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THE IMPACT OF MACRONUTRITIONAL COMPOSITION AND KETOSIS ON COGNITIVE HEALTH: FROM NORMAL AGING TO ALZHEIMER'S DISEASE

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The Impact of Macronutritional Composition and Ketosis on Cognitive Health: From Normal Aging to Alzheimer's Disease

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Abstract

Ketogenic diets (KD) are increasingly investigated for the prevention of cognitive decline and Alzheimer's disease (AD). Without explicitly investigating a KD, this thesis disentangles two of its hallmarks: a reduced dietary carbohydrate/fat-ratio (CFr) and the metabolic state ketosis. Whether health effects from KD are primarily driven by ketosis or from other pathways related macronutritional changes, is not fully understood. Beyond CFr, KD may optionally be modified regarding protein, fat-subtypes, plant/animal-based food proportions, the timing of nutrient intake, and ketogenic supplements.

Strategies to induce ketosis in the absence of a carbohydrate restricted diet (Study I) and subsequent associations between induced ketosis and a biomarker essential for brain function (Study II) was investigated in a randomized clinical trial planned and performed within this doctoral project: In a 6-arm cross-over design, 15 healthy older adults (age 65-73, following their usual diet) were exposed to intake of oils with various composition of medium-chain triglycerides (MCT), with and without glucose. Blood levels of ketones (β-hydroxybutyrate, BHB) and brain-derived neurotrophic factor (BDNF) were thereafter monitored for 4 hours. Mature BDNF (mBDNF) and its precursor proBDNF are essential for brain plasticity, and their concentrations in serum have been associated with cognitive health. A methods comparison for measuring blood ketones (Study III) supports the internal validity of Study I and II.

The impact of self-reported CFr—in the non-ketogenic range—on cognitive performance (Study IV/V) was investigated by panel analyses on data (year 0, 1, and 2) from the Finnish Geriatric Intervention Study to Prevent Cognitive Impairment and Disability (FINGER). The sample (n=1259, age 60–77, 47% females) had no substantial cognitive impairment but had risk factors for developing dementia, and cognition at mean level or slightly lower than expected in screening test. Study V added stratified analyses based on genetics (*APOE*) and insulin status.

Study I: A 16-hour non-carbohydrate window and intake of 20 g caprylic acid (C8) contributed roughly equally to induce transient ketosis (0.45 mmol/L, AUC/time venous BHB hour 0-4, when combining the strategies). Coconut oil (which has a ≈7% fraction constituted by C8 and is dominated by lauric acid) did not share the ketogenic properties of purified C8 (difference –0.22 mmol/L, $p<0.001$).

Study II: Contrary to our expectations, change in mBDNF was lower (z-score: $\beta=-0.88$, $p<0.001$) after intake of C8 (higher ketosis) compared to sunflower oil (lower ketosis). Since associations between BHB and mBDNF appeared unrelated ($p=0.43$) on the individual level, alternative explanations to ketosis as a driver were discussed. In contrast, proBDNF increased more ($\beta=0.25$, $p=0.007$) after intake of C8 compared to

sunflower oil, and individual associations between BHB and proBDNF (β=0.40, p=0.006) supported ketosis as a mechanistic link.

Study III: A handheld ketone meter correlated well with the laboratory method (*r*=0.91) and agreement was high when applied to venous whole blood (which was our primary outcome). However, absolute values were systematically higher in capillary blood, which should be considered in comparisons between studies.

Study IV: A lower CFr (log, z-score) estimated a higher composite z-score on a Neuropsychological Test Battery (β=−0.022, p=0.011) in linear mixed regression. Methodological advantages of analyzing intake of carbohydrates and fat as a ratio compared to single variables were discussed. No significant associations were found for protein, and the saturated/total fat ratio had non-linear associations with cognitive performance.

Study V: *APOE* (ε-2/3/4), which is the most important AD risk gene, modified estimates between diet parameters (CFr, protein, saturated/total fat ratio, fiber, composite score) and cognitive performance in a sub-sample with insulin data, excluding diabetics (n=676). By increasing values of a continuous APOE-gradient [−1 (ε-23), −0.5 (ε-24), 0 (ε-33), 1 (ε-34), 2 (ε-44)], a less favorable estimate (p<0.0001 for interaction) was found for a *Higher-carbohydrates-fiber-Lower-fat-protein* composite score. Estimates for ε-33 were relatively close to zero whereas ε-44 (with some ambiguity for females) typically had an antagonistic estimate to ε-23. Relative hypo- and hyper-insulinemia significantly magnified several estimates *diet➔cognition* in a dose dependent manner, primarily among ε-34/44. The plant/animal-based proportion of macronutrients was discussed as a potential unmeasured confounder.

Conclusions: *Macronutritional changes* may be an alternative explanation to *ketosis* for what may drive potential cognitive effects from KD. Time-restricted carbohydrate intake may be considered as an alternative, or a complement, to C8-enriched MCT-oils for achieving mild ketosis. Signaling functions of ketones may be at work in transient mild/moderate ketosis, but whether our BDNF results have any cognitive implications requires further studies. To guide further research, our *diet➔cognition* analyses have strengthened the case for: (1) a precision nutrition approach based on APOE-genotype and insulin status; (2) not limiting interventions on carbohydrate restriction to the ketogenic range of CFr; (3) considering both ends of the insulin spectrum as representing distinct at-risk types susceptible to diet modifications. APOE-34/44 carriers may be optimal targets for studying potential benefits on brain health from CFr-reduction, and higher protein intake. The concept of universal macronutrient targets may be questioned, and stratified analyses may be encouraged in further studies.

Keywords: macronutrients, gene-diet interaction, apolipoprotein E, dementia, neurocognitive disorder, global cognition, RCT, nutritional epidemiology

List of scientific papers

I. **Ketosis After Intake of Coconut Oil and Caprylic Acid—With and Without Glucose: A Cross-Over Study in Healthy Older Adults**.
Norgren J, Sindi S, Sandebring-Matton A, Kåreholt I, Daniilidou M, Akenine U, Nordin K, Rosenborg S, Ngandu T, Kivipelto M
Frontiers in Nutrition 2020;7:40.

II. **Serum proBDNF Is Associated With Changes in the Ketone Body β-Hydroxybutyrate and Shows Superior Repeatability Over Mature BDNF: Secondary Outcomes From a Cross-Over Trial in Healthy Older Adults.**
Norgren J, Daniilidou M*, Kåreholt I, Sindi S, Akenine U, Nordin K, Rosenborg S, Ngandu T, Kivipelto M, Sandebring-Matton A; * shared 1st author
Frontiers in Aging Neuroscience. 2021;13.

