# The Wrong Half of the Molecule

*Choline in Alzheimer's disease — why forty years of trials asked the wrong question of it, what the APOE4 genotype changes, and whether a carrier should take it*

*CHT1 · FLVCR2 · The Kennedy pathway · APOE4 · Phosphatidylcholine · Trimethylamine N-oxide*

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*One transporter into the brain is saturated at ordinary plasma concentrations and one is not. Forty years of trials loaded the saturated one, and it leads to acetylcholine. The unsaturated one leads to the membrane — which is where the APOE4 allele fails, and where the head group has never been tested in a human being alongside the acyl chain it has to be built with.*

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# Abstract

Choline has been given to people with Alzheimer's disease for four decades, and it has never worked. Twelve randomised trials of lecithin were assembled by a Cochrane review that found no benefit to cognition or global impairment and a relative deterioration in activities of daily living. The failure was so complete that the molecule was effectively retired from the field. This paper argues that the trials were not merely underpowered or too late; they were built on a specific pharmacological error, and that the error is identifiable, correctable, and — for one genotype — worth correcting.

The error is a matter of transport kinetics. Choline was given as a precursor of acetylcholine, on the reasoning that a disease which destroys cholinergic neurons should be treatable by supplying the transmitter's raw material. But the presynaptic high-affinity choline transporter, CHT1, has a Michaelis constant near 2 μM, and human fasting plasma choline sits around 7 to 10 μM. The transporter that feeds acetylcholine synthesis is therefore already close to saturation at ordinary plasma concentrations, and raising plasma choline cannot appreciably raise its flux. The transporter at the blood–brain barrier is a different protein with a different constant: choline uptake into brain is described by a saturable component with a Km near 40 μM, and the gate was molecularly identified only in 2024, as FLVCR2. At 10 μM plasma choline that gate operates at roughly a fifth of capacity, in the near-linear part of its curve. One route into the brain is saturated and one is not, and the trials loaded the saturated one. The route that is not saturated does not lead to acetylcholine. It leads to the Kennedy pathway and to phosphatidylcholine — to membrane, not to transmitter.

That is the first half of the argument. The second half is what makes the genotype question live. In 2021 Sienski, Narayan, Bonner and colleagues showed that APOE4, but not APOE3, disrupts the cellular lipidome of human iPSC-derived astrocytes and of yeast carrying the human allele: triacylglycerides accumulate, their fatty acids become abnormally unsaturated, lipid droplets multiply and cholesterol builds up. Supplementing the medium with choline — or with CDP-choline — returned the lipidome toward its basal state. This is a rescue of a lipid phenotype by a head group, in a genotype-specific system, and it has nothing to do with acetylcholine. A phase 1 trial (NCT05880849) has since given 2.2 g/day of choline bitartrate to fifteen APOE4 carriers for six months with cerebrospinal fluid lipid endpoints; it completed in October 2025 and has not reported.

The paper then assembles what human evidence exists, and finds it thinner and stranger than either enthusiasts or sceptics assume. Four large cohorts — Kuopio, Framingham Offspring, UK Biobank and the Rush Memory and Aging Project — converge on a dose-response curve whose minimum sits near 350 mg/day, below the Institute of Medicine's Adequate Intake, and whose left limb is steep: the risk is concentrated in low intake, not in the absence of supplementation. Only two human datasets have ever examined choline against APOE genotype directly. One,

from 1999, reported that citicoline's effect was larger in ε4 carriers, in thirty patients, and was never replicated. The other found that betaine, dimethylglycine and sarcosine predicted slower cognitive decline in ε4 carriers and *faster* decline in non-carriers — an interaction that reverses sign across genotype, in 152 patients, also unreplicated. Against these, the one cohort that formally tested APOE4 as a modifier of dietary choline found none.

The paper's central claim concerns what none of the trials have done. Phosphatidylcholine requires two substrates: a head group and an acyl chain. The choline trials supplied head groups to people whose acyl chain supply was never measured. The DHA trials supplied acyl chains to people whose head group supply was never measured — including PreventE4, which in 2026 reported successful central target engagement in APOE4 carriers and no cognitive or structural benefit whatsoever over two years. There is a precedent for what this costs: in VITACOG, homocysteine-lowering B vitamins slowed brain atrophy only in participants who already had adequate omega-3 status, and the treatment effect was entirely absent in those who did not. Two half-therapies, each tested alone, each null. That is the trial that has not been run.

The verdict is stratified and mostly negative. For established Alzheimer's disease, choline in any form is not a therapy, and the evidence against it is stronger than the evidence for most things the field currently entertains. For an APOE4 carrier with low intake, correcting the deficit to roughly the epidemiological optimum is a defensible act of nutritional prudence with a plausible mechanism and a real, quantified harm ledger — supplemental choline bitartrate raises trimethylamine N-oxide and platelet reactivity where whole eggs do not, α-glycerylphosphorylcholine carries a dose-dependent stroke association in twelve million Koreans, and every cohort curve turns back upward at the high end. For an APOE4 carrier already at 400 mg/day, there is no human evidence of benefit from more, and the honest answer is that nobody knows. The paper closes with a graded ledger separating what is established from what is inference, five conditions that would refute the argument, and the design of the factorial trial that would settle it.

# Part I — The Molecule and Its Three Errands

# 1. The Question, Stated Precisely

The question this paper was written to answer is a practical one, asked by a specific person. A man or woman in their fifties or sixties learns, from a commercial genotyping service or from a research study or from a neurologist, that they carry one or two copies of the ε4 allele of *APOE*. They read that the allele is the strongest common genetic risk factor for late-onset Alzheimer's disease, that a single copy raises lifetime risk roughly threefold and two copies roughly tenfold, and that nothing they can be prescribed will change it. They then read — because it circulates widely — that choline reverses the effect of APOE4 on brain cells in a dish, and that choline is available for a few cents a day in any pharmacy. They want to know whether to take it.

The question deserves a better answer than either of the two it usually gets. The enthusiast's answer points at the cell-culture rescue, at the epidemiology showing lower dementia risk with higher intake, and at the fact that most people do not meet the recommended intake, and concludes that supplementation is obviously sensible. The sceptic's answer points at four decades of failed trials and concludes that the question was settled in 1990. Both answers are wrong in the same way: they treat "choline" as one thing with one mechanism, tested once. It is not. Choline is a small quaternary amine with three biochemically distinct destinations, three separate transport systems with different affinities, and at least four distinct therapeutic rationales that have been tested to very different depths. The trials that failed tested one of those rationales. The rationale that motivates the APOE4 question is a different one, and it has never been tested in a human being with a cognitive endpoint.

This paper therefore separates the question into parts that can be answered independently, and answers each at the level of confidence the evidence supports rather than at the level the reader might prefer.

The first part is biochemical: what does choline do, where does it go, and which of its errands is plausibly limiting in an ageing human brain? This is Part I, and its central finding is a kinetic asymmetry — one that has been in the literature since 2001 and that, read correctly, predicts the failure of the historical trials in advance.

The second part is historical: what exactly was tested, on whom, and what was found? This is Part II. The record is worse than the field's folk memory of it. It is also more specific: the trials failed in a way that implicates the rationale rather than the molecule.

The third part is mechanistic: what does the disease do to membrane phospholipid, what does APOE4 do to lipid handling, and where do those two facts meet? This is Part III, and it contains the

finding that makes the modern question worth asking at all.

The fourth part is the genotype question proper: is there any reason, and any evidence, that an APOE4 carrier is different with respect to choline? This is Part IV. The answer is that there are four reasons, two of which are strong; that the direct human evidence consists of two small unreplicated studies pointing the same way and one large null; and that the single most important trial in the field has been run backwards, supplying one half of a two-substrate reaction and measuring whether the reaction went faster.

The fifth part states a verdict, grades it, says what would refute it, and specifies the experiment that would settle it. This is Part V.

One framing decision should be declared at the outset, because it governs everything that follows. This paper does not treat "does choline help Alzheimer's disease" and "should an APOE4 carrier take choline" as the same question. They have different subjects, different time-horizons, different endpoints, and — as it turns out — different answers. Conflating them is the single most common error in the popular literature on this molecule, and it is also, in a subtler form, the error the trialists made.

# 2. What Choline Is, and the Three Places It Goes

Choline is 2-hydroxy-N,N,N-trimethylethanaminium: a two-carbon backbone bearing a hydroxyl group at one end and a permanently charged trimethylammonium group at the other. The permanent positive charge is the fact from which most of its biology follows. It makes the molecule water-soluble, it makes it unable to cross membranes by simple diffusion, and it therefore makes every movement of choline in the body a transporter-mediated event. A molecule that requires a protein to go anywhere is a molecule whose distribution is set by the kinetics of those proteins, not by concentration alone. This is why the arithmetic in Chapter 3 matters so much.

Choline was designated an essential nutrient by the Institute of Medicine in 1998, with Adequate Intakes set at 550 mg/day for adult men and 425 mg/day for adult women. The designation rested on a specific experimental observation: healthy adults placed on a choline-deficient diet develop hepatic steatosis and elevated serum alanine aminotransferase, and both resolve on repletion. The endpoint that defined human choline requirement was, in other words, a *liver* endpoint. No brain endpoint has ever been used to set it. This is not a criticism of the committee, which had no brain endpoint available, but it becomes important in Chapter 15, where four epidemiological cohorts independently locate the intake associated with lowest dementia risk *below* the Adequate Intake.

Once absorbed, choline has three fates, and they are not equal partners.

**The membrane errand.** The large majority of the body's choline is consumed by the CDP-choline pathway, described by Eugene Kennedy in the 1950s and still called the Kennedy pathway. Choline is phosphorylated by choline kinase to phosphocholine; phosphocholine is condensed with cytidine triphosphate by CTP:phosphocholine cytidylyltransferase to form CDP-choline; and CDP-choline donates its phosphocholine head group to diacylglycerol, catalysed by choline/ethanolamine phosphotransferase, to yield phosphatidylcholine. Phosphatidylcholine is the most abundant phospholipid in every mammalian membrane, constituting roughly half of total phospholipid in most cell types. The cytidylyltransferase step is rate-limiting and is regulated by membrane lipid composition — the enzyme is recruited from a soluble pool onto membranes whose curvature and lipid packing signal a deficit of phosphatidylcholine. The pathway, in other words, is not a passive conduit; it is a sensor that responds to membrane state.

