A Decrease in Brain Mass and a Decline in Cognitive Function Are Commonly Seen with Advancing Age. While these changes may reflect a general deterioration of the central nervous system, some features of brain structure and function appear to be more prominently affected than others. Notably, at the level of neural circuitry, evidence from animal models and postmortem studies in human brain suggest changes in the number, size, and function of cholinergic neurons with age, even in otherwise healthy individuals. In the pathologic case of Alzheimer's-type dementia, a substantial loss of cholinergic cells, over and above the loss of other types of neurons, is well documented. A factor contributing to the special vulnerability of cholinergic neurons to loss with age or in dementia may be their need to use a limited supply of choline for multiple and competing purposes (Figure 1). In these cells, choline is not only the precursor of the neurotransmitter acetylcholine, it is also the precursor of phosphatidylcholine and sphingomyelin, phospholipids that are both essential structural constituents of all cell membranes and sources of second-messenger molecules used in receptor-mediated intraneuronal signaling. Should the availability of choline decrease with age or illness, cholinergic neurons might be unable to maintain all their essential functions. Despite its key role in neuronal metabolism, little, if any, choline is made in the brain. Rather, it is almost entirely obtained through the diet. Animal studies suggest that when choline is elevated in blood following a meal, it is transported into cells by low-affinity transport pump or, in the case of cholinergic neurons, by low-affinity and high-affinity pumps. Once in cells, choline is rapidly phosphorylated and subsequently incorporated into phospholipids.

In animal studies, both deficiencies and supplementation of dietary choline affect the level of choline-containing compounds in the brain. These changes appear to modify the functioning of cholinergic neurons, with the effect of deficiency being especially notable during illness, stress, or treatment with drugs that require the increased firing of cholinergic neurons. Of particular interest, one animal study has documented a reduction in choline uptake into the brain with age, with the implication that the aging brain, even under normal circumstances may be relatively starved for this essential nutrient. Studies in human subjects have suggested that in illness, perhaps analogous to similar findings in animals under stress, dietary choline or its precursor lecithin (phosphatidylcholine) may cause modest changes in cholinergic function in the brain as manifest in altered mood, movement, cognition, or behavior. However, until recently, there have been no studies in
living human subjects of choline uptake or metabolism in the brain. Such studies are made possible by the development of in vivo proton magnetic resonance spectroscopy (1H-MRS), which can detect choline and choline-containing compounds in the brain.32,33 Using 1H-MRS, we have confirmed that in younger adults, as in animals, choline ingested in quantities large enough to raise the plasma concentration leads to an increase in choline-containing compounds in the brain.4,34,35 The current study extends this examination to older adults. In particular, we have tested whether choline uptake into the brain remains robust with age or whether a decrease in choline uptake is observed in older human subjects. Such a decrease might contribute to the degeneration of neurons observed in the brain in later life.

METHODS

Subjects

Subjects were volunteers consecutively chosen from lists at McLean Hospital, Belmont, Mass, or through the Clinical Research Center, Massachusetts Institute of Technology, Cambridge. Subjects were screened by medical history, physical examination, and laboratory tests, including a complete blood cell count, blood chemistry analysis, and urinalysis, and for older subjects, a Mini-Mental State Examination (MMSE) and single photon emission computed tomography scan, and were free of serious medical, neurological, or psychiatric illness. Dementia was excluded in that all subjects had MMSE scores of 26 or greater. All subjects were white. Younger subjects were between the ages of 20 and 45 years; older subjects were between the ages of 60 and 85 years. No subjects drank alcoholic beverages on a more than an occasional basis (fewer than two drinks per time). Three of the younger subjects and five of the older subjects took one or a combination of thyroid supplements, B-blockers, or cholesteryl synthesis inhibitors (three subjects each); angiotensin-converting enzyme inhibitors or diuretics (two subjects each); and calcium channel blockers or anxiolytics (one subject each). No subject was receiving a medication known to have a profound effect on gastrointestinal absorption, in general, or on the absorption and metabolism of choline, in particular.36

