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Alzheimer's Disease: Brain Desmosterol Levels

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Abstract. Desmosterol is a C27 sterol intermediate in cholesterol synthesis generated during the metabolic pathway that transforms lanosterol into cholesterol. It has become of particular interest in the pathogenesis of Alzheimer's disease (AD) because of the report that the activity of the gene coding for the enzyme DHCR24, which metabolizes desmosterol to cholesterol, is selectively reduced in the affected areas of the brain. Any change in the pattern of C27 sterol intermediates in cholesterol synthesis merits investigation with respect to the pathogenesis of AD, since neurosteroids such as progesterone can modulate the tissue levels. We therefore analyzed the C27 sterol composition using a metabolomics approach that preserves the proportion of the different sterol intermediates. In AD, the proportion of desmosterol was found to be less than that of age-matched controls. The findings do not directly support the focus on Seladin-1, although they could reflect different stages of a slowly progressive disease.

Keywords: Alzheimer's disease, C27 sterol intermediates, 8-dehydrocholesterol, desmosterol, DHCR24, Seladin-1

INTRODUCTION

Several genes involved in cholesterol metabolism have been linked to the pathogenesis of sporadic late onset Alzheimer's disease (AD) [1]. Inheritance of the apolipoprotein E4 allele is the strongest genetic risk factor identified thus far for late-onset AD. Carrying an apolipoprotein E4 allele increases the incidence and decreases the age of onset of amyloid plaques as well as enhancing tau pathology [2–4].

Understanding of the biologic roles for cholesterol in amyloid processing and the development of AD [5] expanded with the report that its local synthesis was affected by the decreased levels of expression of the gene (*DHCR24)* that governs the metabolism of desmosterol to cholesterol (Fig. 1) in specific areas of the brain [6, 7]. DHCR24 has been shown to counteract the β-secretase cleavage of AβPP and the formation of amyloid-β [6, 7]. In addition, down regulation of DHCR24 in vulnerable AD brain regions has been shown to parallel an increase in the amount of hyperphosphorylated tau [8]. These findings imply a biologic role for the high levels of desmosterol in the brain of a mouse model of the disease.

In humans and other species, desmosterol concentration relative to cholesterol is known to be highest in early fetal life and then to progressively fall [9, 10]. The findings are generally interpreted as reflecting the relatively high rates of cholesterol synthesis to support myelin production [11] and rapid brain growth during fetal life [9, 12]. Continual synthesis of desmosterol and other sterol intermediates in adult human brain was established when desmosterol was found to represent 2.1% of the sterol intermediates generated following incubation of tissue with mevalonate-2-C14. The others were mostly delta8 unsaturated sterols [13]. This study and other data [12] establish that a low level of cholesterol synthesis in the brain occurs throughout life