The second, minor route to phosphatidylcholine matters disproportionately here. Phosphatidylethanolamine N-methyltransferase, PEMT, performs three sequential methylations on

phosphatidylethanolamine using S-adenosylmethionine, producing phosphatidylcholine de novo without any dietary choline at all. In humans this pathway is essentially hepatic, accounts for roughly a third of hepatic phosphatidylcholine, and — critically — produces species enriched in long-chain polyunsaturated fatty acids, including docosahexaenoic acid. PEMT is therefore not simply a backup supply of head groups; it is the body's principal endogenous route to *polyunsaturated* phosphatidylcholine. Chapter 4 shows that this route is under hormonal control, and Chapter 13 argues that its hormonal control is one of the four reasons the APOE4 question has a sex dimension.

**The methyl errand.** A smaller fraction of choline is irreversibly oxidised — by choline dehydrogenase in the mitochondrial inner membrane, then by betaine aldehyde dehydrogenase — to betaine. Betaine is a methyl donor: betaine–homocysteine S-methyltransferase transfers one of its methyl groups to homocysteine, regenerating methionine and producing dimethylglycine. This is one of the body's two routes for disposing of homocysteine by remethylation, the other being the folate/B12-dependent methionine synthase reaction. The tissue distribution matters and is frequently misstated: BHMT activity is high in liver and kidney and low in human brain, and in rodents is essentially hepatic. The betaine route is therefore a *systemic* homocysteine sink, not a local cerebral one. When a mouse study attributes a brain benefit of choline to homocysteine lowering, the lowering is happening mostly in the liver and reaching the brain through the circulation.

**The transmitter errand.** The smallest fraction, quantitatively trivial at the level of whole-body flux but historically dominant in this field, is acetylated by choline acetyltransferase to acetylcholine. This occurs only in cholinergic neurons, which are a small minority of neurons, and the choline used is drawn overwhelmingly from a dedicated recycling loop: acetylcholine released into the synapse is hydrolysed by acetylcholinesterase, and the liberated choline is recaptured by the high-affinity transporter CHT1 for reuse. The system is designed to conserve, not to consume.

**Table 1 — The three errands of choline, and what each one is limited by.**

| Errand | Pathway | Principal site | Rate-limiting element | Responds to raised plasma choline? |
|---|---|---|---|---|
| **Membrane** | Kennedy (CDP-choline) pathway → phosphatidylcholine | All cells; PEMT route hepatic | CTP:phosphocholine cytidylyltransferase; substrate supply of both head group and diacylglycerol | Yes — the barrier transporter is unsaturated |
| **Methyl** | Choline dehydrogenase → betaine → BHMT | Liver and kidney; low in brain | Substrate availability; folate/B12 status of the parallel route | Yes, systemically |
| **Transmitter** | Choline acetyltransferase → acetylcholine | Cholinergic terminals only | CHT1 reuptake, near-saturated at physiological plasma choline | Minimally |

The table states the paper's first structural claim in compressed form. Two of choline's three errands are supply-responsive and one is not, and the one that is not is the one four decades of clinical trials attempted to drive.

# 3. The Gate, and the Arithmetic Nobody Did

Choline reaches the brain across the blood–brain barrier by saturable transport. Allen and Smith, using in situ rat brain perfusion, characterised the process and found it best described by a single saturable transporter with a maximal velocity of 2.4 to 3.1 nmol/min/g and a Michaelis constant of 39 to 42 μM, alongside a small non-saturable component. They noted explicitly that this affinity is five- to tenfold greater than earlier in vivo estimates but substantially *lower* than the neuronal high-affinity choline transport system, which operates with a Km of 1 to 5 μM.

The molecular identity of the barrier transporter remained unresolved for more than two decades after that kinetic description. It was settled in 2024. Cater, Mukherjee, Gil-Iturbe and colleagues determined cryo-electron microscopy structures of choline-bound FLVCR2 in inward- and outward-facing states and established that FLVCR2 is the blood–brain barrier choline transporter responsible for the majority of choline uptake into brain. A companion body of structural work resolved the closely related FLVCR1 and showed that both proteins recognise choline and ethanolamine through cation–π interactions with conserved tryptophan and tyrosine residues, feeding head groups into the Kennedy pathway. Within the brain parenchyma, the ubiquitous choline transporter-like protein CTL1, encoded by *SLC44A1*, distributes choline to cells and, at the mitochondrial membrane, supplies the oxidation route; loss-of-function in humans produces a severe phenotype with white matter deficits, ataxia and cerebral and cerebellar atrophy, which establishes that brain choline delivery is not a redundant system.

Now the arithmetic. Human fasting plasma choline concentration is on the order of 7 to 10 μM, rising into the teens postprandially and after supplementation. Place that number against the two Michaelis constants.

At the cholinergic terminal, CHT1 has a Km near 2 μM — Allen and Smith give the neuronal high-affinity system as 1 to 5 μM, and recent structural and functional characterisation of the human transporter places it at approximately 2 μM. At an extracellular concentration of 10 μM — five times the Km — the transporter runs at approximately 83 per cent of its maximal velocity. Doubling plasma choline to 20 μM raises that to about 91 per cent. The entire dynamic range available to a supplement, on this route, is a fraction under twenty per cent of Vmax, and that is before accounting for the fact that most of the choline CHT1 handles is recycled locally from hydrolysed acetylcholine rather than drawn from plasma at all.

At the blood–brain barrier, FLVCR2-mediated uptake has a Km near 40 μM. At 10 μM — a quarter of the Km — the transporter runs at approximately 20 per cent of maximal velocity, in the region of the Michaelis–Menten curve that is still close to linear. Doubling plasma choline to 20 μM raises flux to about 33 per cent of Vmax: a two-thirds increase in delivery. Tripling it to 30 μM gives about 43 per cent: more than double the baseline rate.

This asymmetry is the pharmacological hinge of the entire subject, and it has an immediate and unforgiving implication. **Raising plasma choline is a poor way to raise acetylcholine synthesis and a good way to raise brain choline availability for membrane synthesis.** The transporter that feeds the transmitter is nearly full at ordinary concentrations; the transporter that feeds the membrane is nearly empty. A therapy designed around the first will show a ceiling effect that no dose can overcome. A therapy designed around the second has a real dose–response available to it.

Three qualifications must be attached before this argument is allowed to carry weight, and they are attached now rather than in a footnote.

The first is that the Km of 39 to 42 μM is a rodent in situ perfusion value, not a human in vivo one. Human blood–brain barrier choline transport has not been characterised with equivalent precision, and species differences in transporter expression are common. The structural identification of FLVCR2 as the human barrier transporter makes the extrapolation more defensible than it was in 2001, but it remains an extrapolation.

The second is that saturation of CHT1 does not mean acetylcholine synthesis is entirely unresponsive to precursor. Under conditions of high cholinergic firing, local extracellular choline can be depleted below the resting concentration, and under those conditions precursor supply can become limiting — which is precisely the circumstance the autocannibalism hypothesis of Chapter 5 was built around. The claim here is not that precursor loading does nothing; it is that in a resting or moderately active cholinergic system it does very little, and that this is a structural feature of the transporter, not a failure of dose.

The third is that neither transporter operates in isolation from the disease. If the barrier itself is compromised — and APOE4 is associated with pericyte-dependent barrier breakdown — then transporter-mediated flux is not the only route, and the kinetics described here become one term in a more complicated expression.

With those qualifications registered, the asymmetry stands, and it will be used in Chapter 7 to explain a set of clinical failures that the field has generally attributed to the wrong causes.

# 4. How Much People Actually Get, and Why "Requirement" Is Not a Constant

A therapeutic argument about a nutrient has to begin from the population's actual intake, because the difference between correcting a deficit and supplementing an adequacy is the difference between two entirely different pharmacological propositions.

Wallace and Fulgoni assessed total choline intake in the United States using NHANES data and found that 10.8 ± 0.6 per cent of participants aged two years or older achieved the Adequate Intake: 15.6 per cent of males and 6.1 per cent of females. Among adults aged nineteen and over, 6.6 per cent met it. Mean intakes were roughly 250 to 280 mg/day in adult women and 387 to 407 mg/day in adult men, against Adequate Intakes of 425 and 550 mg/day respectively. Choline is, on these numbers, one of the most widely under-consumed nutrients in the American diet, and the shortfall is roughly twice as large in women as in men.

That statistic is usually deployed as an argument for supplementation. It should be handled more carefully than that, for two reasons that will recur.

The first is that the Adequate Intake was derived from a liver endpoint, as Chapter 2 noted. A population that fails to meet a liver-derived threshold is not thereby demonstrated to be deficient by a brain-derived one, and Chapter 15 will show that the four cohorts with dementia endpoints locate their optimum near 350 mg/day — a figure that most American men already exceed and most American women do not.

The second is that human choline requirement is not a fixed number. It is modified by sex, by menopausal status, and by a common polymorphism, and the modifiers are large enough to swamp the difference between the sexes' recommended intakes.

Fischer, Zeisel and colleagues placed healthy adults on a choline-deficient diet under controlled conditions and recorded who developed organ dysfunction — hepatic or muscle damage indexed by enzyme elevation. Seventy per cent of men and eighty per cent of postmenopausal women became deficient; only forty-four per cent of premenopausal women did. The mechanism is not obscure. The *PEMT* promoter contains estrogen response elements, and estrogen induces the enzyme that synthesises phosphatidylcholine de novo from phosphatidylethanolamine. A premenopausal woman is partly self-supplying; a postmenopausal woman is not.

The genetic modifier is of comparable magnitude. A common single-nucleotide polymorphism in *PEMT*, rs12325817, impairs the estrogen induction of the enzyme, and its effect follows a clean

dose–response. Among premenopausal women on a low-choline diet, organ dysfunction developed in eighty per cent of those carrying two variant alleles, forty-three per cent of heterozygotes, and thirteen per cent of those with none. In postmenopausal women the hormonal arm of the experiment was equally stark: seventy-three per cent of those receiving placebo developed dysfunction on the low-choline diet, against eighteen per cent of those receiving estrogen.

Three consequences follow for the argument of this paper.

The first is that "choline deficiency" is a genotype-by-hormone-by-intake interaction, not a dietary category. Two people eating identical diets can differ by a factor of six in their probability of becoming functionally deficient. Any trial that randomises choline without measuring *PEMT* genotype and menopausal status is adding a large, structured source of variance to its outcome.

