micrograph of disease-specific storage bodies (SB) in a cerebral cortical neuron of a +Carn animal after 25 weeks of treatment. The fine structure of the storage bodies was not altered by the carnitine treatments.

tor cell death had already progressed too far to be affected by intervention by the time the carnitine supplements were initiated at 4 weeks of age. Indeed, morphologic evidence of photoreceptor abnormalities have been reported in the *mnd* mice as early as 15 days of age (Messer et al., 1993). Perhaps by the time carnitine supplementation was initiated, the molecular changes responsible for these abnormalities had progressed too far for carnitine intervention to be effective.

The genetic defect in humans with juvenile Batten disease has been identified (International Batten Disease Consortium, 1995). The disease results from alterations in a gene designated CLN3. The function of the protein coded for by this gene is unknown. It has recently been reported that the CLN3 gene maps to mouse chromosome 7 (Lee et al., 1996), whereas the disease phenotype in *mnd* mice maps to chromosome 8 (Messer et al., 1992). Thus, the disease in *mnd* mice does not appear to be due to a mutation in the CLN3 gene. The conclusion that juvenile ceroid-lipofuscinosis in humans and the disease in *mnd* mice are genetically distinct is supported by the findings that blood carnitine levels are depressed in affected humans (Katz, 1996), whereas they are elevated in affected mice.

Despite the fact that the precise genetic defect in the *mnd* mice is not the same as that in humans with the juvenile disease, the lysosomal accumulation of subunit c in both cases suggests that the diseases are related. In humans, the accumulation of TML-containing subunit c is accompanied by decreased plasma TML and carnitine levels, suggesting that there is an impairment in subunit c degradation. In the *mnd* mice, an elevation in blood carnitine levels accompanies subunit c accumulation. This may reflect an overproduction of subunit c, or as suggested above, an overcompensation in turnover of other TML-containing proteins due to impaired subunit c degradation. In either case, a treatment that would reduce subunit c biosynthesis would be expected to slow the accumulation of storage material and thus the progression of the disease. Carnitine had such an effect in the *mnd* mice. It therefore appears that carnitine supplements may also be beneficial in slowing storage body accumulation and disease progression in humans with the forms of ceroid-lipofuscinosis in which subunit c is a major constituent of the storage bodies.

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