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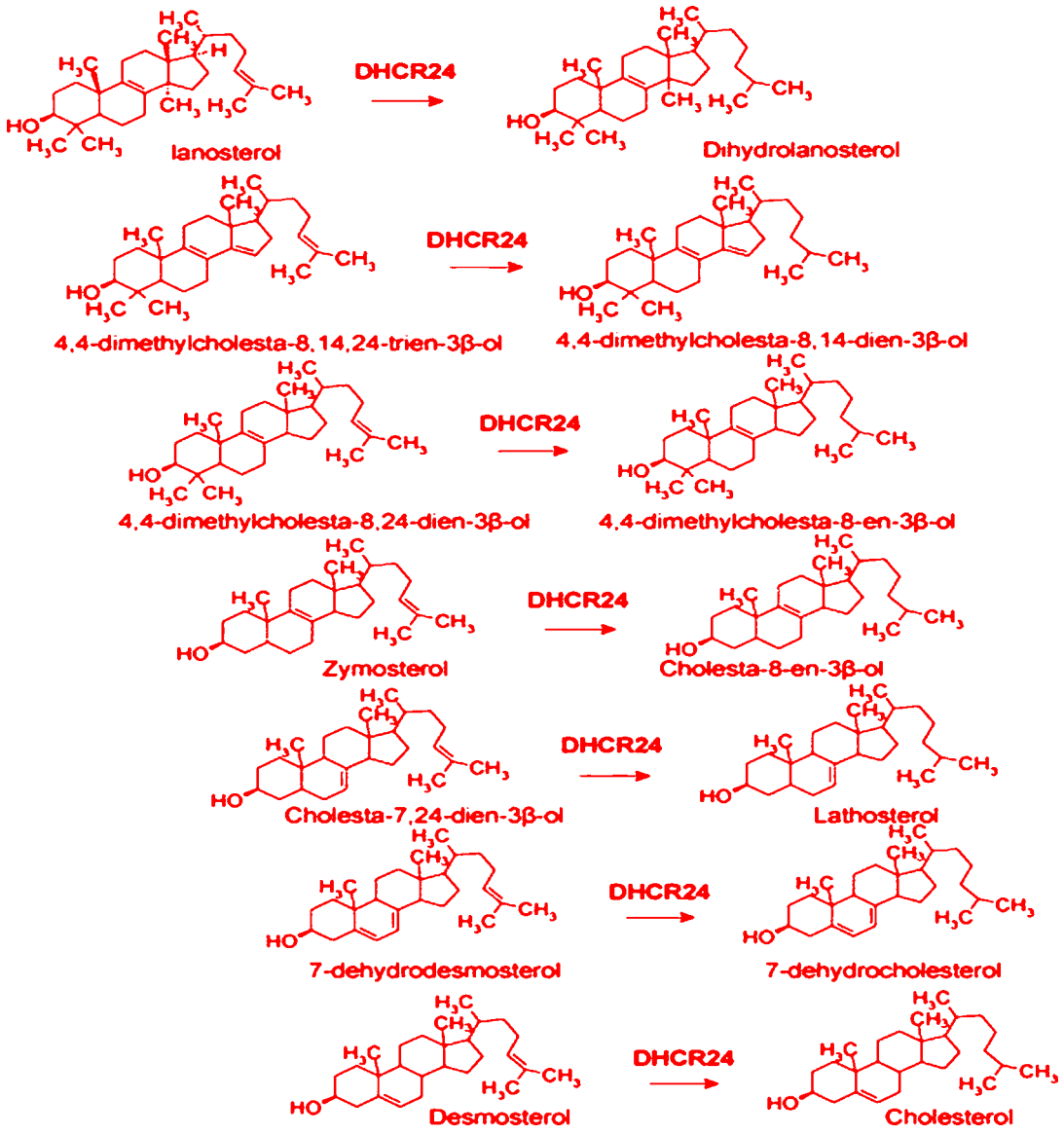

Fig. 1. Role of 24-dehydrocholesterol reductase (DHCR24) in the transformation of lanosterol to cholesterol. Beginning with lanosterol, two groups of sterol intermediates progress toward cholesterol, those with an unsaturation at C24 = C5 and those where the reduction occurs early in the pathway. Desmosterol is considered to be the preferred substrate, see [26], from which this illustration has been taken with permission.

and that desmosterol is not normally the predominant sterol intermediate indicating continuing cholesterol synthesis.

MATERIALS AND METHODS

Tissues

Frozen specimens taken from the gray matter of the frontal lobe obtained within 24 h postmortem. The study was approved and tissues provided by the BioRepository Center of NYU-Langone Medical Center (funded by NIH grants 5P30CA016087-33 and 5P30AG051-23). Patients with histories of dementia, whose age ranged from 50 to 102 years, were divided into non-AD and AD groups (Table 2) based on the neuropathology. None of the specimens had visible blood vessels and the amounts taken for analysis were free of visible heme.