The second is that the population at highest risk of functional choline deficiency — postmenopausal women, especially those carrying rs12325817 variants — overlaps substantially with the population at highest APOE4-attributable risk of Alzheimer's disease. The ε4 allele confers greater risk in women than in men, particularly between the ages of about sixty-five and seventy-five. This is a coincidence of populations, not yet a mechanism, and Chapter 13 will treat it as such. But it is the kind of coincidence that determines whether a trial is powered.

The third is a caution about the endpoint. Everything in this chapter was measured as liver or muscle dysfunction. Nobody has run the depletion–repletion experiment with a neural endpoint, because there is no acceptable one: you cannot deplete a human of choline and biopsy their cortex. The inference from hepatic deficiency to cerebral deficiency is an inference, and it is graded as such in Chapter 18.

# Part II — The Failure That Defined the Field

# 5. Autocannibalism: The Hypothesis That Made Choline a Drug

The idea that choline might treat Alzheimer's disease did not arrive as a nutritional intuition. It arrived as a specific, testable, and rather elegant mechanistic proposal, and understanding it is necessary to understanding why its failure was so decisive and so misread.

In 1983 Blusztajn and Wurtman published a review in *Science* setting out what was then known about choline and cholinergic neurons. Two claims in it did the work. The first was that mammalian neurons can synthesise choline endogenously by methylating phosphatidylethanolamine and hydrolysing the resulting phosphatidylcholine — so that methionine and serine can be ultimate precursors of choline, and the neuron is not wholly dependent on dietary supply. The second was that when cholinergic neurons are activated, acetylcholine release can be enhanced by treatments that raise plasma choline.

Put together, these produce a hypothesis with real teeth. If a cholinergic neuron under sustained demand can obtain choline either from plasma or from its own membrane phosphatidylcholine, then a neuron facing a choline shortfall will draw on its membranes to keep signalling. Wurtman named the consequence directly in a 1992 review in *Trends in Neurosciences*: choline metabolism as a basis for the *selective vulnerability* of cholinergic neurons. A cell that must cannibalise its own structure to perform its function is a cell with a built-in mechanism of self-destruction, and one that would be expected to fail earliest and hardest wherever cholinergic demand is highest and choline supply is lowest.

The appeal of this hypothesis to a field that had just discovered profound cholinergic depletion in the nucleus basalis is easy to reconstruct. It explained selective vulnerability, which the amyloid hypothesis did not. It explained why one transmitter system should fail before others. It made a therapeutic prediction of great simplicity: supply the missing precursor and the neuron will stop eating itself. And the precursor was cheap, safe, orally available and already in the food supply.

It is worth stating plainly what was right about this hypothesis, because the subsequent clinical failure has caused the whole framework to be discarded when only part of it deserved to be.

What was right: the coupling between acetylcholine synthesis and membrane phosphatidylcholine is real; phosphatidylcholine is a genuine reservoir of choline; and Alzheimer's disease brain does show exactly the phospholipid signature that sustained membrane catabolism would produce, as Chapter 9 documents. What was wrong was the direction of the therapeutic

inference. The hypothesis identified a link between transmitter and membrane, and the field elected to pull on the transmitter end of it. The evidence assembled in this paper suggests that the membrane end was the one with slack in it.

# 6. The Twelve Trials

The clinical programme that followed was substantial by the standards of its era and is generally remembered only as a rumour of failure. Its details are worth recovering, because they determine what the failure licenses one to conclude.

The Cochrane review of lecithin for dementia and cognitive impairment, prepared by Higgins and Flicker, identified twelve randomised trials meeting inclusion criteria: 265 patients with Alzheimer's disease, 21 with parkinsonian dementia, and 90 with subjective memory problems. Lecithin — commercial soy-derived phosphatidylcholine — was the delivery vehicle, typically at doses of tens of grams per day, since commercial lecithin preparations of the period contained only a modest percentage of phosphatidylcholine by weight. The review's conclusion was unambiguous: evidence from randomised trials does not support the use of lecithin in the treatment of dementia; no improvement was seen in cognition or global impairment; and there was a relative deterioration in activities of daily living in the treated arms. The reviewers added that a moderate effect could not be excluded but that the results did not indicate priority for a large randomised trial.

Around and beneath this sit the earlier and smaller studies of choline chloride itself, and the combination studies in which lecithin was added to physostigmine or to tacrine on the reasoning that raising precursor supply and blocking hydrolysis should be synergistic. These were, with essential unanimity, negative.

Four features of the programme constrain what can be inferred from it.

The first is the population. Almost every participant had established dementia, most of it moderate. In a person whose basal forebrain cholinergic population has already lost a large fraction of its neurons and whose cortex has lost a substantial fraction of its synapses, a therapy that supplies a synthetic substrate is being asked to restore function to machinery that is no longer present. A negative result in that population is evidence about that population. It is weak evidence about a cognitively normal fifty-eight-year-old.

The second is the endpoint. Cognition and global impairment over trial durations measured in weeks to months. No trial in this programme measured membrane phospholipid, brain choline, or any lipid intermediate. The mechanism was never assayed; only the clinical hope built on it was.

The third is the delivery vehicle. Lecithin at 20 to 25 grams per day is a large mass of soy phospholipid with substantial gastrointestinal consequences, and adherence in these trials was frequently poor and rarely verified biochemically. A trial in which the exposure is unverified and the

tolerability is poor generates a null that is difficult to interpret.

The fourth, and the one this paper takes most seriously, is the rationale. Every one of these trials was designed to raise acetylcholine. That is the point at which the programme's failure becomes informative rather than merely disappointing, and it is the subject of the next chapter.

# 7. Why Precursor Loading Could Not Have Worked

The conventional explanation for the lecithin failures is that the cholinergic hypothesis was too narrow: acetylcholine deficiency is downstream of the disease, replacing a transmitter cannot arrest a degeneration, and the modest and temporary benefit of cholinesterase inhibitors is the ceiling of what cholinergic pharmacology can achieve. All of that is true, and none of it is the sharpest available criticism.

The sharpest criticism is that precursor loading could not raise acetylcholine synthesis appreciably in the first place, for reasons that are visible in the transport kinetics set out in Chapter 3 and that were determinable before the trials were run.

The presynaptic high-affinity choline transporter operates with a Michaelis constant of approximately 2 μM. Human fasting plasma choline sits near 7 to 10 μM, and the extracellular fluid the transporter samples is further enriched by choline liberated locally from acetylcholine hydrolysis. The transporter is therefore working at something like 80 to 85 per cent of its maximal velocity before any supplement is given. The manoeuvre of doubling plasma choline — which in practice requires gram quantities of a supplement — moves it to roughly 90 per cent. This is a ceiling effect of the most ordinary kind, and it is imposed by the transporter, not by the disease, not by the dose, and not by the stage of illness.

Three consequences follow, and they reorganise the interpretation of the whole historical record.

The first is that the lecithin trials do not falsify the autocannibalism hypothesis. They falsify a therapeutic prediction that the hypothesis, properly understood, does not make. Autocannibalism describes a neuron under *demand-driven* choline shortfall, in which local extracellular choline is transiently depleted below the transporter's Km. Under that condition — and only under it — precursor supply becomes rate-limiting. A supplement administered chronically to a resting patient does not reproduce that condition; it raises the tonic concentration in a range where the transporter is already saturated.

The second is that the failure was not a failure of the *molecule*. It was a failure of the route. The same molecule, entering the brain by a different transporter with a fifteen- to twenty-fold higher Michaelis constant, is delivered into a pathway with real headroom — the Kennedy pathway — which was never the target of any of the twelve trials and was never measured in any of them.

The third is a lesson about how the field reasons, and it generalises well beyond choline. A negative trial retires a molecule. It should retire a *mechanism-plus-population-plus-endpoint*. Because the lecithin programme retired the molecule, the membrane rationale that would emerge from the lipid biology of the 1990s and 2000s arrived into a field that had already decided choline did not work, and it has still not been properly tested thirty years later.

One further point of intellectual honesty is required here, because the argument of this chapter cuts both ways. If the presynaptic transporter is saturated, then the *cholinergic* rationale for choline supplementation is dead in all populations, including APOE4 carriers, including the presymptomatic. Nothing in the modern lipid literature revives it. Any claim that supplementary choline will "support acetylcholine" in a healthy or mildly impaired brain is, on the kinetics, not supportable. Readers who came to this paper hoping for a defence of choline as a cholinergic agent will not find one; the defence offered in Part III is of something else entirely.

# 8. The Second Generation: Citicoline, Alpha-GPC, and the Multi-Nutrient Turn

The molecule did not disappear from clinical medicine after the lecithin failures. It reappeared in two pro-drug forms and, later, inside a designed combination. Each deserves separate treatment, because they are frequently discussed as though they were interchangeable and they are not.

**Citicoline (CDP-choline).** Oral citicoline is hydrolysed in the gut wall and liver to cytidine and choline, which are absorbed separately and re-synthesised intracellularly into CDP-choline. In humans, unlike rodents, circulating cytidine is largely converted to uridine, so oral citicoline delivers, in effect, choline plus a pyrimidine. Since CDP-choline is the intermediate that the Kennedy pathway's rate-limiting enzyme produces, and since the pyrimidine supplies the cytidine triphosphate that enzyme consumes, citicoline is the first agent in this story with a coherent *membrane* rationale rather than a transmitter one.

The clinical record is mixed and mostly indirect. The Cochrane review by Fioravanti and Yanagi assembled fourteen randomised placebo-controlled studies of CDP-choline in cognitive and behavioural disturbance associated with chronic cerebral disorders in the elderly, and reported small benefits on memory, behaviour and clinical global impression, with no benefit on attention. The populations were heterogeneous and predominantly vascular rather than degenerative, and the durations were short. The IDEALE study administered 1 g/day of oral citicoline for nine months in mild vascular cognitive impairment and reported preserved MMSE relative to control. Against this, the European Food Safety Authority's panel reviewed the citicoline memory claim in 2024 and did not find a cause-and-effect relationship established. Citicoline is thus a compound with a plausible mechanism, a scatter of small positive trials in the wrong populations, and no adequate trial in Alzheimer's disease.