III. **Capillary Blood Tests May Overestimate Ketosis: Triangulation Between Three Different Measures of β-Hydroxybutyrate.**
Norgren J, Sindi S, Sandebring-Matton A, Kåreholt I, Akenine U, Nordin K, Rosenborg S, Ngandu T, Kivipelto M
American Journal of Physiology Endocrinology and Metabolism. 2020;318(2):E184-E8

IV. **The Dietary Carbohydrate/Fat-ratio and Cognitive Performance: Panel Analyses in Older Adults at Risk for Dementia**
Norgren J, Sindi S, Sandebring-Matton A, Ngandu T, Kivipelto M, Kåreholt I
Manuscript—accepted for publication in "Current Developments for Nutrition", May 3, 2023

V. **APOE-genotype and Insulin Modulate Estimates Between Dietary Macronutrients and Cognitive Performance: Panel Analyses in Non-Diabetic Older Adults at Risk for Dementia**
Norgren J, Sindi S, Sandebring-Matton A, Kivipelto M, Kåreholt I
Manuscript—ready for submission

Contents

List of abbreviations

AD	Alzheimer's disease
AcAc	Acetoacetate (ketone)
ApoA/B/E	Apolipoprotein A/B/E (protein)
APOE	Apolipoprotein E (gene)
APOE+/–	ε-34, 44 dichotomized versus ε-23, 24, 33
BDNF	Brain-derived neurotropic factor (m/pro: mature/precursor)
BHB	β-hydroxybutyrate (ketone)
C8	Caprylic acid
C12	Lauric acid
CI	Confidence interval
CFr	Carbohydrate/fat ratio
DASH	Dietary approaches to stop hypertension
DGA	Dietary guidelines for Americans
e (prefix)	Nutrient by E% (eCarb: carbohydrates, eFat: total fat, eProt: protein, eFib: fiber, eAlc: alcohol
E%	Percentage of total energy intake
HDL	High-density lipoprotein
HOMA-IR	Homeostatic assessment of insulin resistance
HOMA-BCF	Homeostatic assessment of beta-cell function
ICC	Intra-class correlation coefficient
kMCT	Ketogenic medium-chain triglycerides
LDL	Low-density lipoprotein
MCI	Mild cognitive impairment
MCT/MCFA	Medium-chain triglycerides/fatty acids
MeDi	Mediterranean diet
MUFA	Mono-unsaturated fatty acids
NNR	Nordic Nutrition Recommendations
PUFA	Poly-unsaturated fatty acids
RCT	Randomized controlled trial

SAFr	Ratio saturated/total fat
SCFA	Short-chain fatty acids
SD	Standard deviation
SFA	Saturated fatty acids
TE	Total energy intake
TRC	Time-restricted carbohydrate intake
WHO	World Health Organization

1 Introduction

This introduction will include definitions of concepts, and a broader overview of relevant theory. After that, a literature review will give a more focused background to the included studies, and thereafter the overall research aims will be stated.

1.1 Parameters of a Ketogenic Diet

Ketogenic diets (KD) are investigated in relation to cognitive health (1). Without explicitly studying a KD, this thesis focuses on two of its hallmarks: *modification of macronutritional composition* and the metabolic state *ketosis*, while briefly covering other aspects of diet.

Introduced as a treatment for epilepsy in 1921, KD with various modifications is currently applied spontaneously in the population and investigated for conditions including obesity, diabetes, neurodegenerative diseases, depression, bipolar disorder, and cancer (2-6). The primary macronutritional difference between a ketogenic diet and most peoples' habitual diet is a lower *carbohydrate/fat-ratio* (CFr) (6). Other parameters that may be modified—but not necessarily—are the level of *protein* intake, the ratio *saturated/total fat* (SAFr), and the ratio of plant/animal-based food sources (3, 7, 8).

Ketosis is a metabolic state characterized by elevated blood levels of the ketone bodies ("ketones") β-hydroxybutyrate (BHB) and acetoacetate (AcAc), which are mainly produced by the liver (9). A reversible redox reaction determines the ratio between BHB and AcAc, and BHB becomes dominating as ketosis increase (10). Nutritional factors like fasting and carbohydrate restriction are the main drivers of ketosis (9). However, it is also possible to achieve ketosis independent of dietary habits, either by intake of ketogenic medium-chain triglycerides (kMCT) which stimulate endogenous ketogenesis (11, 12), or by supplementation with exogenous ketones (13). Ketosis should not be confused with the pathological state ketoacidosis (9).

Importantly, ketosis does not increase by a linear gradient when carbohydrate intake is reduced but is practically absent until intake falls below a threshold at 20-50 g/day (14). While strictly defined ratios between macronutrients constitute one way of defining a diet as "ketogenic" (15), a more pragmatic definition may be any diet that induces sustained nutritional ketosis, conventionally defined as 0.5–3 mmol/L BHB in blood (16). Underlying mechanisms of ketogenic diets include effects on glucose metabolism, lipid metabolism, inflammation, gut microbiota, and endocrine functions (17). Although certain mechanisms related to brain health, e.g., improved brain energetics (1) and epigenetic signaling (18), are directly related to ketosis, the relative importance of such pathways in relation to mechanisms that might be at work even in the non-ketogenic range of macronutrient changes are not fully understood. In fact, already in 1931—looking back on the first decade of KD treatment for children with epilepsy, it was noticed that the

treatment effect often remained when patients resumed to a more liberal low-carbohydrate-high-fat diet (LCHF) after 9-12 months on a strict KD (2).

Knowledge on the relative importance of ketosis versus moderate macronutrient changes is important to further develop optimal dementia risk reduction strategies and for the promotion of cognitive health. If ketosis is the main driver, it would strengthen the case for testing and developing ketogenic supplements, which do not need to be used in combination with dietary changes. It would further motivate studying diet interventions on *strict* LCHF (very-LCHF, VLCHF), with high demands on discipline and motivation—making the target group limited. On the other hand, if brain health benefits would be achievable by moderate carbohydrate restriction (or modulation of other macronutrient parameters), it could have broader implications for dietary guidelines over lifetime from a public health perspective.

1.2 A Definition of Cognitive Health in Aging

Within this thesis the term *cognitive health* will be used as an umbrella term for status of cognitive performance, neurocognitive diagnoses, and biomarkers associated with cognitive function or diagnoses.

1.2.1 Cognitive Performance

Cognitive performance refers to how various cognitive functions are reflected by cognitive tests. Such validated test batteries typically target various cognitive sub-domains—e.g., memory, attention, language, inhibition, and decision making—which have their biological base in distinct brain regions interacting in neuronal networks (19). The Cattell-Horn-Carroll model provide one frame work for structuring and combining cognitive abilities and sub-tests (20). Magnetic Resonance Imaging (MRI), electroencephalogram (EEG), and Positron Emission Tomography (PET), exemplify methods to link cognitive function with brain structure and/or brain activity (21). The term *global cognition* (primary outcome in *Study IV/V*) may refer to a composite score calculated as the average of z-scores (normalized to have the mean = 0 and the standard deviation [SD] = 1) from the various sub-domains. The Neuropsychological Test Battery (NTB) and ADAS-Cog exemplify validated test batteries for older adults with potential cognitive impairment or dementia (22).

According to good reporting practice, e.g., following CONSORT or STROBE guidelines (23, 24), the predefined primary outcome—typically a composite score— should always be reported, and post-hoc analyses should clearly be defined as such, with consideration of a potential multiple-comparisons issue (25). How this aspect is handled should be an important quality indicator when studies are interpreted. The literature review identified several articles where a few retrospectively selected sub-tests were highlighted while the composite score was skipped, and that complicates a synthesis of the evidence.

When repeated measures (separated by hours or years) are applied to cognitive testing, *learning-effects* must be considered: Due to familiarity with the test (and the testing context), the score may become higher without reflecting a true difference in cognitive function. When learning effects are assumed to be equally (randomly) distributed among individuals, *relative* differences in cognitive change may give information on their progress.