After an overnight fast, subjects underwent an initial 1H-MRS session to determine the intensity of the baseline brain choline resonance signal, and blood was drawn for the determination of baseline plasma choline concentration. Immediately following these procedures, the subjects ingested capsules of choline bitartrate with 400 mL of apple juice to yield free choline equal to 50 mg/kg of body weight. This dose was chosen on the basis of a prior study in our laboratory that showed it produced at least a 50% increase in plasma choline; an increase comparable to that observed in human subjects after a choline-rich meal.37 Subjects then underwent repeated 1H-MRS and blood sampling at 3 hours after ingestion of choline. The 3-hour sampling point was chosen on the basis of past experience documenting the time course of the rise of plasma choline concentration in young and old subjects38-40 and the concomitant increase in brain choline-containing compounds in young subjects.41 These increases occur over an extended period of time, from 6 to 8 hours following the ingestion of choline, but the 3-hour point is both practical for subjects and near the peak increase, on average.34-36

This protocol was approved by the McLean Hospital Institutional Review Board, and all subjects gave written informed consent for these procedures. Analyses of MR spectra and plasma choline concentrations were performed by research staff blinded to the origin of the source of the data or specimen.

MR Imaging for Brain Volumetric Measurements

The MR images were acquired on a 1.5-Sigma whole-body scanner (GE Medical Systems, Milwaukee, Wis). Sagittal scout images were obtained first, followed by spin-echo 3-mm axial slices of the whole brain. The imaging parameters included a 2500-Hz spectral width, 2500-Hz spectral width, and a 2500-Hz spectral width. The voxel (volume of pixel) dimensions were $0.975 \times 0.975$ mm by 3 mm. This resulted in 54 contiguous sets of images through the entire brain for each TE.

Image processing was carried out on workstations (Sun Microsystems Mountain View, Calif) using MRX software.42 This software includes a segmentation algorithm to assign voxels to distinct classes, including voxels representing cerebrospinal fluid (CSF), gray matter, and white matter. Following classification, voxels for each tissue class are summed to compute estimates of brain tissue volume. Two raters, blinded to age, measured total intracranial volume. Cortical volume was then divided into total CSF volume, total gray matter volume, and total white matter volume. Preliminary data on segmentation of a phantom of a brain slice with these techniques showed that the error of measurement ranged from 4% to 6% for each tissue type.42,43

MRS for Estimation of Brain Choline--Containing Compounds

Human brain choline resonance was determined by 1H-MRS, using a 1.5-T MR imaging device. An 8-cm3 voxel centered on the head of the caudate and the putamen was determined from coronal, T1-weighted, 3-mm contiguous scout images. The basal ganglia were chosen as a representative site because they contain a mixture of white and gray matter and cholinergic and other neuronal types. Choline transport across the blood-brain barrier varies little by brain region, although overall uptake is slightly greater in regions with cholinergic neurons.43 Voxel placement was chosen to contain no or minimal (<2%) CSF. A modified stimulated echo acquisition method (STEAM) pulse sequence was used to acquire the 1H-MR spectra using TR=2 seconds, TE=30 milliseconds, 1024 data points, and 2500-Hz spectral width.44 The total data acquisition time was just over 8 minutes as 256 transients were averaged. Spectra were zero-filled to 2048 data points and filtered with an exponential function to produce a line broadening of 1 Hz after Fourier transformation. The transformed spectra were baseline corrected with a spline function to compensate for residual water signal. Following phase correction, peaks were fit to Gaussian line shapes using a Marquardt algorithm.

Three major resonance signals were observed: choline, creatine, and N-acetylasparrtate. The choline resonance reflects the contribution of several cytosolic choline-containing compounds in the brain,45-47 principally phosphocholine, glycerophosphocholine, as well as choline, acetycholine, and cytidine diphosphate choline (Figure 1). The creatine resonance is composed of creatine and phosphocreatine and is often used as an internal standard, because this signal appears to be stable over time and across a range of physiological states.48,49 A previous study observed no significant age-dependent variation in the T1 and T2 relaxation times of these resonances in the brain.48 Relative concentrations of choline were determined using the ratio of the choline resonance intensity to the creatine resonance intensity. Repeated 1H-MRS studies in the same individual at our center have documented that the reliability of this method is high, with a coefficient of variation of 9.3% for the ratio of choline-to-creatine. The choline in the blood does not substantially contribute to the choline resonance measured in the brain both because blood makes up only a few percent of brain tissue by volume44 and cho-
phosphorylated to phosphocholine (PhC) and incorporated into the membrane lipids phosphatidylcholine (PC). 