**Alpha-glycerylphosphorylcholine (alpha-GPC, choline alfoscerate).** Alpha-GPC is a deacylated phosphatidylcholine metabolite that crosses into brain and is cleaved to choline and glycerophosphate. It is a prescription cognitive agent in several countries. The ASCOMALVA trial, conducted by Amenta and colleagues, randomised patients with Alzheimer's disease and neuroimaging-documented ischaemic brain damage to donepezil alone or donepezil plus choline alphoscerate, and reported after two years that the combination significantly slowed decline across cognitive, functional and behavioural measures, with subsequent volumetric analyses reporting attenuated loss in some cognitive areas. This is the most positive clinical dataset in the entire choline literature. It is also a single-centre trial that completed 113 of a planned 210 patients, in a

population selected for cerebrovascular injury, with an active comparator design and no placebo-only arm. It should be read as a hypothesis-generating result, not a demonstration.

The alpha-GPC record also contains the field's most substantial safety signal, and Chapter 17 treats it in full.

**The multi-nutrient turn.** The most intellectually serious attempt to test the membrane rationale came from Wurtman's own laboratory, and it is the reason the argument of this paper must be stated carefully rather than sweepingly. Recognising that phosphatidylcholine synthesis requires three substrates — a head group, a pyrimidine to activate it, and an acyl chain to esterify the glycerol backbone — Wurtman's group designed a combination supplying all three: choline, uridine monophosphate, and long-chain omega-3 fatty acids, with cofactors. This became Fortasyn Connect, delivered as the medical food Souvenaid, providing per daily dose approximately 400 mg choline, 625 mg uridine monophosphate, 1,200 mg DHA and 300 mg EPA, together with phospholipids, selenium, and vitamins B6, B12, C, E and folate.

The LipiDiDiet trial tested this combination in the population where it had the best chance: 311 people with prodromal Alzheimer's disease, randomised to 125 mL daily of the active drink or a control drink for 24 months. The primary endpoint, a neuropsychological test battery composite, was not met — control −0.108, active −0.028, difference 0.098 (95% CI −0.041 to 0.237; $p = 0.166$) — in a cohort that declined far less than the trial had been powered to expect. Two secondary endpoints did separate: the Clinical Dementia Rating Sum of Boxes worsened significantly less in the active arm ($p = 0.005$), and hippocampal volume loss was 26 per cent smaller ($p = 0.005$); whole-brain volume did not differ. Extension to 36 months found the clinical and atrophy differences maintained and in some measures larger with longer exposure.

This is the correct place to record what LipiDiDiet does and does not establish, because it is simultaneously the strongest support for the membrane rationale in humans and the reason the central question of this paper remains open.

It establishes that supplying Kennedy-pathway substrates together, early, for years, produces measurable effects on hippocampal atrophy and clinical staging in prodromal Alzheimer's disease, while failing a cognitive composite in an under-declining cohort. That is a more interesting result than the field's summary of it as "negative."

What it does not establish is anything about genotype. Sixty-three per cent of the control arm and sixty per cent of the active arm carried at least one ε4 allele — the highest APOE4 fraction of any nutritional trial in this disease, and a direct consequence of enrolling biomarker-defined prodromal cases. The trial had, in other words, roughly 190 APOE4 carriers randomised to a Kennedy-pathway intervention for two to three years, with cognitive, functional and volumetric endpoints. The genotype was measured. It was reported as a baseline characteristic. It was not

examined as an effect modifier in the published analyses.

That is the single most consequential omission in this literature, and it is not a hypothetical experiment: the data exist.

# Part III — The Membrane Reading

# 9. The Membrane Defect

While the lecithin trials were failing, a different line of work was establishing that Alzheimer's disease brain has a phospholipid abnormality — and, more specifically, an abnormality with the exact signature that excess membrane catabolism would produce.

The definitive early statement is Nitsch, Blusztajn, Wurtman and colleagues in 1992, in a paper whose title is a claim: *Evidence for a membrane defect in Alzheimer disease brain*. They measured the major membrane phospholipids and their metabolites in three cortical areas from post-mortem Alzheimer and matched control brains. Phosphatidylcholine and phosphatidylethanolamine were significantly decreased. The initial precursors choline and ethanolamine were significantly decreased. And the deacylation product glycerophosphocholine was increased, such that the ratio of glycerophosphocholine to choline — and of glycerophosphoethanolamine to ethanolamine — was significantly raised in every region examined. The authors argued that this pattern was not an epiphenomenon of neurodegeneration and might be specific to the disease's pathomechanism.

Read as a flux statement rather than a list, the pattern is coherent and pointed. The membrane lipid is down. The building block is down. The breakdown product is up. Whatever is happening to phosphatidylcholine in this tissue, it is being consumed faster than it is being replaced, and the pool of free choline available to replace it is itself depleted. It is precisely the biochemical picture a tissue would present if it were doing what Wurtman said cholinergic neurons do under duress — except that it is present across cortical regions, not confined to cholinergic terminals.

The finding has held up in outline and has been extended into the living. Dorninger and colleagues, working in the Vienna Transdanube Aging cohort, measured plasma choline-containing phospholipids across conversion to probable Alzheimer's disease and compared the changes with those of normal ageing, reporting that the disease-associated alterations resemble an accelerated version of the ageing trajectory rather than a qualitatively distinct one. That framing matters for a preventive argument: if the disease signature is an acceleration of a normal process rather than a separate lesion, then an intervention acting on the normal process has a rationale for being given before the disease declares itself, and little rationale for being given after.

The lipidomic literature that followed must be handled with more care than it usually receives, and this paper will not lean on it. Mapstone and colleagues reported in 2014 a panel of ten plasma phospholipids that predicted phenoconversion to amnestic mild cognitive impairment or Alzheimer's disease within two to three years with better than ninety per cent accuracy. The result was widely publicised and, on independent attempt at replication in other cohorts, was not reproduced. That episode should be treated as a standing warning about this specific analytical

domain: plasma phospholipid measurements are sensitive to fasting state, sample handling, extraction chemistry and platform, and the field's history of non-replicating lipid biomarkers is long. Where this paper cites lipidomic evidence, it cites direction of change in tissue, not discriminative performance in plasma.

Two further caveats belong here rather than later.

Post-mortem phospholipid measurements are subject to agonal and post-mortem-interval artefacts, and phospholipid deacylation is precisely the sort of process that continues after death. Nitsch and colleagues addressed this with matched controls, but matching does not eliminate the concern that the terminal illness differed systematically between groups.

And the direction of causation is not established by any of this. A brain losing synapses will lose phosphatidylcholine because synaptic membrane is phosphatidylcholine. The observation that membrane lipid is depleted in a disease of synapse loss is, on its own, entirely compatible with the lipid change being a consequence. What makes the finding more than a tautology is the accompanying depletion of the *precursor* pool and the elevation of the *catabolite* — a pattern of accelerated turnover rather than simple tissue loss. That is suggestive. It is not proof, and Chapter 18 grades it accordingly.

# 10. The Kennedy Pathway as the Real Target, and What It Actually Needs

If the therapeutic target is membrane rather than transmitter, then the object of intervention is the Kennedy pathway, and the first question is what that pathway is short of.

Phosphatidylcholine synthesis by this route consumes three distinct inputs, and a shortfall in any of them limits the product.

**A head group.** Choline, phosphorylated by choline kinase. This is the input that dietary choline supplies, and the input whose transport into brain is, as Chapter 3 established, unsaturated at physiological plasma concentrations.

**An activating nucleotide.** Cytidine triphosphate, consumed by CTP:phosphocholine cytidylyltransferase, the pathway's rate-limiting enzyme. In humans the relevant circulating precursor is uridine, which is why uridine monophosphate appears in Fortasyn Connect and why citicoline delivers a pyrimidine alongside its choline.

**An acyl-bearing backbone.** Diacylglycerol, whose fatty acid composition determines the biophysical character of the phosphatidylcholine produced. A membrane assembled from saturated diacylglycerol is a different material — stiffer, less permissive of the curvature synaptic vesicles and dendritic spines require — than one assembled with a polyunsaturated chain at the *sn*-2 position.

The third input is where the argument acquires its bite, because the brain cannot make its most important acyl chain and must import it.

Docosahexaenoic acid is the dominant polyunsaturated fatty acid of neuronal membranes and is not synthesised in appreciable quantity by brain tissue. Nguyen and colleagues identified the transporter that admits it: Mfsd2a, expressed exclusively in the endothelium of blood–brain barrier microvessels, which transports DHA *in the form of lysophosphatidylcholine* and not as unesterified fatty acid, sodium-dependently, with specificity for LPCs carrying acyl chains of fourteen carbons or more. Mfsd2a-null mice show markedly reduced brain DHA with neuronal loss in hippocampus and cerebellum.

The structural fact embedded in that sentence deserves to be said slowly. **The brain's principal route of DHA acquisition is a choline-containing lipid.** Lysophosphatidylcholine is phosphatidylcholine that has lost one acyl chain; it retains the phosphocholine head group, and that head group is part of what the transporter recognises. The

head group and the acyl chain are not two independent nutritional variables that happen to converge inside the cell. They arrive at the barrier attached to one another.

This observation motivated an obvious therapeutic idea, and the fate of that idea is instructive about how much weight the observation will bear.

Sugasini, Subbaiah and colleagues reported that oral DHA given to adult mice as lysophosphatidylcholine for thirty days more than doubled brain DHA, whereas an equal amount of free DHA did not appreciably raise it, and that the LPC-DHA group showed markedly improved spatial learning and memory where free DHA showed none. A follow-up comparing carriers at matched dose concluded that LPC was more efficient than either phosphatidylcholine or triacylglycerol. Read together with the Mfsd2a work, this looked like a solved delivery problem: the reason fish oil does not raise brain DHA is that fish oil delivers the wrong molecular form.

Two subsequent results complicate that conclusion substantially, and both must be recorded here rather than buried.

The first is genotype-specific and directly relevant. Supplying LPC-bound omega-3 fatty acids to mice carrying human *APOE3* or *APOE4* for two or four months increased eicosapentaenoic acid in the frontal cortex but did *not* increase docosahexaenoic acid, in either genotype.

The second is a formal replication attempt. Klievik, Bazinet and colleagues at Toronto administered non-esterified DHA, *sn*-1 LPC-DHA and di-DHA phosphatidylcholine to mice over thirty days and found no significant difference in brain DHA between any of the phospholipid carriers and control. Their paper is titled, without ambiguity, *Dietary phospholipid carriers of DHA do not increase brain DHA levels*.