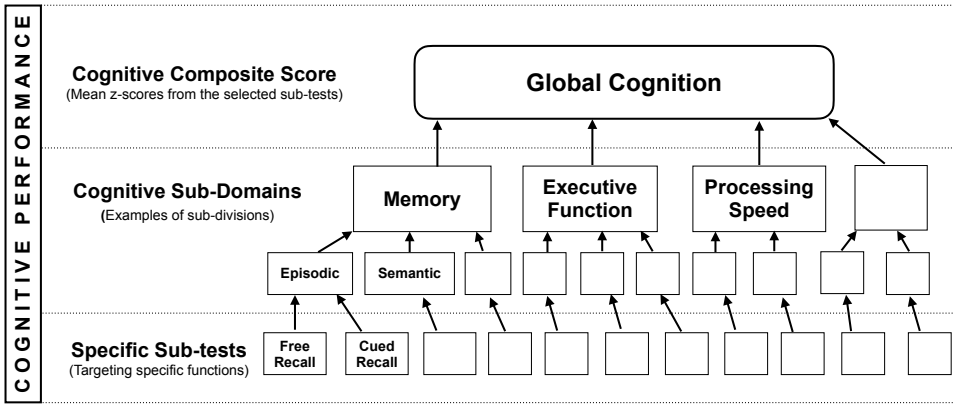


Fig. 1: Examples on how cognitive performance may be conceptualized and measured.

1.2.2 Cognitive Diagnoses

Average cognitive performance declines in some cognitive domains in aging, but there are large inter-individual differences (26). In a proportion of the population, the cognitive decline may exceed expected ranges (subjectively or objectively by tests), and diagnosed as subjective cognitive decline (SCD), mild cognitive impairment (MCI), or Alzheimer's disease (AD) and other dementia diagnoses (27, 28). Dementia—meaning that the cognitive decline is severe enough to substantially affect daily living—is absent in SCD and MCI. The terms subjective cognitive impairment (SCI) may be used almost synonymously with SCD in the literature (27, 28). While a proportion of SCD and MCI cases will progress to dementia within a few years, many will remain stable or improve their cognitive status (27). Amnesic MCI (affecting memory) has a substantially higher risk for dementia conversion compared to non-amnesic MCI (29). In the updated diagnosis manual DSM-5, the term dementia was replaced by *major neurocognitive disorder* while *minor neurocognitive disorder* represents a state almost synonymous with MCI (29), but these newer terms are rarely applied in the literature reviewed within this thesis.

Decline in episodic memory, which relay on functions of the *hippocampus* in the medial temporal lobe, is a specific hallmark of AD (30). While AD is the most common disease causing dementia, it can also be identified for research purposes in the preclinical or prodromal stage based on biomarkers, but in the absence of dementia (30-32).

Biomarkers defining AD include the proteins beta-amyloid (Aβ42 or preferably the ratio Aβ42/40) and phosphorylated tau, typically measured in cerebrospinal fluid (CSF) (33). It has been estimated globally that 22% of all persons at age ≥50 years have AD-pathology when the preclinical state (which may last for decades) is included (34).

Tab. 1: Description of stages of cognitive decline

	DIAGNOSIS / LABELLING					
CHARACTERISTICS	**Cognitively Normal (CN)**	**Subjective Cognitive Decline (SCD)**	**Mild Cognitive Impairment (MCI)**	**Preclinical Alzheimer's Disease**	**Prodromal Alzheimer's Disease**	**Alzheimer Dementia**
Subjective cognitive complaints	(very minor)	X	X	(X)	(X)	(X)
Objective cognitive decline (by tests)			X		X	X
Alzheimer pathology in biomarkers				X	X	X
Impaired daily function						X

Screening tools for dementia include for example Mini Mental State Examination (MMSE), The Montreal Cognitive Assessment (MoCa) (35) and Clinical Dementia Rating (CDR). Of all dementia cases, 60–80% may be attributed to AD, 5–10% to vascular dementia alone, 3–10% to frontotemporal lobar degeneration (FTLD), and 5% to Lewy Body Disease (LBD) alone, but diagnoses are commonly overlapping and mixed dementia is common especially among older persons (36). Approximately 50 million people on our planet live with dementia, and this number is expected to be tripled by 2050 as the older fraction of populations increases; AD is now the fifth cause of death among Americans of age ≥65 years (36, 37).

Familial (*early onset*) AD, which accounts for <5 % of total AD cases and is caused by a limited number of dominant genes (amyloid precursor protein, presenilin 1 & 2) (38) will not be distinctly covered within this thesis. For the remaining cases (labelled *sporadic* or *late onset* AD), the etiology is multifactorial and about 40 different risk genes have been identified (39) which act in combination with modifiable risk factors for dementia (37). Of those, Apolipoprotein E (APOE) is the most important risk gene, as described below.

1.2.3 Biomarkers Associated with Cognitive Health

Beyond the AD-defining biomarkers, Aβ and Tau, several other biomarkers have been associated with cognitive disorders. Neurofilament light (NFL) may reflect AD-pathology (40), but is also related to a broader range of neurodegenerative disorders (41). Glial fibrillary acidic protein (GFAP) is an inflammatory marker associated to Aβ-pathology, but not for tau pathology, in AD (42). To support clinical diagnosing, CSF measures are typically used, but in recent years Aβ, tau, NFL, and GFAP have also been validated as blood biomarkers and they may increasingly be incorporated in clinical practice (43). Such blood markers may already be an important scientific tool to monitor disease progression in trials as repeated measuring is more feasible in blood compared to CSF. Moreover, compared to cognitive testing blood biomarkers should be less biased by learning effects, placebo effects, and language problems. Particularly for diet and other lifestyle interventions—which rarely can be blinded—blood biomarkers may promote the validity of scientific studies. Further research is however needed to optimize their interpretation.

Biomarkers analyzed or discussed within this thesis are described below, and they were all measured in peripheral blood. The peripheral measures are considered relevant for studying brain function, either as a proxy for brain levels—when the marker is known/assumed to pass through the blood-brain barrier (BBB)—or as a marker for metabolic health status which at least indirectly may be related to cognitive health.

1.2.3.1 Brain-Derived Neurotrophic Factor (BDNF)

Among its several functions, the mature form of BDNF (mBDNF) is important for strengthening synapses and consolidation of memories—a process called long-term potentiation (LTP)—while the precursor (proBDNF) facilitates long-term depression (LTD) and apoptosis (44). Those complementary processes are essential for brain plasticity. Only a small fraction (≈2%) of the total BDNF literature has reported results on proBDNF (45), which may encourage further investigations on how these two forms may interact.

Altered blood levels of BDNF have been observed in several psychiatric and neurological diseases and is one of the most studied biomarkers in AD research (46, 47). The interplay between blood, CSF, and brain BDNF levels, is not fully understood, and while many studies have investigated measured levels, more knowledge is warranted regarding dynamic changes after various exposures. The ketone BHB may modify expression of the *bdnf* gene (48-50), suggesting that BDNF could be one mediating link between ketosis and cognitive function. Human studies on BDNF in ketogenic contexts are few (51, 52), and proBDNF was not analyzed in those.

1.2.3.1 Insulin

Peripherally, the peptide hormone insulin is essential for broad metabolic functions, including uptake of glucose in muscle and adipose cells (45). In the brain, insulin has distinct signaling functions while its potential role for glucose uptake and transport is not fully understood (1, 46).