Neurotransmitter (cholinergic cells) is performed by both low- and high-affinity transport pumps. Intracellular Cho uptake into neurons and glia by low-affinity transport pumps. Transport into neurons that use acetylcholine (ACh) as their neurotransmitter (cholinergic cells) is performed by both low- and high-affinity transport pumps. Intracellular Cho is phosphorylated to phosphocholine (PhC) and incorporated into the membrane lipids phosphatidylcholine (PC). ACh and CDP-Chol are precursors for ACh (dotted line). PhC and CDP-Chol release Cho or its derivatives (e.g., glyceroxophosphocholine [GPC]) during membrane remodeling, breakdown, or the production of stimulus-induced second-messenger molecules. Compounds in unshaded boxes are visible by magnetic resonance spectroscopy. CDP-Chol indicates cytidine diphosphate choline; LysO-PtdCho, lysophosphatidylcholine; FA, fatty acid; and ECF, extracellular fluid.

**Figure 1.**-Transport and metabolism of choline (Cho) in the brain. The passage of Cho between blood and brain is mediated by facilitated diffusion processes located within capillary endothelia. Cho is carried into neurons and glia by low-affinity transport pumps. Transport into neurons that use acetylcholine (ACh) as their neurotransmitter (cholinergic cells) is performed by both low- and high-affinity transport pumps. Intracellular Cho is phosphorylated to phosphocholine (PhC) and incorporated into the membrane lipids phosphatidylcholine (PC). ACh and CDP-Chol are precursors for ACh (dotted line). PhC and CDP-Chol release Cho or its derivatives (e.g., glyceroxophosphocholine [GPC]) during membrane remodeling, breakdown, or the production of stimulus-induced second-messenger molecules. Compounds in unshaded boxes are visible by magnetic resonance spectroscopy. CDP-Chol indicates cytidine diphosphate choline; LysO-PtdCho, lysophosphatidylcholine; FA, fatty acid; and ECF, extracellular fluid.

**Plasma Choline Determination**

Plasma choline level was determined by high-performance liquid chromatography coupled to electrochemical detection. Blood was drawn into heparinized tubes and centrifuged, and the plasma was stored at -70°C. For the analysis of choline, 100 μL of plasma were added to 600 μL of ice-cold methanol in a polystyrene tube and centrifuged at 4°C for 15 minutes at 15,000 rpm to precipitate the proteins. The protein pellet was discarded, and the supernatant (approximately 400 μL) was placed in a tube, to which 240 μL of water and 640 μL of chloroform were added. The mixture was vortexed for 10 seconds and then centrifuged for 5 minutes at 4000 rpm. All of the aqueous phase (approximately 600 μL) was placed in a fresh tube and dried to a powder using a vacuum concentrator. To remove any protein residue, the samples underwent a third extraction in a mixture of methanol, water, and chloroform (1:1:2). The dried powder was reconstituted and analyzed for choline.

Choline level was determined by high-performance liquid chromatography (Waters model 710B, Waters Associates, Milford, Mass) with a choline oxidase column (Waters model 710B, Waters Associates, Milford, Mass) coupled to an electrochemical detector. The mobile phase (flow rate, 1.2 mL/min) contained 50 mmol/L sodium phosphate, dibasic and 0.005% Kathon (combination of methylisothiazolinone and methylchloroisothiazolinone) at a pH of 8.5. Choline was enzymatically converted to hydrogen peroxide, and the peroxidase was measured electrochemically on a platinum electrode at +800 mV (vs a palladium reference electrode).

**RESULTS**

**Brain Choline Uptake in Younger and Older Adults**

Values for plasma choline and brain choline-containing compounds in younger and older subjects before and after ingestion of choline are shown in the Table. The baseline plasma choline concentrations of older adults were slightly higher (15%) than those of younger adults. This difference was not statistically significant. In each group, there was a marked and statistically significant (P<.001) increase for younger vs older, analysis of variance (ANOVA) increase in plasma choline concentration 3 hours after choline ingestion. These increases were of similar magnitude (76% and 50%, on average) in both younger and older subjects.