Sample preparation

Specimens ranging in weight from 60 to 137 mg were homogenized for 0.5 min in 3.0 ml of cold chloroform/methanol (2 : 1) using a Tissuemiser (Fisher Scientific Cat # 15-338-420) and were filtered following classical Folch techniques [14]. To each homogenate, 250 ng of D6-desmosterol (Avanti Polar Lipids, Alabaster AL) and 10 μg of D6-cholesterol (Sigma-Aldrich St. Louis, MO) were added. The washed chloroform (sterol) fraction was taken to dryness by speed vacuum evaporation. Although brain tissue contains little if any esterified cholesterol, the dried residue was redissolved in 2.0 ml of freshly prepared ethanolic KOH [15] to remove lipids and phospholipids and was saponified for 1 h at 60°C, followed by partition between hexane and water with back-washing until neutral. The hexane was then taken to dryness, redissolved in 100 μl of ethanol, and placed in a 200-μl insert within an amber vial for automatic injection.

Sample analysis

A metabolomics approach [16, 17] to sterol analysis was utilized to avoid initial fractionation of the sterol extract or derivative formation. As reported previously [17], a reverse-phase C18 column separates zymosterol, desmosterol, 8-dehydrocholesterol, and 7-dehydrocholesterol from other sterol intermediates and from cholesterol without the need for silver nitrate-impregnated media [13]. By using two reverse-phase columns in tandem (Ascentis Express C18 columns 15 cm × 4.6 mm 2.7 μm particle size Cat# 53829-U, Supelco, Bellefonte, PA), we obtained baseline separations. Other minor changes from those reported [17] were the use of isocratic 100% methanol as a solvent instead of 98% aqueous methanol, a flow rate of 0.5 ml/min instead of 1.0 ml/min, and a column temperature of 35°C. These modifications increased the separation time between each sterol intermediate but did not change their order of elution. Utilizing an APCI (atmospheric pressure chemical ionization) source in positive-ion mode avoids the need to prepare derivatives. These studies were done on a Shimadzu 2010-EV with a SIL- 20A HT auto sampler. The instrument was operated in simultaneous SIM and SCAN modes. For all the sterols, the M-17(OH) fragments were monitored and used for area analysis. Thus 367.3 m/z was monitored for the sterol diene intermediates, 369.3 m/z for cholesterol, and 373.3 m/z and m/z 375.3 for the deuterated (D6) desmosterol and cholesterol standards, respectively.

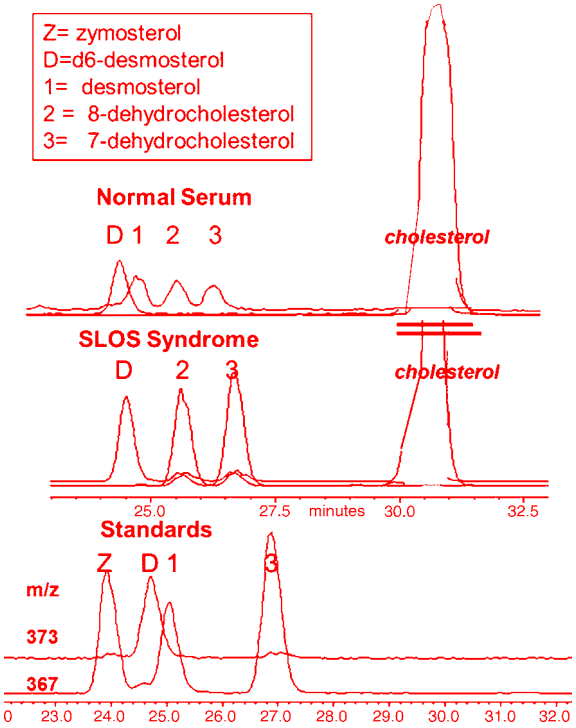

Fig. 2. LC-MS analysis of C27 sterol intermediates in cholesterol synthesis. Reverse-phase (C18) chromatography separates isomers of C27 sterol intermediates of cholesterol as zymosterol, desmosterol, 8-dehydrocholesterol, and 7-dehydrocholesterol based on the different sites of the unsaturation in the molecule. Because it is established that levels of both 8- and 7-dehydrocholesterol are increased in the plasma of individuals with Smith-Lemli-Opitz syndrome, see [21], and desmosterol is undetectable, this plasma is a very useful reference for establishing relative retention times. Loss of the 3-OH group (M-17), common to cholesterol and the sterol intermediates, that occurs with an APCI detector provides fragments, 369 m/z and 367, respectively, in high abundance that permits detection at the nanogram level.