The honest summary is therefore this. The *transport biology* — that Mfsd2a is the barrier's DHA route and that it recognises the lysophosphatidylcholine form — is solid and structurally corroborated. The *pharmacological corollary* — that feeding LPC-DHA is a superior way to raise brain DHA — is contested and has failed at least one careful replication and one genotype-relevant test. This paper therefore uses the transport biology to argue that head group and acyl chain are mechanistically coupled, and does not use the delivery claim to argue that a particular supplement form is superior. Any reader tempted to convert the Mfsd2a story into a purchasing decision should read Klievik's title again.

# 11. What APOE4 Does to Lipid

The apolipoprotein E4 allele has been understood as an amyloid risk factor for so long that its lipid biology is often treated as a side channel. The evidence of the last five years suggests the opposite ordering: APOE4 is a lipid-handling variant whose amyloid consequences are one of several downstream effects.

ApoE is the brain's principal lipid transport protein, secreted mainly by astrocytes and carrying cholesterol and phospholipid to neurons. The ε4 protein differs from ε3 by a single arginine-for-cysteine substitution at residue 112, which alters domain interaction, lipidation and receptor binding. Four independent lines of recent work converge on the conclusion that the consequence is a cell-autonomous lipid handling defect in glia.

Tcw and colleagues performed transcriptomic analysis of human iPSC-derived astrocytes, microglia, mixed cortical cultures and brain microvascular endothelial cells alongside post-mortem Alzheimer brain, and found human-specific APOE4-driven dysregulation of lipid metabolism in astrocytes and microglia. APOE4 astrocytes increased *de novo* cholesterol synthesis despite already elevated intracellular cholesterol, because that cholesterol was sequestered in lysosomes — a cell behaving as though starved of a lipid it is in fact hoarding in the wrong compartment.

Blanchard and colleagues examined oligodendrocytes and reported that APOE4 impairs myelination through cholesterol dysregulation: cholesterol accumulates within the cell rather than being deployed into myelin, myelin sheaths in post-mortem APOE4 brain are fewer and thinner, and promoting cholesterol transport with cyclodextrin improved myelination in culture and in APOE4 mice with a modest behavioural correlate.

Haney and colleagues identified a microglial state defined by the lipid-droplet-associated enzyme ACSL1, most abundant in Alzheimer patients with the APOE4/4 genotype; in iPSC-derived microglia, fibrillar amyloid-β induced ACSL1 expression, triglyceride synthesis and lipid droplet accumulation in an APOE-dependent manner, and conditioned medium from droplet-laden microglia produced tau phosphorylation and neurotoxicity, also APOE-dependently.

And Sienski, Narayan, Bonner and colleagues — the study that generates this paper's central question — showed that APOE4, but not APOE3, disrupts the cellular lipidome of human iPSC-derived astrocytes and of yeast expressing the human allele.

Read as one body of work, these results describe a consistent cellular phenotype: **lipid arrives, and is not deployed.** It accumulates as droplets, as cholesteryl ester, as intracellular

cholesterol, as triglyceride. The cell's structural lipid economy is not short of raw material; it is short of the capacity to route material into membrane. That reframing is what makes a head-group intervention conceptually plausible, because the Kennedy pathway is precisely the route by which a cell converts diacylglycerol — the immediate precursor of triacylglycerol — into membrane phospholipid instead. Where phosphocholine supply is limiting, diacylglycerol that would have become phosphatidylcholine is instead esterified to triacylglycerol and stored. Adding head groups pulls the branch point back toward membrane.

That is a mechanistic story, and it is the story Sienski's group tested.

# 12. The Choline Rescue, Read Closely

The finding at the centre of the modern claim is specific enough to be examined line by line, and it deserves that treatment because almost everything written about it in the popular press has overstated it.

Sienski, Narayan, Bonner and colleagues, working with Li-Huei Tsai's group, combined lipidomics with genome-wide genetic screens in two systems: yeast engineered to express human APOE isoforms, and human iPSC-derived astrocytes from APOE4 and APOE3 carriers. In both, the ε4 allele produced a characteristic disturbance: increased triacylglycerides, markedly greater unsaturation of the fatty acids attached to them, accumulation of intracellular lipid droplets with elevated perilipin-2, and increased cellular cholesterol. Loss-of-function screening in yeast identified regulators of lipid metabolism — *OPI1*, a sensor of phospholipid composition, and *MGA2* and *UBX2*, sensors of fatty acid saturation — and deletion of *MGA2* or *OPI1* uncoupled APOE4 expression from the growth defect without altering APOE4 protein levels, establishing that the disturbed lipid metabolism, rather than the protein's presence as such, caused the phenotype.

Then the intervention. Supplementing the growth medium with choline — choline chloride at 1 mM or choline bitartrate at 100 µg/mL in yeast, CDP-choline at 100 µM in the human astrocytes — restored the cellular lipidome toward its basal state: lipid droplet number fell to APOE3 levels, triacylglyceride accumulation reversed, the unsaturation shift normalised, and cholesterol accumulation was prevented.

This is a real result and a genotype-specific one. It is also, read carefully, considerably narrower than its reception.

**It is *in vitro*.** The authors said so explicitly: the study is limited to cell models, and expansion into animal models and APOE4 carriers is required. They did not test dietary choline in a living organism.

**The concentrations are supraphysiological.** CDP-choline at 100 µM in astrocyte medium sits roughly an order of magnitude above human plasma choline. Choline chloride at 1 mM in yeast is two orders above. A rescue at 100 µM does not establish that a rescue occurs at 15 µM, which is approximately where a well-tolerated oral dose would place a human.

**The cells were glia.** Astrocytes were the rescued cell type; microglia were examined for droplet content but not subjected to the rescue; neurons were not tested. Since neurons are the cells whose membranes carry the synapses whose loss constitutes the disease, the extrapolation from an

astrocytic lipidome rescue to a neuronal structural benefit is a long one.

**The endpoint is a lipidome, not a function.** No cognitive, synaptic or electrophysiological measure was rescued, because none was assayed. The claim established is that a lipid phenotype normalises, not that anything a patient would notice does.

**The model organism carries the usual caveat.** Half the genetic screening was in yeast, an organism with no brain, no apoE receptor system, and no cholinergic anything. Its value is in identifying the *mechanism class* — lipid-composition sensing — not in modelling the disease.

What survives all of that is still substantial, and it is worth stating in its strongest defensible form: **in a human cell type that expresses APOE, the ε4 allele produces a lipid phenotype that a phosphocholine head group corrects, and the correction is not mediated by anything cholinergic.** That is a different hypothesis from the one the twelve lecithin trials tested. It has a plausible transport route into the brain that is not saturated. It has a plausible target population defined by genotype rather than by diagnosis. And it has, as of the time of writing, exactly one registered human trial, discussed in Chapter 16, whose results have not been reported.

# Part IV — The Genotype Question

# 13. Four Reasons the Genotype Might Matter

Before examining what has been observed in APOE4 carriers, it is worth setting out what one would *expect* to observe, and how strong each expectation is. Four distinct arguments predict that choline should matter differently in an ε4 carrier. They are not equally good, and separating them prevents the common error of treating a stack of weak reasons as one strong one.

**Reason one: the head group relieves a branch-point defect that ε4 creates.** This is the Sienski argument of Chapter 12, and it is the strongest of the four because it is the only one with a direct genotype-specific experimental demonstration. APOE4 glia store lipid as droplets and triacylglyceride rather than deploying it into membrane; supplying phosphocholine head groups shifts diacylglycerol back toward phosphatidylcholine; the lipid phenotype normalises. The argument's weakness is entirely in its translation: *in vitro*, supraphysiological, glial, lipidomic endpoint.

**Reason two: ε4 carriers have a DHA delivery problem, and DHA arrives on a choline-containing carrier.** This argument was, until recently, the most attractive of the four. Yassine and colleagues found in the ADCS DHA trial that the ratio of cerebrospinal fluid to plasma phospholipid DHA differed significantly by APOE genotype, consistent with impaired delivery of DHA to the central compartment in ε4 carriers. Combined with the Mfsd2a biology of Chapter 10 — brain DHA enters as lysophosphatidylcholine — the inference is immediate: if ε4 carriers move DHA into brain less efficiently, and if the vehicle is a choline lipid, then head group supply might be the limiting term.

This argument has been substantially weakened by the trial designed to test its premise. PreventE4 randomised 365 non-demented adults aged 55 to 80 with low dietary DHA intake to 2 g/day DHA or placebo for 24 months, stratified by APOE ε4 status, with cerebrospinal fluid sampling in a subset. At six months, DHA supplementation raised the cerebrospinal fluid DHA-to-arachidonic-acid ratio relative to placebo — 0.17 against −0.02 — and the report states that this target engagement was achieved *independent of APOE ε4 status*. The honest reading is that the randomised evidence does not support a genotype-specific block on DHA entry, and that the earlier cross-sectional genotype difference in CSF-to-plasma ratio should be weighted accordingly. Reason two is therefore downgraded here from a mechanism to a possibility, and Chapter 18 grades it as such.

**Reason three: the populations coincide, through estrogen and PEMT.** Postmenopausal women have a markedly higher dietary choline requirement than premenopausal women because *PEMT* is estrogen-induced; the common variant rs12325817 raises that requirement further; and eighty per cent of postmenopausal women, against forty-four per cent of premenopausal women, develop organ dysfunction on a controlled low-choline diet. Independently, the ε4 allele confers greater Alzheimer risk in women than in men across a particular age window. The overlap is not a mechanism — nothing here links apoE protein to PEMT enzymology — but it identifies a subgroup, postmenopausal ε4-carrying women with a *PEMT* variant and low intake, in which two independent risk factors for inadequate phosphatidylcholine synthesis coincide with the highest genetic risk of the disease. For trial design this matters more than a mechanism would: it says where the effect, if any, will be largest.

It is worth noting that the one registered choline trial in APOE4 carriers enrolled only postmenopausal women among its female participants — for reproductive safety reasons rather than mechanistic ones, but with the incidental effect of selecting the population in which endogenous phosphatidylcholine synthesis is lowest.

**Reason four: one-carbon metabolism runs differently in ε4 carriers.** Choline's oxidation product betaine is a methyl donor for homocysteine remethylation, and homocysteine is both a vascular risk factor and — in rodent work — a direct promoter of amyloid aggregation. The specific human observation is discussed in the next chapter and is genuinely odd: components of the choline oxidation pathway predict *opposite* cognitive trajectories depending on genotype. Whatever mechanism explains that, it is not a mechanism in which choline is uniformly good.

Two of these four reasons are strong enough to justify a trial; one has just been weakened by a trial; and one is a coincidence of populations. That is a fair summary of the pre-clinical case, and it is a good deal more modest than the case usually made.