The brain choline-creatine ratio at baseline was lower (12%) in older subjects than in younger subjects. This difference was not statistically significant. However, at 3 hours after ingestion of choline, the brain choline-creatine ratio rose markedly (60% on average) only in younger adults (P<.001 vs choline-creatine ratio at baseline). In older adults, the brain choline-creatine ratio rose an average of only 16%, significantly less than the rise observed in younger adults (P<.001).

**Brain Choline Uptake by Age and Sex**

Figure 3 shows the individual increases in brain choline-creatine ratio plotted against age for all subjects. It is notable that an increase in the brain choline-creatine ratio was evident in all the younger subjects. Only two of the older subjects had increases great enough to overlap the changes observed in the younger adults. These two subjects were not distinguishable from the other older subjects by age. Both were women. However, for younger and older subjects alike, no significant relationships were found between sex and the level of plasma choline or the brain choline-creatine ratio before or after ingestion of choline (ANOVA, P=.31 to .76), and there was no evidence of a statistically significant interaction of sex and age on these measurements (two-factor ANOVA, P=.28 to .76). On average, the brain choline-creatine ratio increased 0.37 (SD, 0.15) in younger men vs an increase of only 0.062 (0.047) in older men, a difference significant at P<.001. Among women, the brain choline-creatine ratio also increased more in younger subjects than in older subjects (0.283 [0.021] vs 0.10 [0.14]), although this difference did not reach significance (P=.37) when women were analyzed separately (n=10).

**Brain Mass and Brain Choline Uptake**

Brain mass and, in particular, the volume of gray matter decrease with age. In our subjects, gray matter was lower by 29% in the older subjects than in the young subjects (507 mL vs 712 mL; P<.001 by ANOVA), while white matter (724 mL vs 883 mL, 6% greater in older subjects; P=.37) and total brain volume (1459 mL vs 1512 mL, 4% lower in older subjects; P=.44) showed only modest differences. These values are consistent with those observed in previous studies. Brain choline uptake is similar in gray matter and white matter, suggesting that a reduction in gray matter did not explain the reduction in brain choline uptake. Consistent with this suggestion, no significant association was observed within the older subjects between the proportion of gray to white matter and apparent brain volume.
uptake of choline, as measured by the change in the brain choline-creatine ratio following ingestion of choline ($R^2 = 0.007$ by linear regression; $P = 0.80$).

**Time Course of Brain Choline Uptake in Older Adults**

Plasma choline level rises and falls within several hours of choline ingestion in young animals or in human subjects, and brain choline uptake follows a similar course. Because it is possible that brain choline uptake is delayed in older adults, we monitored plasma choline level and the brain choline-creatine ratio in four older subjects (aged 66, 69, 76, and 80 years; mean [SD], 73 [6.4] years) at baseline and 3, 5, and 7 hours after the ingestion of choline, 50 mg/kg. As previously observed in younger subjects, plasma choline was significantly elevated over baseline at 3 hours (178% elevation; $P = .06$), peaked near 5 hours (233% elevation; $P = .008$), and appeared to be declining by 7 hours (160% elevation; $P = .11$) after ingestion of choline. However, despite these large increases in plasma choline level, the brain choline-creatine ratio was not substantially elevated at any time after choline ingestion. At 3 hours after ingestion, the increase in these four older subjects (mean [SD], 16% [8.5%]; $P = .10$ vs baseline) was similar to that observed in our larger group of older subjects (16%, Table). Also, the brain choline-creatine ratio was not further elevated over baseline at 5 hours (9.5% [10%]; $P = .64$) or at 7 hours (14% [11%]; $P = .14$) after ingestion of choline and never achieved the levels of increase seen in younger subjects (60% at 3 hours, Table).