Insulin dysfunction may be related to availability—by functions of synthesis, transport, and degradation—or to the receptor response, i.e., insulin resistance (or inversely insulin sensitivity). Homeostatic assessment of insulin resistance (HOMA-IR) and beta-cell function (HOMA-BCF) is an estimation method based on imputing fasting levels of glucose and insulin in a standard formula (47). Insulin function can be reflected by an oral glucose tolerance test (OGTT) where blood glucose levels are measured 120 minutes after glucose intake, preceded by an overnight fasting. One method to assess insulin resistance in the brain is via neural-derived exosomes in plasma (53).

1.2.3.2 Glucose

Blood glucose is a major energy provider to all tissues in the body. Keeping adequate levels of circulating glucose is not dependent on intake of dietary carbohydrates since normal levels may be maintained by gluconeogenesis, where the glycerol backbone from triglycerides or amino acids from protein provide the substrate (45). A shift from supply-driven (dietary carbohydrates followed by insulin release) to demand-driven (gluconeogenesis) glucose homeostasis is a hallmark of ketogenic diets and fasting. Increased adaptation to utilization of fatty acids and ketones under such conditions may be described as a metabolic switch (49).

1.2.3.3 Lipids and Lipoproteins

In contrast to glucose which passes relatively smoothly through the BBB, the pools of lipids and lipoproteins are more separated between the brain and the periphery (54). Nevertheless, peripheral lipid status may be relevant to address here since middle age hypercholesterolemia may be predictive of dementia (55) and lipid status is an important topic in relation to safety discussions regarding ketogenic diets (56).

The variables underlying a typical lipid panel can metaphorically be categorized as *cargo*, *ships,* or *flags on ships*: The lipid cargo is either *cholesterol* (C, structural material) in free or esterified form, or *triglycerides* (TG, energy) (57). Common ships are, in descending size, *chylomicrons* (transporting dietary TG from the intestines to tissues), *very-low-density-lipoproteins* (VLDL, transporting TG synthesized in the liver to tissues), *"remnants"* (shrunken chylomicrons or VLDL, after delivery of their TG), *low-density-lipoproteins* (LDL) or *high-density-lipoproteins* (HDL). Although being the same molecule, cholesterol is labelled differently depending on what ship it is transported in: HDL-C, LDL-C, or remnant-C. The ships are equipped with "flags" which act as ligands

and determine their function. For HDL, one important flag is apolipoprotein A1 (ApoA), whereas LDL, VLDL, and chylomicrons have apolipoprotein B (ApoB). For ApoB, there is commonly just one flag per ship, which makes ApoB a proxy for the particle number of that group of lipoproteins. Since the residence time in blood is much shorter for chylomicrons (≈20 minutes) and VLDL (2-3 hours) compared to LDL (2-4 days), in a blood sample most ApoB belong to LDL (57). Thus, ApoB is primarily a proxy for LDL particle number (LDL-P). (Analogous with describing the heart as a pump, the ship/cargo metaphor is so widely used that I have not identified any original reference. The flag metaphor is my own suggestion, although it does not refer to any suggestion that apolipoproteins would be small.)

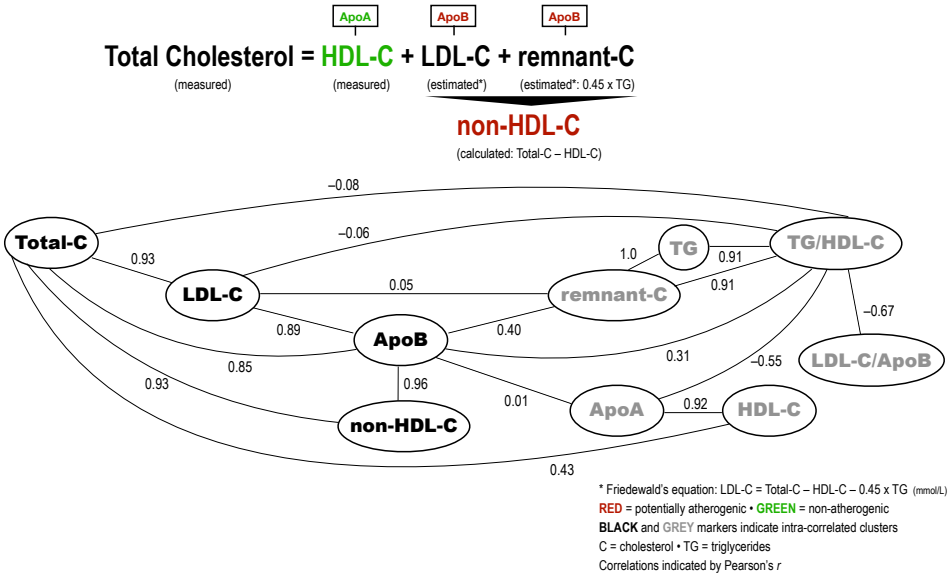


Fig. 2: Correlations between lipid markers in the FINGER sample

Cholesterol is essential as a structural component of cell membranes, and as a precursor for steroid hormones and bile acid. Most cholesterol is synthesized locally in cells, whereas the lipoproteins provide supportive supply and reverse transport of excess cholesterol to the liver (58). Blood cholesterol levels are highly genetically determined, and dietary cholesterol makes only a minor contribution to blood levels in most individuals, whereas a higher ratio SFA/PUFA typically increases levels (59, 60). ApoB is the primary marker, over LDL-C and total cholesterol, for predicting cardiovascular disease (CVD) risk (61).

ApoE is not typically measured in a peripheral lipid panel but since the different isoforms (ε-2, ε-3, ε-4) of the APOE-gene (APOE2, APOE3, APOE4) constitute the most important genetic risk factor for Alzheimer's disease (62), it may be relevant to introduce it here. In contrast to ApoA and ApoB which are tied to specific types of lipoproteins, ApoE can be transfered between various types and modulate their function

and clearance (63). A functional difference between the isoforms is their relative preference of binding to VLDL versus HDL: ApoE4 has a stronger affinity to VLDL, while ApoE3 and ApoE2 prefers HDL (64). Homeostatic levels of HDL vary by APOE-genotype 22>23>24>33>34>44, with an opposite direction for LDL (64). In the brain, ApoE is the primary cholesterol transporter (54).

Global prevalence and AD risk for the iso-forms are for APOE3: 79%, normal risk; APOE4: 14%, increased risk; APOE2: 7%, decreased risk, but large regional variations exist for both prevalence and risk ratios (65).

1.3 Approaches To Study The Impact of Diet on Health

This thesis will primarily address nutrition with focus on macronutritional composition, as mentioned in the title. To put this approach in context, some other conceptualizations of diet are described below.

1.3.1 Micronutrients, Single Foods, and Dietary Patterns

While early nutritional studies often focused on deficiencies in nutrients, current research more often study how the compositional level of diet is related to health (66). This thesis will cover nutrition from the perspective when malnutrition is assumed not to be a major issue. The focus is rather on food composition on a systemic level in relation to chronic disease.