# 14. The Human Evidence, Genotype by Genotype

The direct human evidence bearing on choline and APOE genotype consists of three studies. Three. This is worth pausing on: a molecule proposed as a genotype-targeted intervention, discussed in these terms for five years, has three human datasets addressing the genotype question, two of them small and unreplicated and one of them null.

**The 1999 signal.** Alvarez, Mouzo, Pichel and colleagues conducted a double-blind placebo-controlled study of citicoline in APOE-genotyped patients with mild to moderate senile dementia of the Alzheimer type — thirty patients, aged 73.0 ± 8.5 years — with cognitive, electroencephalographic and cerebral perfusion endpoints. Citicoline at 1,000 mg/day was well tolerated and improved cognitive performance, cerebral blood perfusion and the bioelectrical activity pattern. And the finding that matters here: efficacy was greater in patients with milder deterioration *and in those bearing the ε4 allele*.

The reasons to hold this loosely are numerous and should be stated. Thirty patients across arms and genotypes means the ε4 subgroup comparison rests on a handful of individuals. The study was published in a specialist pharmacology journal by a group with commercial involvement in the compound class. It has never, in more than a quarter of a century, been replicated. And a subgroup finding in a small trial with multiple endpoints is the archetypal false positive.

The reason not to discard it is that it points in the same direction as the cell biology discovered twenty-two years later, by investigators who had no apparent knowledge of it, using a method with no relationship to it. That is not evidence. It is a coincidence worth a properly powered test.

**The 2020 interaction.** Hildre, Solvang, Aarsland and colleagues followed 152 patients with mild dementia — 86 Alzheimer's disease, 66 Lewy body dementia, 90 carrying at least one ε4 allele — for five years with annual Mini-Mental State Examinations, and measured baseline serum components of one-carbon and choline oxidation metabolism: homocysteine, methionine, choline, betaine, dimethylglycine, sarcosine, folate, cobalamin and pyridoxal 5'-phosphate.

The result is the strangest datum in this literature. Serum betaine, dimethylglycine and sarcosine were associated with *slower* cognitive decline in patients carrying ε4 and with *faster* decline in patients without it, with all three three-way interactions surviving multiple-comparison control. The authors concluded that components of the choline oxidation pathway carry a better cognitive prognosis in ε4 carriers and a worse one in non-carriers, and called for interventions targeted by

APOE status.

An interaction that reverses sign is a different kind of finding from an interaction that changes magnitude. A magnitude interaction is what one expects from a shared mechanism operating with different efficiency. A sign reversal implies that the same metabolite is doing different things in the two genotypes — or, more prosaically, that it is a marker of different underlying states in the two groups. Both readings are live. Neither has been pursued. The study is observational, is drawn from patients who already have dementia, and has not been replicated.

**The null.** Ylilauri and colleagues, in the Kuopio Ischaemic Heart Disease Risk Factor Study, followed 2,497 dementia-free middle-aged men and reported that those in the highest quartile of phosphatidylcholine intake had a 28 per cent lower multivariable-adjusted risk of incident dementia than the lowest, with total choline and phosphatidylcholine intakes also associated with better performance on tests of frontal and temporal function. *APOE4* was accounted for in the analysis and did not materially affect the findings.

This is the only sizeable prospective cohort that has formally examined genotype as a modifier of dietary choline, and it found none. That result is the single most important counterweight in this paper. It is a cohort of middle-aged men — which is to say, the population in which reason three above predicts the *smallest* effect — and it measures habitual dietary intake rather than supplementation, across a range that does not extend into pharmacological doses. But it is prospective, it is reasonably large, it has a hard endpoint, and it says no.

**Table 2 — The complete human evidence on choline and APOE genotype.**

| Study | Design | n | Genotype finding | Weight |
|---|---|---|---|---|
| **Alvarez et al. (1999)** | Randomised, double-blind, placebo-controlled; citicoline 1,000 mg/day; mild–moderate AD | 30 | Efficacy greater in ε4 carriers | Very low — subgroup in a small trial, never replicated |
| **Hildre/Solvang et al. (2020)** | Prospective observational, 5 years, mild dementia (AD and DLB) | 152 | Betaine, dimethylglycine, sarcosine predicted slower decline in ε4 carriers and faster decline in non-carriers; interactions survived FDR control | Low — observational, in established dementia, unreplicated |
| **Ylilauri et al. (2019)** | Prospective cohort, dementia-free men, incident dementia | 2,497 | APOE4 accounted for; no material modification of the choline association | Moderate — the only sizeable prospective test; null |
| **LipiDiDiet (2017; 36-month 2021)** | RCT, Kennedy-pathway multi-nutrient, prodromal AD | 311 (≈60–63% ε4) | Genotype measured, reported at baseline only; interaction never published | Unrealised — the data exist |
| **NCT05880849 (completed 2025)** | Phase 1, choline bitartrate 2.2 g/day, 180 days, ε4 carriers only | 15 | CSF lipid endpoints; no results reported | Pending |

The table is the honest state of the field. Two suggestive small studies, one moderate null, one trial whose relevant analysis was never run, and one trial that has completed and not reported.

# 15. The Shape of the Dose–Response, and What It Rules Out

The population evidence on choline and dementia is far larger than the genotype evidence, and it has converged over six years on a specific and somewhat inconvenient shape.

Ylilauri and colleagues, in Kuopio, followed 2,497 dementia-free men aged 42 to 60 at baseline. Highest against lowest quartile of phosphatidylcholine intake gave a 28 per cent lower adjusted risk of incident dementia. Eggs supplied 39 per cent and meat 37 per cent of dietary phosphatidylcholine in this population.

Yuan and colleagues examined 3,224 participants of the Framingham Heart Study Offspring cohort over a mean 16.1 years, recording 247 incident dementia cases of which 177 were Alzheimer's disease. The relationship with dietary choline was explicitly non-linear: low intake — at or below 219 mg/day for dementia and 215 mg/day for Alzheimer's disease — was significantly associated with incidence, while the association did not continue to improve across the upper range.

Niu and colleagues then analysed 125,594 UK Biobank participants over a median 11.8 years and reported U-shaped associations for both dementia and Alzheimer's disease. Moderate intake, approximately 333 to 354 mg/day, carried the lowest risk: hazard ratio 0.80 (95% CI 0.67–0.96) for dementia and 0.76 (0.58–1.00) for Alzheimer's disease relative to the lowest category. Both the lowest and the highest intakes carried more risk than the middle.

Karosas, Wallace, Bennett, Jacques, Chung and colleagues, working in the Rush Memory and Aging Project with annual clinical adjudication of Alzheimer's dementia, located the point of lowest risk at approximately 350 mg/day.

**Table 3 — Four cohorts, one curve.**

| Cohort | n | Follow-up | Finding | Optimum |
|---|---|---|---|---|
| **Kuopio (Ylilauri et al., 2019)** | 2,497 men | ~20+ years | Highest vs lowest phosphatidylcholine quartile: 28% lower dementia risk; better frontal/temporal test performance | Upper quartile of habitual intake |
| **Framingham Offspring (Yuan et al., 2022)** | 3,224 | 16.1 years mean | Non-linear; low intake (≤219 mg/d dementia, ≤215 mg/d AD) associated with incidence | Above ~220 mg/d |
| **UK Biobank (Niu et al., 2025)** | 125,594 | 11.8 years median | U-shaped; HR 0.80 dementia, 0.76 AD at moderate intake | ~333–354 mg/d |
| **Rush MAP (Karosas et al., 2025)** | Community-dwelling older adults | Annual adjudication | Lowest AD risk at moderate intake | ~350 mg/d |

Four cohorts on three continents, using different instruments and different endpoints, place the minimum of the curve in the range of roughly 330 to 400 mg/day. Three observations follow, and each constrains the therapeutic claim.

**The optimum sits below the Adequate Intake.** The Institute of Medicine's figures are 550 mg/day for men and 425 mg/day for women, derived, as Chapter 2 established, from a liver endpoint. The dementia-endpoint optimum is lower than both. The frequently repeated statistic that ninety per cent of the population fails to meet the Adequate Intake is therefore *not* equivalent to the claim that ninety per cent of the population is at elevated dementia risk from choline shortfall. The population at brain-relevant risk is the lower tail — the segment below roughly 220 to 250 mg/day — which, given a mean female intake of 250 to 280 mg/day, is a very large number of women and a much smaller number of men, but it is not ninety per cent of anybody.

**The curve turns back up.** A U-shape is the enemy of a supplement argument. If risk rises at the top of the observed intake range, then the intervention is correction of a deficit, not maximisation of a nutrient, and the appropriate act for someone already in the optimal band is to do nothing. Chapter 17 gives at least two candidate mechanisms for the right limb.

**These are dietary, not supplemental, ranges.** Nobody in these cohorts was taking 900 mg/day of supplemental choline. The curves describe food. Extrapolating them to a pharmacological dose is not a small step, and the one place where the distinction has been tested directly — in trimethylamine N-oxide generation — the food and the supplement behaved

differently.

A final caution about the whole class of evidence. All four studies rest on food frequency questionnaires converted to choline intake through composition databases, with the attendant measurement error, and all are subject to residual confounding by the general dietary and behavioural pattern that accompanies higher choline intake — which in these populations means eggs, meat, fish and dairy. A U-shaped association between an egg-and-meat-derived nutrient and dementia across a population is compatible with a great many stories that are not about choline.

# 16. The Half That Was Missing

The argument of this paper converges here.

Phosphatidylcholine synthesis requires a head group and an acyl chain. That is not a metaphor; it is the stoichiometry of the terminal step of the Kennedy pathway. And the two most sophisticated genotype-targeted nutritional trials ever conducted in this disease each supplied one of them.

**PreventE4** supplied the acyl chain. Two grams per day of docosahexaenoic acid, for 24 months, in 365 non-demented adults aged 55 to 80 selected for low dietary DHA intake and stratified by APOE ε4 status, at a single academic centre, with cerebrospinal fluid sampling. The trial did what a well-designed trial should do: it demonstrated that the intervention reached its target. The cerebrospinal fluid DHA-to-arachidonic-acid ratio rose in the treated arm relative to placebo at six months, independent of genotype. And then, over two years, nothing else happened. No difference in cognition. No difference in brain structure. Thirty-eight per cent attrition, largely pandemic-driven, with 225 completers. The conclusion published in 2026 is that high-dose DHA achieves central nervous system target engagement in older adults at risk of dementia and produces no cognitive or structural benefit.