Utilizing the whole sterol extract preserves the quantitative relationships of the different constituents. Although sensitivity is less than can be obtained by other techniques [18, 19], it is sufficient for sterol constituents, present at nanogram levels. Because an authentic sample of 8-dehydrocholesterol was not available, we used serum from a patient with Smith-Lemli-Opitz syndrome [20], since the finding that it contains increased amounts of 8- and 7-dehydrocholesterol in plasma with no detectable desmosterol is well-established [21] (Fig. 2 middle panel). D6-desmosterol elutes slightly ahead of desmosterol, a known isotope effect, but because of the constant relationship to desmosterol it provides certainty with respect to identity, irrespective of slight variations in retention time that may occur.

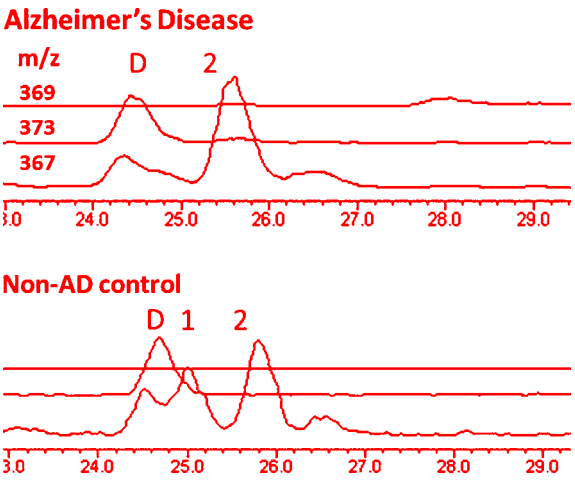

Fig. 3. C27 sterol intermediates in the sterol extract of brain tissue from AD and non-AD age-matched controls. Using an internal standard of deuterated (373 m/z) desmosterol (D) the location of endogenous desmosterol, 367 m/z [1], can always be ascertained regardless of slight changes that may occur in the retention time. We chose for NMR analysis samples with barely detectable desmosterol to highlight the difference between AD and non-AD patients. 8-Dehydro-cholesterol was the predominant C27 sterol intermediate found in all specimens.

Statistics

The differences between groups were analyzed using univariate analysis of variance (ANOVA) with age and gender as covariates.

RESULTS

AD patients were slightly younger than non-AD patients, although the difference was not statistically significant (Table 2). Gender distribution was similar (M/F; AD patients: 5,4; non-AD: 5,5).

Figure 3 illustrates the difference in the proportions of the different sterol intermediates in brain tissue obtained from a patient with AD and from an age-matched control. With the D6-desmosterol peak as a reference, it is apparent that the area of the desmosterol peak is proportionally much smaller than that of the 8-dehydrocholesterol in the AD patient and relatively greater in the age-matched control patient. An unidentified 367 m/z fragment peak with a retention time slightly later than that of zymosterol but earlier than that of desmosterol is present in all samples. Based on NMR analysis (Table 1) of the fraction shown in Fig. 3 and reports in the literature [10, 13], it is likely that the unidentified peak is an 8-dienol. The predominant 367 m/z peak in all samples had the retention time of 8-dehydrocholesterol (cholesta-5, 8-dien-3beta-ol) (Fig. 4). Although the mean area for 8-dehydrocholesterol was greater in the AD group, the difference between the two groups was not statistically significant ($p \leq 0.1$).