Various micronutrients (e.g., vitamins and minerals) have been associated with cognitive health, as reported from several reviews (67, 68). One aim official dietary guidelines, e.g., the Nordic Nutrition Recommendations (NRR) (ref), is to guide food choices providing adequate micronutrient intake. Estimations and empirical evidence indicate that sufficient micronutritional intake is also achievable from low-carbohydrate and ketogenic diets (69, 70), i.e., even when the macronutritional composition deviate from official dietary guidelines. Furthermore, it has been suggested that micronutritional needs might be different in a low versus a high carbohydrate context (71). Nutritional supplements may be added to diet for achieving micronutrients. A 2018 Cochrane review however found "no good evidence that middle-aged or older people can preserve cognitive function or prevent dementia by taking vitamin or mineral supplements" (63). In the *Guidelines for risk reduction of cognitive decline and dementia* by the World Health Organization (WHO), micronutrient supplementation is not recommended in people without deficiencies (72). Research and debates on the micronutritional level will not be covered within this thesis.

Some studies have reported associations with cognitive health for intake of single foods or food groups, e.g., vegetables, fruits, olive oil, coffee, fish, or nuts (67). Single foods are not a prioritized target of this thesis, beyond coconut oil which is compared for its potential ketogenic effect against sunflower oil and a ketogenic supplement in *Study I*.

Dietary pattern approaches typically use several aspects of nutrient or food composition—defined either *a priori* or *a posteriori*—to define a pattern that can be investigated in relation to health outcomes (73, 74). The most studied dietary pattern in the cognitive field is the Mediterranean Diet (MeDi), while some studies addressed the Dietary Approaches to Stop Hypertension (DASH), MIND (a hybrid of MeDi and DASH) or the Nordic Diet (67). Those diets may be compared against a traditional "Western" diet as being higher in fruit, vegetables, grains, and other plant-based foods, while being lower in animal-based foods, particularly red, fatty, and processed meat (74). All these patterns are relatively low in *ultra-processed food*, as can be classified according to the four categories of the NOVA system, depending on "physical, biological and chemical processes used after foods are separated from nature, and before being consumed or prepared as dishes and meals" (75). It is debated whether the concept *ultra-processed food* adds substantial information that cannot be captured by conventional nutrient metrics and classification systems (76).

It is also wort considering that dietary parameters may affect health negatively both by *loss* of function (at low levels of the dietary parameter) and by *gain* of toxic or anti-nutrient functions (at high levels of the dietary parameter). For a full understanding of such factors, and of bioavailability, flavor perception, and satiety factors, the *food matrix* (77) should be considered when individual nutrients are analyzed.

1.3.2 Macronutritional Composition

The term *macronutrient* primarily refers to carbohydrates, fat, and protein, which are the predominant energy providers in human diets (78). When referring to their proportional contribution of total metabolized energy (E%), I will use the abbreviations eCarb, eFat, and eProt, respectively. Dietary fiber (eFib), and optionally alcohol (eAlc), contribute with some additional energy and will here also be considered as macronutrients. Since *exogenous ketones* provide energy (79) it may be relevant to add it as a separate macronutrient category, although it is not naturally present in food.

While fiber from a chemistry perspective constitute carbohydrates, they are metabolized fundamentally differently from the other macronutrients by not being taken up in the intestine. Instead, fiber is fermented to short-chain fatty acids (SCFA: butyrate [C4], propionate [C3], acetate [C2]) by microbes in the colon (80). On American food labels, *carbohydrates* refers to eFib+eCarb and the term *net carbs* is used to specify eCarb. In contrast, on European labels (and within this thesis, and *Study IV/V*) *carbohydrates* refers exclusively to eCarb, which may make more sense from a metabolic perspective. When used as predictor variables, those differing definitions of carbohydrates should however give almost identical results since they correlate highly (by 0.999 in *Study IV/V*) because of the low range for eFib. Alongside its widely assumed health promoting properties, fiber may have antinutritive effects by inhibiting

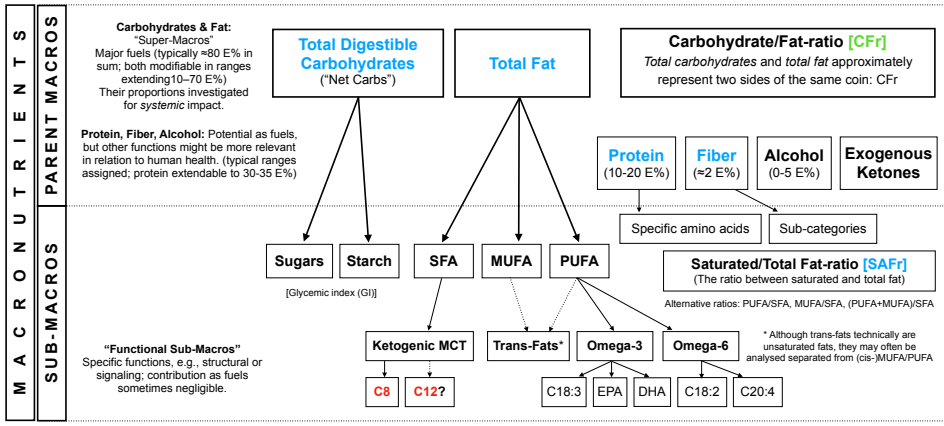
uptake of other macronutrients as well as micronutrients (71, 80). How alcohol by various pathways may impact cognitive health is beyond the aims of this thesis to address, but its energetic impact on the statistical interpretation of other diet variables will be discussed.

No standard approach has been established for analyzing and reporting macronutritional composition in relation to health, in the meaning of combining a statistical method with a pre-defined variable selection. Various methods for accounting for total energy intake as a confounder were discussed in the 1990's and—alongside a two-step method where macronutrients by weight where "energy adjusted"—the *nutrient density model* (analyses by E% instead by weight) was established as one common method (81). However, while macronutrient intake expressed by weight constitutes *unrestricted data*, values by E% constitute *compositional data* (summing up to 100%) which requires specific methodological considerations, according to Aitchison (82). Methods for compositional data analysis, which are based on *ratios* between the components, have received remarkably little attention within the nutrition field, as pointed out by Correa Leite (83). Others have further argued for the use of dietary ratios because it corresponds to a biological way of thinking, i.e., pathways of metabolism can often be described in terms of balances between components (84).

Since weak or inconsistent methodology has been acknowledged as areas for improvement of the nutrition field (85-88), it became a necessary aim of this thesis to develop a structured method for analyzing macronutritional composition. That method will be more extensively described in further sections, validated in *Study IV*, and applied in *Study IV/V*. A short introductory background is given here.

The relations between various macronutrients are illustrated in Fig. 3. Notably, it is easy to come up with >20 different predictor variables to select from, of which many may be highly correlated. This may induce a risk for selective reporting and complicates a synthesis of accumulating evidence. In the process of creating an analysis framework that is valid and optimized from a biological as well as a statistical perspective, I introduced some categorical concepts. The point with using those is to acknowledge the huge differences in modifiable ranges (as described in *Study IV*), and to make a conceptual distinction between *compositional* impact (which is my primary research focus) and *functional* ("micronutritional") impact. *Parent-macros* refers to the main categories which are metabolized by distinct pathways (78, 80) and sum up to 100 E%: eCarb, eFat, eProt, eFib, and eAlc. Of those, I refer to eCarb and eFat as *super-macros* since they provide roughly 80 E% in broad human diets (89, 90) and should be expected to have a highly reciprocal relation.