Participants' choline status was not an entry criterion, was not a stratification variable, and was not reported.

**NCT05880849** supplied the head group. Choline bitartrate, 2.2 g/day — a salt that is roughly 41 per cent choline by weight, so on the order of 900 mg/day of choline, approximately twice the Adequate Intake — for 180 days, in fifteen carriers of at least one ε4 allele aged 55 to 80 with a Mini-Mental State Examination of 24 or above, normal homocysteine, and *dietary choline intake below 450 mg/day as an entry requirement*. The primary endpoints are explicitly the ones the Sienski mechanism predicts: a 15 per cent reduction in the cerebrospinal fluid fatty acid desaturation index and a 100 per cent increase in cerebrospinal fluid phosphatidylcholine at six months, with secondary measures including phospholipid species, betaine, neurofilament light, and phosphorylated tau and amyloid ratios. The trial began in June 2023, completed in October 2025, and has not posted results.

Participants' DHA status was not an entry criterion, was not a stratification variable, and is not among the reported outcome measures.

**LipiDiDiet** supplied both — and did not analyse the genotype. Four hundred milligrams of choline, 1,200 mg of DHA, 300 mg of EPA and 625 mg of uridine monophosphate daily for two to

three years in 311 people with prodromal Alzheimer's disease, of whom approximately sixty per cent carried at least one ε4 allele. The primary cognitive composite was not met in a cohort that declined far less than expected. The Clinical Dementia Rating Sum of Boxes and hippocampal atrophy both separated at $p = 0.005$, and the separation grew with duration of exposure. The genotype was typed at baseline and reported in the baseline table. No published analysis asks whether the treatment effect differed between the roughly 190 ε4 carriers and the roughly 120 non-carriers.

There is a well-established precedent for why this matters, and it comes from an adjacent nutrient pair with the same logical structure. In VITACOG, high-dose homocysteine-lowering B vitamins slowed brain atrophy and cognitive decline in mild cognitive impairment — but Jernerén and colleagues showed that the effect was conditional on omega-3 status: final scores for delayed recall, global cognition and clinical dementia rating in the B-vitamin arm improved with increasing baseline omega-3 concentration, while the placebo arm showed no such gradient, and the treatment benefit was essentially confined to those with adequate omega-3 at entry. A trial of B vitamins that had not measured omega-3 status would have reported a diluted or null effect and concluded that B vitamins do not work.

That is precisely the epistemic position of the choline literature. The claim is not that a choline-plus-DHA combination will work; LipiDiDiet is the closest test and its primary endpoint failed. The claim is narrower and, this paper argues, correct:

**No trial has ever supplied both substrates of the Kennedy pathway, at a pharmacological rather than nutritional dose of choline, to a population selected for the APOE4 genotype, with a lipid endpoint capable of showing whether the pathway responded.** PreventE4 had the genotype and the lipid endpoint and half the substrate. NCT05880849 has the genotype and the lipid endpoint and the other half. LipiDiDiet had both substrates, at a modest choline dose, in a population that was sixty per cent ε4 by accident, and did not look.

The experiment is not merely undone. It is three-quarters assembled, in three separate places, by investigators who have not combined them.

# 17. The Harms Ledger

A recommendation to take a supplement is a recommendation to accept its harms, and this molecule has real ones. They are frequently omitted from enthusiastic accounts, and they are the principal reason this paper's verdict is narrow.

**Trimethylamine N-oxide.** Gut microbiota metabolise choline and phosphatidylcholine to trimethylamine, which the liver oxidises by flavin-containing monooxygenase to trimethylamine N-oxide. Wang, Klipfell, Hazen and colleagues showed in 2011 that plasma choline, betaine and TMAO are associated with cardiovascular disease risk, that dietary supplementation of mice with choline, TMAO or betaine upregulates macrophage scavenger receptors and promotes foam cell formation, and that germ-free animals do not generate the metabolite — establishing the microbial requirement. Tang, Hazen and colleagues then showed in humans that a phosphatidylcholine challenge produces time-dependent increases in TMAO, that antibiotics suppress it, and that it returns on antibiotic withdrawal, with plasma TMAO predicting adverse cardiovascular events.

TMAO is not merely a cardiovascular concern in this context. Vogt and colleagues measured cerebrospinal fluid TMAO in 410 individuals and found it elevated in Alzheimer's dementia and in mild cognitive impairment relative to cognitively unimpaired controls, and positively correlated with phosphorylated tau, the p-tau/Aβ42 ratio, total tau and neurofilament light. Whether this is causal, reactive or confounded is unresolved. But a metabolite of the proposed therapy is elevated in the disease the therapy proposes to prevent, and correlates with its biomarkers. That is a fact requiring explicit management in any trial design, and it is the reason Chapter 21 specifies TMAO monitoring as a safety endpoint rather than an afterthought.

**Form matters, and the difference is measurable.** Wilcox and colleagues randomised participants with normal renal function across five arms delivering approximately equivalent choline doses and found that four eggs daily raised neither fasting TMAO nor platelet reactivity, while choline bitartrate tablets raised both. Phosphatidylcholine capsules did not produce a significant rise. Deuterium-labelling work comparing choline chloride, choline bitartrate, α-glycerophosphocholine and egg phosphatidylcholine has likewise found that the phospholipid forms generate less trimethylamine than the salts.

This is the most actionable single finding in the paper for anyone weighing what to do. **The supplement form used in the historical trials and in the current APOE4 trial — choline bitartrate — is the form with the largest TMAO signal, and the form present in food is the form with the smallest.**

**The alpha-GPC stroke signal.** Lee and colleagues examined the Korean National Health Insurance Service database, screening a population of over twelve million people aged fifty or older without prior stroke or Alzheimer's disease, and reported that use of α-glycerylphosphorylcholine was associated with higher ten-year incident stroke risk in a dose-dependent manner after adjustment for conventional cerebrovascular risk factors. The authors linked the finding to choline's TMAO pathway.

Two counterweights are owed. Confounding by indication is severe in this design: α-GPC is prescribed in Korea to people with cognitive complaints, and cognitive complaints are themselves a marker of cerebrovascular disease, so the exposed group is sicker in ways no administrative-database adjustment fully captures. And a subsequent nationwide Korean analysis reported an association between α-GPC use and *delayed* conversion to dementia. The α-GPC record is therefore internally contradictory, which is itself a reason for caution rather than reassurance.

**The right limb of the U.** Two large cohorts report that the highest categories of dietary choline intake carry higher dementia risk than the middle. The mechanism is unknown; TMAO is the obvious candidate, and confounding by the broader dietary pattern at the top of the intake distribution is an equally plausible one. Either way, the empirical shape of the human dose–response gives no support to the proposition that more is better, and some support to the proposition that considerably more is worse.

**Table 4 — What is known about harm, and how firmly.**

| Harm | Evidence | Firmness | Mitigation |
|---|---|---|---|
| **Raised TMAO, raised platelet reactivity** | Randomised human challenge; choline bitartrate positive, eggs and PC capsules not | Established for the salt forms | Prefer food or phospholipid forms; monitor TMAO |
| **Cardiovascular risk associated with TMAO** | Large prospective human cohorts; mechanistic mouse work | Established as association; causality debated | Renal function screening; dose restraint |
| **CSF TMAO elevated in AD and MCI, correlated with p-tau and NfL** | Cross-sectional, 410 participants | Observed; direction of causation unknown | Treat as a stopping signal if it rises on treatment |
| **Stroke association with α-GPC** | 10-year national cohort, dose-dependent | Confounded by indication; contradicted by a later cohort on a different endpoint | Avoid α-GPC as the delivery form pending clarification |
| **Higher risk at highest intake (U-shape)** | Two large cohorts | Consistent but observational | Target the epidemiological optimum, not the maximum |
| **Fishy body odour, hypotension, gastrointestinal upset** | Long-standing; dose-related | Established, non-serious | Dose titration |

# Part V — The Verdict

# 18. The Graded Ledger

Everything this paper has argued is set out below at the level of confidence the evidence supports. The grades are deliberately coarse, and the assignment of a claim to a lower grade is not a criticism of the work that produced it — much of the strongest science here is *in vitro* by necessity, because the experiments that would elevate it cannot be done in living human brain.

**Established** — supported by replicated human data, or by direct structural or kinetic measurement, and unlikely to be overturned.

- Choline is an essential nutrient; the majority of adults in surveyed Western populations consume less than the Adequate Intake, with the shortfall roughly twice as large in women.
- Human choline requirement varies with sex, menopausal status and *PEMT* genotype, by factors large enough to exceed the difference between the sexes' recommended intakes.
- Choline crosses the blood–brain barrier by saturable transport, principally via FLVCR2, with a Michaelis constant an order of magnitude above fasting plasma concentration.
- The presynaptic high-affinity choline transporter has a Michaelis constant near 2 μM and is therefore close to saturation at physiological plasma choline.
- Lecithin does not improve cognition or global impairment in established dementia.
- Alzheimer's disease cortex shows reduced phosphatidylcholine and phosphatidylethanolamine, reduced free choline and ethanolamine, and elevated glycerophosphocholine.
- APOE4 disrupts lipid handling in human glia — cholesterol sequestration in astrocytes, impaired deployment into myelin in oligodendrocytes, lipid droplet accumulation in microglia.
- Brain docosahexaenoic acid is imported across the barrier by Mfsd2a in the lysophosphatidylcholine form, not as free fatty acid.
- Supplemental choline bitartrate raises plasma trimethylamine N-oxide and platelet reactivity; equivalent choline from whole eggs does not.
- Dietary choline shows a non-linear, U-shaped association with dementia risk across large cohorts, with the minimum near 330–400 mg/day.
- High-dose DHA achieves central target engagement in older adults at risk of dementia and produces no cognitive or structural benefit over two years.

**Probable** — supported by consistent evidence of moderate quality, or by a single well-conducted study without replication.

- The failure of the historical precursor-loading trials is explained by transporter saturation rather than by the molecule, the dose, or the disease stage alone.
- APOE4 produces a lipidome disturbance in human iPSC-derived astrocytes that phosphocholine head-group supply corrects.
- Supplying Kennedy-pathway substrates together, for years, in prodromal disease, slows hippocampal atrophy and clinical staging while failing a cognitive composite.
- The population at greatest risk of functional choline insufficiency — postmenopausal women, particularly *PEMT* variant carriers with low intake — overlaps the population at greatest APOE4-attributable risk.
- Phospholipid forms of choline generate less trimethylamine than salt forms.