Table 1
NMR analysis of sterol fraction from AD brain tissue collected from 23 to 30 min

Sterol	Relative abundance (%)
Cholesterol	99
Cholestanol	0.3
8-Dehydrocholesterol	0.2
Lathosterol	0.2
Cholest-8-enol	0.1
Cholesta-8,14-dienol	0.05
Desmosterol	0.05

Table 1 shows the results of a 1D and 2D NMR analysis that was done at Rice University on a sterol fraction from an AD patient collected from 23 to 30 min during the HPLC analysis utilizing techniques previously published in detail, which established the NMR characteristics of 8-dehydrocholesterol and all the C27 unsaturated sterol intermediates that can occur during the transformation of lanosterol to cholesterol [22]. At most, the proportion of desmosterol represents 0.05% in contrast to that of 8-dehydrocholesterol, which is at least 4-fold greater. Other sterol intermediates for which we have no standards were also present, and one, cholesta-8, 14-dienol, may be the unknown peak. Its abundance in the AD samples was not significantly different from that in the age-matched controls.

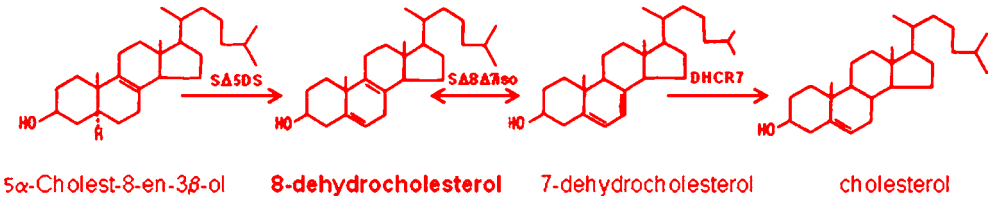

Fig. 4. Determinants of 8-dehydrocholesterol levels in tissues. Evidence for active cholesterol synthesis is the finding of relatively high levels of sterol intermediates in tissues. Other than the endogenous rate of cholesterol synthesis, the other determinants of the tissue level of 8-dehydrocholesterol are the relative activities of sterol delta 5 desaturase (SΔ5DS), delta 8-delta 7 isomerase (SΔ8Δ7iso), and 7-dehydrocholesterol 7 reductase (7DHCR7).

Table 2
LC-MS analysis of brain tissue for desmosterol and cholesterol

Sample	Amount mg	Age & gender	d6desmos 373 m/z area	Desmos 367 m/z area	Desx250/des ng desmos	Desmosterol ng/mg tissue	d6chol 375 m/z area	Chol 369 m/z area	Cholx10/d6chol cholesterol	Cholesterol μg/mg tissue	Des/chol ng/μg
Non-AD											
1	88	50 m	373	374	250	2.84	20546	1204339	586	6.7	0.426
2	60	64 f	318	318	250	4.17	17091	1030540	603	10.0	0.415
3	131	80 f	321	782	610	4.66	43589	2071216	475	3.6	1.284
4	125	80 m	834	374	110	0.88	27322	2530266	926	7.4	0.119
5	73	84 f	96	106	280	3.84	17486	657370	376	5.1	0.745
6	103	86 m	103	70	170	1.65	7915	1311229	1657	16.1	0.103
7	69	90 m	270	424	390	5.65	40707	2108334	518	7.5	0.753
8	91	90 f	350	387	280	3.08	51472	2117131	411	4.5	0.681
9	63	92 m	336	574	430	6.83	48822	1635659	335	5.3	1.283
10	83	102 f	951	807	210	2.53	39046	1595410	409	4.9	0.514
Average	89	83				3.61				7.1	0.632
SD	23.4	15.2				1.71				3.50	0.39-
AD											
1	70	62 f	166	99	150	2.14	26632	1255462	471	6.7	0.318
2	80	67 m	340	65	50	0.63	14538	1774180	1220	15.3	0.041
3	65	75 f	849	358	110	1.69	33446	1750130	523	8.1	0.210
4	73	78 f	302	93	80	1.10	16443	1390098	845	11.6	0.095
5	80	79 m	166	118	180	2.25	12808	957430	748	9.3	0.241
6	137	81 m	386	283	180	1.31	15177	2071470	1365	10.0	0.132
7	124	84 m	424	258	150	1.21	19208	1877311	977	7.9	0.153
8	131	88 m	484	577	300	2.29	21882	2552843	1167	8.9	0.257
9	87	91 f	224	187	210	2.39	11161	1810351	1622	18.6	0.128
Average	94	78				1.67				10.7	0.175
SD	26.7	8.8				0.60				3.67	0.083
p value	0.67	0.70				0.008				0.102	0.007