Each parent-macro can be divided into sub-categories, which I refer to as *sub-macros*. This includes the classification of fatty acids (FA) by the number of double-bonds in the carbon-chain: 0=saturated FA (SFA), 1=mono-saturated FA (MUFA), >1=poly-unsaturated FA (PUFA). In another dimension, FA may be classified by the *length* of their carbon chain as SCFA (C2-C4), medium-chain FA (MCFA, C6-12), long-chain FA (LCFA, C≥14), and sometimes separating C>20 as very-long-chain (VLCFA). By specifying the position of the first double-bond in the carbon chain, PUFA may be further sub-divided as omega-3 or omega-6.



- CFr was the primary predictor variable of interest in Study IV/V.
- Blue indicate secondary/exploratory predictors in Study IV/V.
- In Study I, the ketogenic properties of C8 and C12 were compared and their associations with serum BDNF were investigated in Study II.

Fig. 3: My conceptualization of macronutrients—and their role within this thesis. The parent macro level should sum up at 100 E%. Examples of sub-categories are shown below. E%: percentage of total metabolized energy; SFA: saturated fatty acids; MUFA: mono-unsaturated fatty acids; PUFA: poly-unsaturated fatty acids; MCT: medium-chain triglycerides; C8: caprylic acid; C12: lauric acid; C18:3: α-linolenic acid; EPA: eicosapentaenoic acid (C20:5); DHA: docosahexaenoic acid (C22:6); C18:2: linoleic acid; C20:4: arachidonic acid; BDNF: brain-derived neurotrophic factor

1.3.3 Types of Trials and Methods for Measuring Food Intake

Some RCT—labelled *efficacy* or *explanatory* trials—prioritize control over actual food intake by keeping participants in a metabolic ward, or by providing food and/or intense counseling among free-living individuals (86, 88). Another type of RCT—labelled *effectiveness* or *pragmatic* trials—may deliver dietary recommendations with less intense control of actual food intake, which may allow longer follow-up times and a larger number of participants (91). Rather than distinct categories, explanatory vs. pragmatic trials may be viewed as endpoints on a spectrum, described by the PRECIS tool (92), where the explanatory component (evaluating hypotheses on biological causality under ideal conditions) may have a trade-off against generalizability to a real-life context in longer term. In trials among free-living participants and epidemiological research, diet intake can be monitored by *food records* or retrospectively for longer

timespans by *food frequency questionnaires* (FFQ) (66). Biological methods reflecting food intake are increasingly investigated, e.g., analyses of blood, urine, stool (microbiome), and hair, as well as continuous glucose monitoring (93-95).

1.4 Clarifying Research Aims: Prediction or Causal Inference?

To guide decision making on prevention or treatment strategies, it is not sufficient to identify a risk factor as *modifiable* and *predictive* of the outcome—it must be identified as *causal* (96). Whereas randomized controlled trials (RCT) constitute the gold standard for establishing causality, decision making on dementia prevention in practice rely on a combination of RCT and observational evidence (97). Causal inference (also referred to as counterfactual prediction) in observational contexts refers to studies which ask causal research questions and use adequate methods to answer those; by a convention to avoid causal language in observational research it may be difficult to distinguish such studies from those which have *description* or *prediction* as their aim (98, 99). Shmueli (100) uses the terms *explanatory* versus *predictive* to make a similar distinction between research aims, but Hernán et al. point out that explanation is not a criteria for causality, e.g., an RCT can establish that treatment A is more effective than treatment B without necessarily giving a clue on the mechanisms (99).

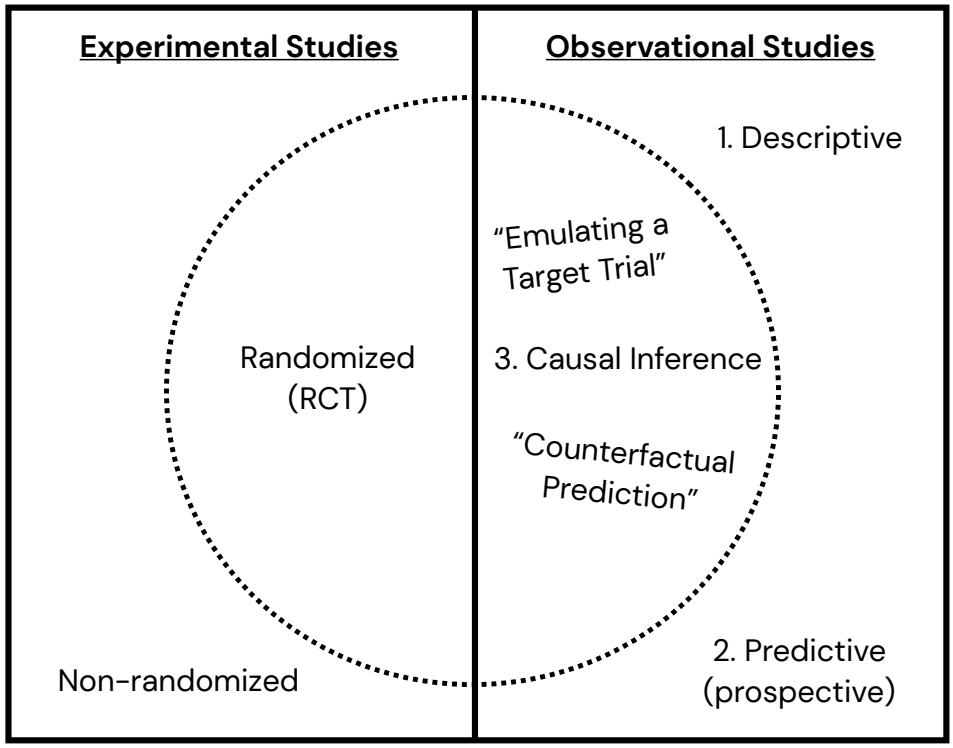


Fig. 4: The circle captures the fractions of experimental and observational research which—by their research questions and methodology—aim at guiding decisions on prevention strategies.

An observational study can apply the term *target trial* to define an imaginary RCT which would target the same research question, thereby aligning its results to the relevant evidence base for decision making (98). A meta-epidemiological study on nutrition research indicated good agreement between evidence from RCT compared to observational studies and concluded that it is motivated to base decisions on a synthesis from both categories of evidence (101). My conceptualization of the evidence base relevant for making decisions on preventive strategies is illustrated within the circle in Fig. 4.

Since methods—e.g., regression models—for prediction and causal inference are similar in many ways, erroneous conclusions may be drawn if β-coefficients (and confidence intervals, CI) are interpreted as causal estimates when the research aim was prediction, or when the aim was causal but predictive methods were mistakenly applied. A key difference between a predictive and a causal approach is the principles for covariate selection: A prediction model may be chiseled out by data driven strategies like stepwise addition or subtraction of covariates in response to p-values or values from the Bayesian Information Criterion (BIC), alternatively the Akaikes Information Criterion (AIC) (100). In contrast, when the research question is how an exposure of interest (X) *causes* the outcome (Y), covariate selection should be made *a priori* based on assumptions on the causal relations between the variables. Theories of *Causal Inference* give guidance on such decisions and Directed Acyclic Graphs (DAG) provide one method to develop an analysis framework (102, 103). Those decisions are based on field specific knowledge (and assumptions) rather than statistics. A key task in the development of a DAG is to identify the functional role of a covariate as a *confounder*, a *mediator*, or a *collider* since that function will decide whether inclusion of the covariate in the model will increase or decrease bias (i.e., make the causal estimate for the predictor of interest either *more* or *less* true). Inclusion of a confounder will *decrease* bias while a collider/mediator will *increase* bias (103). However, a mediator may be included if the aim is to separate out different pathways from the *total* causal effect of X (104). The three DAG in Fig. 5 illustrate how assumed directions of causality define the functional role of a covariate (Z).