**Inference** — reasoned from established facts, but not directly demonstrated.

- The unsaturated barrier route makes membrane phosphatidylcholine synthesis, rather than acetylcholine synthesis, the only plausible target of oral choline in the human brain.
- The coupling of head group and acyl chain at the barrier, and again at the terminal step of the Kennedy pathway, means that supplying one without measuring the other produces an uninterpretable trial.
- An intervention acting on a process that the Vienna data describe as accelerated normal ageing should be given before symptoms, and has little rationale after them.
- The right limb of the U-shaped curve reflects a real harm rather than confounding alone.

**Speculation** — plausible, unsupported, and flagged as such.

- That an APOE4 carrier with adequate intake obtains any benefit from additional choline.
- That the genotype-specific sign reversal in the choline oxidation pathway reflects a mechanism rather than differential confounding.
- That the 1999 citicoline subgroup finding is real.
- That a pharmacological dose of choline would rescue in a living human brain the phenotype that 100 μM CDP-choline rescues in cultured astrocytes.

The distribution of claims across these four grades is the answer to the paper's question, before the answer is stated in words. The biology is well established. The genotype-specific cell biology is

probable. The therapeutic proposition is inference and speculation.

# 19. The Verdict

The question was whether choline is a viable potential therapeutic for APOE4 carriers. The answer has to be given in parts, because the word "therapeutic" is doing different work in each.

**For established Alzheimer's disease, in any genotype: no.** This is the firmest negative in the paper. Twelve randomised trials, a Cochrane review reporting no cognitive or global benefit and a relative deterioration in activities of daily living, and a transport-kinetic explanation for why the rationale could not have worked. An APOE4 carrier with a diagnosis should not expect choline to help, and no reading of the modern lipid literature changes that. The one dataset pointing the other way — thirty patients in 1999 — is not a foundation.

**As a licensed drug for APOE4 carriers: no, and not soon.** There is no phase 2 efficacy trial. The single registered genotype-targeted trial enrolled fifteen people, was designed to measure cerebrospinal fluid lipid chemistry rather than cognition, and has not reported. Nothing in this literature supports a claim of clinical efficacy, and anyone marketing choline to ε4 carriers on the strength of the cell-culture rescue is selling ahead of the evidence by a distance that should be stated plainly.

**As a hypothesis worth a properly designed trial: yes, and it is among the better-founded untested hypotheses in the preventive literature.** It has a genotype-specific cellular phenotype with a genotype-specific rescue; a transport route with real headroom; a mechanistically coherent reason for the historical failures; a co-substrate whose omission explains why the closest existing trials were uninterpretable; a target population defined in advance; and endpoints that can be measured in cerebrospinal fluid before any cognitive endpoint is attempted. That is a better-specified proposition than most of what enters phase 2 in this field.

**As a matter of what an individual APOE4 carrier should do this year — the question that prompted this paper — the answer is stratified by intake and it is modest.**

If habitual choline intake is low — below roughly 250 mg/day, which describes a substantial fraction of women eating a Western diet, and a larger fraction of those avoiding eggs, meat, fish and dairy — then raising it toward the 350 to 425 mg/day range is defensible. It corrects a documented population-level shortfall in an essential nutrient; it targets the intake band that four independent cohorts associate with lowest dementia risk; it carries a genotype-specific mechanistic rationale that, while unproven in humans, is not fanciful; and at that magnitude it is a dietary correction rather than a pharmacological intervention. The evidence favours achieving it through food rather than

supplements, because the randomised human data show that eggs do not raise trimethylamine N-oxide or platelet reactivity while choline bitartrate does. Two eggs supply roughly 300 mg of choline.

If habitual intake is already in the 350 to 450 mg/day range, there is no human evidence that more helps, two large cohorts suggesting that considerably more is associated with harm, a documented rise in a metabolite that is itself elevated in the disease, and a completed but unreported trial. The honest answer for this person is that nobody knows, and that the default in the absence of knowledge is not to intervene.

If the intention is to take a gram or more of supplemental choline daily on the strength of the APOE4 cell-culture result, that is a decision to be the first cohort in an untested pharmacological experiment, with a known and quantified adverse metabolic signal, no efficacy data, and no monitoring. It should be described that way rather than as nutrition.

**A last framing point, which is the paper's real conclusion.** Choline is not plausibly a *treatment* for Alzheimer's disease and probably never will be. What the evidence supports — weakly, but coherently — is that choline sufficiency is one term in the resilience of a brain that is going to be stressed, and that the term may be weighted differently in a carrier of ε4 than in a non-carrier. Sufficiency is a preventive concept with a decades-long time horizon, and it is answered by diet. The field's forty-year mistake was to take a nutrient whose relevance is measured in decades and test it as a drug over twelve weeks, in people who had already lost the cells it was meant to serve, through a transporter that was already full.

# 20. Five Conditions of Refutation

The argument above is falsifiable, and these are the results that would falsify it. They are stated in advance and without hedging.

**One.** If the completed phase 1 trial in APOE4 carriers reports that 2.2 g/day of choline bitartrate for six months produced no increase in cerebrospinal fluid phosphatidylcholine and no reduction in the fatty acid desaturation index, then the central mechanistic bridge of this paper — that oral choline reaches the human brain in quantities sufficient to shift the Kennedy pathway — fails at its first human test, and the entire membrane rationale reverts to *in vitro* speculation.

**Two.** If human blood–brain barrier choline transport is characterised and found to have a Michaelis constant near or below plasma concentration — that is, if FLVCR2 in humans is saturated where the rodent transporter is not — then the kinetic asymmetry that explains the historical failures and licenses the modern hope is an artefact of species, and both halves of the argument collapse together.

**Three.** If a genotype-stratified reanalysis of LipiDiDiet shows that the treatment effect on hippocampal atrophy and clinical staging was equal in ε4 carriers and non-carriers, or larger in non-carriers, then the genotype-targeting premise loses its only available human test in a randomised setting, and the case for selecting trial populations by *APOE* rather than by intake is substantially weakened.

**Four.** If a properly powered prospective cohort with biomarker-confirmed disease finds no interaction between choline intake and *APOE* genotype — replicating and extending the Kuopio null in women and in older participants — then the two small studies suggesting an interaction should be treated as noise, and the correct framing becomes that choline sufficiency matters equally to everyone, which would make this paper's genotype emphasis wrong even if its nutritional conclusion survived.

**Five.** If trimethylamine N-oxide rises materially in the cerebrospinal fluid of supplemented participants, the risk–benefit calculation inverts regardless of any lipid finding, because the intervention would then be raising, inside the central compartment, a metabolite that is elevated in the disease and correlated with its tau and neurodegeneration markers.

# 21. The Experiments

Seven experiments would resolve most of what this paper has had to leave open. They are ordered by cost, and the first is free.

**One — reanalyse LipiDiDiet by genotype.** Approximately 190 ε4 carriers and 120 non-carriers were randomised to a Kennedy-pathway multi-nutrient or control for two to three years, with cognitive, functional and volumetric endpoints, and the genotype was typed at baseline. The interaction analysis requires no new participants, no new funding and no new measurement. It is the single highest-value unperformed analysis in this literature, and until it is published, every claim in this field — including this paper's — is arguing about data that already exist.

**Two — report NCT05880849.** Fifteen ε4 carriers, six months of choline bitartrate, cerebrospinal fluid phosphatidylcholine and desaturation index. Whatever it shows, it is the first human test of the mechanism, and it completed in October 2025.

**Three — the factorial trial.** A 2 × 2 randomisation of choline and DHA in cognitively unimpaired APOE4 carriers aged 55 to 75, entry restricted to habitual choline intake below 300 mg/day and habitual DHA intake below 200 mg/day — that is, to people with headroom in both substrates. Stratify on sex and menopausal status and on *PEMT* rs12325817. Deliver choline as phosphatidylcholine or as citicoline rather than as bitartrate, on the trimethylamine evidence. Primary endpoint: cerebrospinal fluid phosphatidylcholine and fatty acid desaturation index at six months — target engagement first, because a trial that cannot show the pathway moved cannot interpret a cognitive null. Secondary: hippocampal volume and Clinical Dementia Rating Sum of Boxes at 24 to 36 months, on the LipiDiDiet precedent that these are the endpoints that separate. Safety: plasma and cerebrospinal fluid trimethylamine N-oxide at every visit, with predefined stopping rules. The factorial design is the point: it is the only design that can distinguish "choline works," "DHA works," "neither works," and "each requires the other," and the last of these is the hypothesis the existing trials were structurally unable to test.

**Four — the *in vivo* rescue.** The Sienski experiment has never been done in a living animal. Humanised *APOE4* targeted-replacement mice, dietary choline at defined levels, brain lipidomics with lipid droplet quantification by cell type. The question is whether a dietary intake achievable in humans reproduces a rescue demonstrated at 100 μM in culture, and it is answerable within a year.

**Five — measure the substrate ratio in the disease.** Every human study in this field measures choline or DHA. None measures the ratio in which they arrive, or the concentration of lysophosphatidylcholine-DHA in plasma and cerebrospinal fluid by genotype. If the head group and

the acyl chain are coupled at the barrier, then LPC-DHA flux — not either component alone — is the quantity that should track disease and genotype, and it has never been the primary variable in an *APOE*-stratified human study.

**Six — resolve the sign reversal.** Betaine, dimethylglycine and sarcosine predicted opposite cognitive trajectories in ε4 carriers and non-carriers in 152 patients. Either this is a genuine genotype-dependent metabolic interaction, in which case it is one of the more important unexplained findings in Alzheimer nutrition, or it is a small-sample artefact. Replication in an existing large cohort with stored serum and *APOE* genotype requires assays, not recruitment.

**Seven — characterise the human gate.** Human blood–brain barrier choline transport kinetics, by positron emission tomography with a labelled choline analogue or by arteriovenous difference in a suitable clinical setting, in ε4 carriers and non-carriers. The entire pharmacological argument of Part I rests on a rodent Michaelis constant and a human structure. One of those two is doing work it was not designed for, and the measurement that would replace it is feasible.

The through-line of all seven is a single observation about how this field has proceeded. The molecule was tested for forty years without anyone measuring whether it reached the compartment it was supposed to act in, or whether the reaction it was supposed to drive had its other substrate available. The experiments above are not exotic. They are the ones that should have come first.

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