Table 2 summarizes the amount of desmosterol and cholesterol found in the gray matter of the frontal lobe of 9 patients with AD compared with 10 non-AD patients of comparable age. The mean cholesterol in the AD group (10.7 μg/mg of tissue) was greater than in the non-AD group (7.1 μg/mg of tissue), but the difference was not statistically significant ($p = 0.102$), a finding noted previously by others [23]. In four patients ranging in age from 10 months to 55 years, the cholesterol content of gray matter of the frontal lobe was to be approximately one-half that of the white matter [24].

The amount of desmosterol per mg of non-AD tissue (3.61 ng/mg tissue) agrees well with a previous study [13]. In AD tissue the mean value was found to be 1.67 ng/mg tissue, significantly less ($p = 0.008$) than that in the non-AD group. Utilizing tissue cholesterol as the denominator in place of mg of tissue also indicated a statistically significant difference between the two groups with respect to desmosterol ($p = 0.007$).

DISCUSSION

These studies were undertaken to compare human data with that obtained in a mouse model of AD [25] and with the expectation that desmosterol levels might be increased because of the report regarding Seladin-1 [6], a suggested name for *DHCR24,* the gene that codes for the enzyme that metabolizes desmosterol to cholesterol. Although the enzyme catalyzes the reduction of the C24 = C25 unsaturation in several intermediates in cholesterol synthesis (Fig. 1), the preferred substrate is desmosterol [26], the sterol that accumulates when a deficiency occurs [27, 28].

Initially, using reverse phase C18 columns and solvent systems with less resolving power, we thought the 8-dehydrocholesterol peak was desmosterol, but because it had a slightly longer retention time than the desmosterol standard, we modified our analytic method and used a deuterated internal standard of desmosterol. The serum from a patient with Smith-Lemli-Opitz syndrome served as an authentic standard for 8-dehydrocholesterol. Further confirmation was obtained by NMR analysis.

The low levels of desmosterol in AD tissue are consonant with the recent report in this journal that provides evidence questioning the *DHCR24* gene (*Seladin-1)* as a selective AD indicator [29]. Except for the mutated and non-functional *DHCR24* gene

that accounts for desmosterolosis [28], relatively little is known about the relationship of gene expression to tissue levels of desmosterol. Our finding of significantly less desmosterol in AD tissues compared with those from age-matched controls certainly implies high, rather than low, levels of gene expression. In the recent study [29], no difference was found in *DHCR24* gene expression between AD tissues and those from controls. Thus other determinants of the tissue level of desmosterol also merit consideration.

Of particular interest is the knowledge that steroid hormones affect the synthesis of cholesterol [30]. In this study it was noted that "steroids may preferentially inhibit cholesterol synthesis after mevalonate formation at the step between lanosterol and cholesterol." Of the eight steroid hormones studied, progesterone had the greatest effect, which supported previous findings that demonstrated its direct effect on enzymes regulating the lanosterol-to-cholesterol metabolic pathway [31]. More recently, it was shown that progesterone, pregnenolone, and 17αOH-progesterone are potent inhibitors of Δ24-reduction, which leads to the accumulation of desmosterol [32].