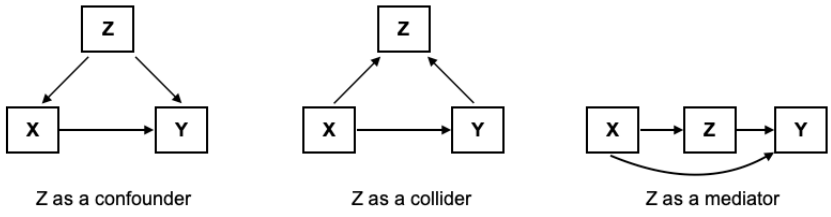


Fig. 5: The role of covariate Z is illustrated by various assumptions on causality.
Adjustment for Z will *decrease* bias for the causal estimate X➔Y if Z is a confounder, while adjustment will *increase* bias when Z is a collider or a mediator.

Tab. 2: Comparison of Data Science Tasks

Research Aim:	**Description**	**Prediction**	**Causal Inference**
Type of research question:	Is dementia more common among those who had a high sugar intake 10 years ago?	What is the likelihood that patient A develops dementia within 10 years?	Does higher sugar intake cause increased risk for dementia? (Should we recommend lower intake?)
Predictor of interest:	One (e.g., sugar intake)	No particular	One (e.g., sugar intake)
Covariate selection:	No other covariates needed	Data-driven selection (p-values, BIC, AIC) Sugar intake might be included, but not as *the* predictor of specific interest.	A priori definition of confounders (included) and mediators/colliders (excluded)
Interpretation of β and CI	"association" "correlation" (if linear)	Unclear (since variables may constitute colliders or mediators in relation to each other, and confounding adjustment might be incomplete)	Predictor of interest: "Causal estimate" (though in practice often referred to as "association") Covariates: Unclear
Validity of causal interpretation of β, and translation into preventive recommendations:	Low	Low	If the assumed causal structure is correct and no unmeasured confounding. (debatable—not testable)
Competence required:	Statistical (Artificial Intelligence)	Statistical (Artificial Intelligence)	Statistical Subject matter knowledge

Note: My interpretation of distinctions made by Hernán (98, 99) and Shmueli (100).

The variable "number of recent hospital visits" may exemplify a collider and should therefore not be adjusted for when the aim is to estimate the causal effect of an exposure of interest on a disease outcome; on the other hand, its inclusion in a (prospective) prediction model is motivated if it improves the precision of the model (98). In the latter case, the error of translating its slope into a preventive recommendation (*to decrease the number of hospital visits)* may be obvious, but for other factors such mistakes may not be intuitively detected. As an analogous example, a specific diet behavior, or a biomarker (or "omics" (105)), might be upregulated as an adaptive (potentially protective) response to preclinical disease. If so, its predictive and causal roles may have opposing directions, and a recommendation based on the predictive estimate may be counterproductive. It will indeed decrease your risk-*score* but not decrease your actual risk. This problem goes beyond *reverse causality*—i.e., misinterpretation that X causes Y when the true direction is Y➔X—since it involves the causal structure of all included (and excluded/unmeasured) covariates. As *big data* and *machine learning* approaches are expected to revolutionize predictive science (106), improved guidance of prevention strategies may not automatically follow; that field requires distinct methods based on causal inference theory (99, 107). Those fields may be complementary for applying *precision medicine*: Predictive and descriptive science

identify people at risk and various risk-subtypes; causal science provides targeted strategies for those subtypes.

View-points for making causal inference on observational data—to guide decisions on taking action or not—were proposed by Bradford Hill in 1965 (108, 109), and the field has been increasingly formalized in recent decades (107). For clarification, I do not interpret causal inference theory as a tool for raising observational evidence to the same hierarchical level as RCT, but as a tool for distinguishing the fraction of observational evidence that is qualified to accompany RCT-evidence in decision making on prevention or treatment strategies. Possibly, a raised awareness of the need to identify that evidence base—distinct from predictive/descriptive science—underly what has been described as a *causal revolution* (104, 107). Application of the concept *target trial*—as a tool to refine the research questions and methods in observational research—has recently been encouraged through leading journals like American Journal of Clinical Nutrition (110), British Medical Journal (111), JAMA (112), and New England Journal of Medicine (113). My understanding on how to distinguish descriptive, predictive, and causal research is structured in Tab. 2. The research questions asked within this thesis are primarily causal.

1.5 Data Sources

1.5.1 The Clinical Trial "Coffee & Cream"

Within this thesis, *Study I* (114), *II* (45) and *III* (115) are based on a cross-over RCT, in healthy older adults (N=15), which was planned and performed within the doctoral project. My role in the clinical trial was to identify knowledge gaps, develop the study design, perform statistical analyses and interpretations, and manuscript writing as first author (in *Study II,* first authorship was shared).

1.5.2 Observational Analyses on Data from the FINGER Trial

Study IV (116) and *V* (manuscript) use an epidemiological approach by analyzing data that was already collected within the Finnish Geriatric Intervention Study to Prevent Cognitive Impairment and Disability (FINGER) (117). Notably, those studies—although using data collected within an RCT—address research questions that are not an evaluation of the trial itself. My role in *Study IV/V* have included development of the analysis framework, performing statistical analyses, interpretations, and manuscript writing as first author—with guidance from my co-authors, which include the principal investigator and others who planned and supervised the FINGER trial.

1.6 Disclosure

This thesis targets the impact of diet *on human health,* from a curiosity perspective. Choices on diet composition on the individual and public health level may additionally depend on ethical, sustainability, economical, ideological, and cultural aspects.

2 Literature Review

2.1 Prevention of Cognitive Decline and Dementia

Considering the high global prevalence, the long preclinical period, and the absence of a drug that substantially can change the course of dementia progression, development and implementation of *prevention strategies* are fundamental tasks for the cognitive field (39, 118). WHO published for the first time in 2019 guidelines for risk reduction of cognitive decline and dementia (72). Evidence and recommendations were compiled regarding physical activity, nutrition, tobacco, alcohol, weight management, social and cognitive activity, and management of weigh, hypertension, diabetes, dyslipidemia, depression, and hearing loss. The strongest recommendations targeted adequate physical activity, tobacco cessation, management of hypertension, diabetes, and *not* recommending dietary supplements (Vitamin B and E, PUFA, and multi-complex).