**COMMENT**

The results confirm our observations in a pilot study that ingestion of choline and the resultant increase in plasma choline levels leads to a marked and rapid increase in choline-containing compounds in the brains of younger adults. Animal studies using direct chemical analyses of choline suggest that this increase is due to facilitated diffusion of choline across the blood-brain barrier. Choline is rapidly converted to phosphocholine in the brain and appears predominantly in this pool in the first hours after choline ingestion. The striking increase in brain choline-containing compounds observed following choline ingestion in younger adults was not seen in older subjects. Rather, older adults showed little increase in brain choline-containing compounds on average, and only two of 16 of the older subjects had apparent increases in brain choline-containing compounds overlapping even the lower end of the range of increase observed in younger subjects. This difference between younger and older adults was not due to a difference in absorption of choline from gut to blood, as blood levels of choline were similar at baseline and rose by similar amounts in both groups of subjects. Also, it is unlikely that the large differences in apparent uptake of choline were due to differences in brain mass because the degree of brain atrophy was modest in the older subjects studied and the tissue sampled in each case contained minimal CSF, the presence of which would have reduced the apparent uptake of choline. Rather, these differences likely indicate an age-related decrease in choline uptake from blood to brain following ingestion. This conclusion is supported by the observation in an animal model of the loss of uptake of choline into the brain with advancing age. The molecular basis for this change is not known, and alternative explanations (eg, reduction or loss of a transport protein or its cofactor or a change in choline phosphorylation and intracellular trapping of the charged molecule phosphocholine) cannot be differentiated on the basis of our human data or available animal data.

This study was performed with a single dose of choline, and it would be of interest to know if blood-to-brain distribution of choline in older subjects was proportion-

Figure 2.—Proton magnetic resonance spectra were obtained from a voxel centered on the basal ganglia, as outlined in white (left). Spectra are shown for a representative subject before (top right) and 3 hours after (bottom right) ingestion of choline (50 mg/kg). Boxes indicate the relative areas under the resonance peaks for choline (red), creatine (green), and N-acetylaspartate (blue).
Older adults

Subject Groups

<table>
<thead>
<tr>
<th>Mean Age, y (SD)</th>
<th>Baseline</th>
<th>After Ingression</th>
<th>% Increase</th>
</tr>
</thead>
<tbody>
<tr>
<td>Older adults</td>
<td>73 (6.9)</td>
<td>10.1 (3.23)</td>
<td>18.2 (3.83)</td>
</tr>
<tr>
<td>Younger Adults</td>
<td>2.0 (1.0)</td>
<td>2.0 (1.0)</td>
<td>2.0 (1.0)</td>
</tr>
</tbody>
</table>

*Twelve younger adults and 16 older adults were studied before and 3 hours after ingestion of choline (see "Methods" section for details).
†P < .001 vs baseline.
‡P < .001 vs baseline and P < .01 vs older adults, after ingestion. All tests are one-way analyses of variance.

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Figure 3.—Change in brain choline-containing compounds after ingestion of choline by age of subject. The relative concentration of brain choline-containing compounds was measured as the ratio of choline and creatine resonances by proton magnetic resonance spectroscopy. Change in brain choline-containing compounds is estimated as the difference between the choline-creatine ratios before and 3 hours after ingestion of choline (see "Methods" section for details).

**Figure 3.** Change in brain choline-containing compounds after ingestion of choline by age of subject. The relative concentration of brain choline-containing compounds was measured as the ratio of choline and creatine resonances by proton magnetic resonance spectroscopy. Change in brain choline-containing compounds is estimated as the difference between the choline-creatine ratios before and 3 hours after ingestion of choline (see "Methods" section for details).

The dose chosen produces at least as great an increase in plasma choline level as does a choline- or lecithin-rich meal, with the implication that in older persons, choline from dietary sources does not lead to an increase in brain choline level comparable with that observed in younger adults.

Might this age-related decrease in choline uptake have physiological consequences? In particular, does this decrease in uptake lead to a decrease in functionally important choline-containing compounds in the brain? In our studies, the apparent baseline concentration of choline-containing compounds in the brain was only modestly lower in older adults compared with younger adults studied before ingestion of choline. Similarly, no difference in the magnitude of the choline resonance was observed in older and younger adults in a previous study. It is worth noting, in this regard, that the choline resonance by MRS is higher on average in white matter than in gray matter in the brain. Therefore, the relatively greater proportion of white to gray matter in older adults may mask an actual decrease in choline-containing compounds with age. However, it is also important to note that most of the choline in the brain is in the form of the membrane lipid phosphatidylcholine. Because the motion of this molecule is restricted, it is largely invisible to MRS, despite being present at a concentration at least 10 times that of the MRS-visible compounds choline, acetylcholine, phosphocholine, cytidine diphosphate choline, and glycerophosphocholine.