These hormones and others, including their sulfate derivatives, are produced in the brain and generically referred to as neurosteroids, a concept introduced by Baulieau [33, 34]. The quantification of neurosteroids in human brain regions in patients with AD compared with those in non-demented patients [35] led to the finding that the high levels of key proteins implicated in the formation of plaques and neurofibrillary tangles were correlated with decreased brain levels of neurosteroids, suggesting a possible neuroprotective role of these neurosteroids in AD. Thus, our finding of a significantly decreased amount of desmosterol may correlate with both low levels of neurosteroids [35] and the level of *DHCR24* gene expression [29]. The molecular basis of the neuroprotective effects remains to be delineated.

Finding significantly less desmosterol in brain tissue of advanced AD may not reflect what is occurring at earlier stages of the disease in AD transgenic models where neuronal loss has not occurred. In heterozygous Seladin-1 deficient mice, which develop normally, desmosterol levels in brain tissue are 5 to 7 times greater than in that from wild-type animals. No neuropathological lesions occur [7]. In the APPSLxPS1 mutant mouse model of AD, increased levels of desmosterol occurred in the cerebellum at 9 months and rose further to 600% greater than that in controls in the hippocampus at 21 months, by which time there were abundant deposits of amyloid [25]. There is no information on other sterol intermediates. Although incomplete with respect to time course, the data indicate that the dysregulation of the lanosterol-to-cholesterol pathway is not static but varies during the development of the neuropathology. A more complete time course is provided in a mouse model of Wilson's disease that was analyzed for desmosterol, 8-dehydrocholesterol, and 7-dehydrocholesterol in both wild-type and mutant animals that lack a copper transporter [36]. When the animals were placed on a copper-containing diet, only the mutant mice developed the neurologic disease. In these animals, 8-dehydrocholesterol levels were 400% greater than those in wild-type animals, which exceeded the increase in both desmosterol and 7-dehydrocholesterol. However, as the brain damage advanced, cholesterol and all the sterol intermediates fell to levels less than those in the wild-type animals on the same diet. Lacking in studies of AD in humans is a time course; nevertheless it appears essential to obtain data at different time points in the progression of AD to evaluate the possible role of changes in neurosteroids and the dysregulation of the lanosterol-to-cholesterol metabolic pathway.

Except for the study in a single 44-year-old adult [13], we are not aware of 8-dehydrocholesterol levels (Fig. 4) in normal brain tissue. A study of desmosterol levels in normal human and AD brain [37] did not provide data with regard to 8-dehydrocholesterol. A comparison was made of the desmosterol content, expressed as percent of total sterol, between 4 normal patients, ages 20 to 38, and AD patients, ages 66–86. Most of the non-cholesterol sterols were unidentified and no significant difference was found in the percent of desmosterol in the frontal cortex.

In the present study, 8-dehydrocholesterol was found to be the major sterol intermediate (Fig. 3). The mean area of 8-dehydrocholesterol in AD patients compared with that of controls did not reach acceptable statistical significance ($p \leq 0.1$) (data not shown). The failure to detect sterol intermediates is an indication of no active cholesterol synthesis. This concept is based on the logic that if cholesterol synthesis ceases, all the intermediates would continue to the end product and is supported by the knowledge that desmosterol levels are greatly diminished in adult brain compared with fetal brain [10] and by studies in animals where the time course can be carefully charted [36]. Also, conversely, when metabolic blocks occur, as in Smith-Lemli-Opitz [38] and Conradi-Hunerman-Happle [39] syndromes, the appropriate sterol intermediate level increases markedly. Thus our finding that 8-dehydrocholesterol levels in AD tissues are not significantly different from

those in controls implies continued active cholesterol synthesis in the affected tissues in late stages of AD.

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Authors' disclosures available online (http://www.j-alz.com/disclosures/view.php?id=1517).

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