Because phosphocholine largely serves as a precursor of phosphatidylcholine, and glycerophosphatidylcholine is largely a product of phosphatidylcholine metabolism, these MRS-visible compounds are transiently affected by changes in choline metabolism and serve as good measures of change in choline-containing compounds in the brain. However, they are not good measures of the steady state level of total choline-containing compounds in the brain.

Direct chemical analyses of the brain in postmortem studies have documented a substantial reduction in phosphatidylcholine in the brain with age. This change contributes to an age-related decrease in fluidity of cell membranes and may be associated with decreased function of membrane-spanning receptor molecules and ion transport channels and an alteration in the efficiency of membrane-bound enzymes, including those necessary for energy metabolism, which have a requirement for interaction with phosphatidylcholine. Our findings raise the possibility that reduced passage of choline from blood to brain is a factor in loss of phosphatidylcholine, as well as some of the age-related changes in neuronal function associated with this loss.

Similarly, the decreased ability of the brain to obtain choline with age may explain why cholinergic neurons appear to be particularly susceptible to degeneration during normal aging and even more profoundly, in the pathologic case of dementias of the Alzheimer's type. In fact, reduced uptake of choline with age might partly explain the remarkable delay in appearance of Alzheimer's disease (AD), even in those genetically predisposed, until late in life. In younger persons, choline levels may be adequate to sustain necessary cell functions even under circumstances of stress or disorder. Decreased uptake with age might lead to a situation in which neurons can no longer compensate for underlying metabolic abnormalities and essential cell functions cannot be maintained. Postmortem brain samples from patients with AD exhibit decreases in choline and phosphatidylcholine levels compared with those of age-matched controls and an increase in glycerophosphocholine, a breakdown product of phosphatidylcholine metabolism. Brains starved for choline will metabolize cell membrane lipids to supply this nutrient for acetylcholine synthesis or other cell needs. The neurochemical findings in AD suggest that a similar process may be occurring.

Decreased choline uptake might be particularly prevalent in older subjects who have experienced a decline in cognitive function or in those with AD. Alternatively, older subjects who maintain a higher level of choline uptake into the brain might be at reduced risk for neurodegenerative or dementing disorders. These possibilities cannot be properly tested in the subjects of the current study, who were chosen to be healthy and have no medical, neurological, or psychiatric impairment.

Finally, supplementation with choline or its dietary precursor, lecithin, has been used in an attempt to ameliorate the symptoms or progression of AD. This hypothesis that additional choline might improve the function or decrease the degeneration of cholinergic neurons. Such trials, whether with dietary supplements alone or with supplements combined with cholinergic drugs, have at best produced modest results. The reasons for these disappointing effects in the face of clearly documented cholinergic deficits in AD are unclear. Our findings suggest that a factor contributing to the limited success of these treatments may be an impairment of carrying circulating choline to the brain with advancing age.

To our knowledge, this is the first study of the effects of aging on the uptake of the nutrient choline into the human brain. At least seven other blood-brain barrier transport systems normally funnel nutrients and metabolites into and out of the human brain and might be similarly affected by aging. The only other system that has been measured in older adults is glucose transport. Studies using fluorodeoxyglucose and positron emission tomography suggest that glucose uptake is sustained with age in healthy adults.
Thus, the reduced brain uptake of choline observed with age is not a general phenomenon affecting all nutrients. The findings reported herein suggest that uptake and, by implication, the supply of brain choline may become limited and inadequate in old age. It will be important to study the relationship between the uptake of circulating choline and brain function in healthy and ill older subjects and to determine by which age-related decrement in uptake might be reversed. Documenting and addressing these changes might lead to the development of effective strategies for slowing age-related deterioration of cognition and the prevention of dementia illness late in life.

This work was supported in part by a 1-year research grant administered by the Clinical Research Center of the Massachusetts Institute of Technology using MIT patent royalties, as well as by National Institutes of Health grants MH31104 (E. L. Spector), MH29785 (E. L. S. W.), and MO1-RR-00088 (MIT Clinical Research Center). Equipment support was provided for the McLean Imaging Center was also provided from a grant from the Virginia B. Tidwell Endowment Fund (B. M. C.), and faculty support was provided by a grant from the JoAnn M. Collins Investigator Endowment Fund (F. F. R.).

References