2.2 The Suggested Top 12 Preventive Factors of the Lancet Comission

The 2020 report of the Lancet commission (37) concluded that up to 40% of dementia cases may be prevented or delayed by targeting *modifiable* [my italics] risk factors along the lifespan. In descending order of importance—at distinct phases of the lifespan—those factors were: untreated hearing loss, less education, smoking, depression, social isolation, traumatic brain injury, hypertension, physical inactivity, air pollution, alcohol overconsumption, obesity, and diabetes. The used method, calculation of the *population attributable fraction* (PAF), depend on that the analyzed associations are *causal*, which was questioned in the 2017 version of the report (119), and still may be for the 2020 report. . Assumptions that 8-9% of global dementia cases would be preventable by treating hearing-loss rely on assumptions of a downstream mechanism (reduced sensory stimulation, or compensatory redistribution of cognitive resources); an alternative hypothesis is that a *common cause* affects both hearing-loss and dementia, and if so there is no prevention potential by adding hearing aids (120). Indeed, the WHO commission concluded that there was insufficient evidence to recommend hearing aids as a dementia prevention strategy (72). The Lancet commission discusses that the direction of causality between depression and dementia may be bidirectional but appear to pay little attention to a possible common cause explanation for depression as well, and for several other of the reported factors (37). The suggested prevention potential for these 12 factors might thus be an overestimation, and a clearer distinction between *predictive* and *causal* risk factors (see 1.4) might prevent misinterpretations.

The Lancet commission attributed 2% of dementia cases to factors (obesity and diabetes) that potentially act downstream diet but, notably, the commission did not include diet as a preventive factor due to insufficient high-quality evidence. The

commission however lists diet as an emerging factor and accumulating evidence supports a link between food habits and cognitive health (37, 67, 121). But a conclusion may be that future research may not only aim at delivering *more* evidence but also at improving research methods to deliver evidence of higher quality.

2.3 Ketosis as a Preventive Factor

2.3.1 Brain-Energy

In the 1960's it was established that ketones are the brain's main alternative fuel to glucose (122). This back-up fuel system is more developed in humans than other species, and has been suggested to be a prerequisite for human brain evolution and development, with the ketogenic response to starvation being specifically fast in children (10). After prolonged fasting, ketones may provide 60% of brain energy for a human adult, which is essential as the brain has a very low capacity to extract energy directly from fatty acids (123).

In the 1990's, positron emission tomography (PET) studies at Karolinska Institutet showed that brain BHB utilization is proportional to plasma BHB levels in humans, indicating that transport across the blood-brain-barrier (BBB) is the rate-limiting step for ketone utilization (124). The hypothesis that energy from ketones can protect the aging brain, especially in AD, has been developed during the last decades, e.g. by Stephen Cunnane (1, 125) and Mark Mattson (126). Impaired brain glucose metabolism is observed in normal aging—mainly in the frontal lobe—and increasingly in MCI and AD, also affecting the parietal lobe, precuneus, and entorhinal cortex (1). In support of the hypothesis that ketones could compensate for glucose hypometabolism, Croteau et al. (127) observed that the slope of the association between brain ketone uptake and peripheral BHB was not different in AD compared to young healthy adults. Those results have also given clues to whether glucose hypometabolism is a result of a transport problem for glucose, or impaired mitochondrial function. Ketones are metabolized to Acetyl-CoA in the mitochondria and then enter the citric acid cycle, like glucose after its metabolization to pyruvate. In contrast to glucose, ketones do not have an alternative metabolic pathway outside the mitochondria. Based on the observation that ketone metabolism is preserved in AD, Cunnane et al. (1) conclude that mitochondrial function seems to be acceptably intact, and glucose *transport* is more likely to be the problem in glucose hypometabolism. Nevertheless, impaired mitochondrial function is also thought to play a role in AD (128).

In contrast to ketones, which are taken up by the brain in proportion to its peripheral concentration (a "push" mechanism), brain glucose uptake from the circulation is driven by neural energy demands (a "pull" mechanism), as described by Cunnane et al. (129). While some have described glucose transporters in brain cells as insulin-insensitive (while insulin has other important functions in the brain) (130), Cunnane et al. argue that

at least GLUT4 is probably insulin dependent for glucose uptake to neurons, consistent with its peripheral function (1). An increased redox ratio (NAD+/NADH), observed in the brain of healthy humans, provide an additional mechanism by which nutritional ketosis may enhance the energetic status of the brain (131). Further, a potential mechanistic link between white matter degeneration and brain energy failure has been suggested: Ketone bodies are used by oligodendrocytes as substrate for lipid synthesis in brain development, especially in early life. An observed reverse process in female mice, where myelin is catabolized to synthesize ketones, has been suggested to be an adaptive response to a shortage of brain fuel (132). Increased ketone uptake in white matter was found after a 6-months intake of ketogenic supplements, and interpreted by the authors that ketones might have a role in myelin integrity among MCI (133).

2.3.2 Epigenetic Signaling

In the recent decade, ketones have been identified as epigenetic signalling molecules, providing new mechanistic pathways alongside their energetic function (18). The ketone BHB is an inhibitor of histone-deacetylases (HDACi), and in animal studies BHB has been shown to mediate reduced inflammation by the NLRP3 inflammasome (134), improved oxidative stress tolerance (135), increased hippocampal levels of brain-derived neurotrophic factor (BDNF) (50), and extended health- and life span (136). All these pathways are well known to be involved in memory, cognition, and dementia. John Newman has presented a paradigm for mechanistic separation of the energy- and the signalling-pathway in the study of ketosis (137): Enantiomers (labelled L-/D-) are molecules that are identical, except for being mirrored like left/right gloves. Normally, BHB implicitly refers to D-BHB, which is the endogenously produced enantiomer. When using exogenous ketones, it is however possible to also include L-BHB. Both enantiomers have the signalling function, but only D-BHB have the enzymatic support to be used as energy (137).

2.3.3 Ketogenic Strategies

After 12-16 hours of fasting, adults may achieve mild ketosis (BHB≈0.5 mmol/L), with levels at >1 mmol/L after 24 hours and >5 mmol/L after a week without food intake; for children, >2 mmol/L may be reached within just a few hours, as described by Cahill (10). The role of ketosis for the infant brain has been linked to human brain evolution (138). Ketosis is primarily counteracted by intake of *carbohydrates* with subsequent insulin secretion (9), meaning that a VLCHF diet (=KD) can mimic the fasting response while maintaining adequate energy supply. Prolonged physical activity stimulates production and disposal of ketones (139).

By two metabolic shortcuts, MCFA have been described as ketogenic even in the absence of carbohydrate restriction: 1. Unlike longer fatty acids which are transported from the intestine to tissues by chylomicrons via the lymphatic system, MCFA can be

transported to the liver via the portal vein. 2. MCFA are not dependent on the carnitine shuttle to enter mitochondria for β-oxidation, but may enter by passive diffusion (11). For a more nuanced description of those two shortcuts, it should be acknowledged that it is a gradual difference in preference of the conventional versus the "short-cut" pathways that distinguishes various LCFA and MCFA. Since C12 has been demonstrated to readily utilize the "shortcut pathways" (11), we assumed C12 to be ketogenic at an early planning stage of this project. As C12 constitutes ≈50% of coconut oil, the initial plan was to use coconut oil as a ketogenic agent to investigate the impact of ketosis on cognitive health outcomes. A closer look at the literature, e.g., (140), however indicated that "MCT" typically referred to a mix dominated by C8 with very low C12 content. Despite C12 was described as ketogenic in the informal health literature, we were not able to identify a scientific paper reporting increased blood ketone levels after intake of coconut oil. Additionally, a paper published at that time showed that neither C10 nor coconut oil were substantially ketogenic among nine younger adults after a single intake in a cross-over trial (141). No study had investigated the ketogenic effect of coconut oil compared to an LCFA oil in older adults, and since the sample size was low in the aforementioned study, the ketogenic potential of coconut oil in older adults remained unclear.

2.3.4 Measurement of Ketosis

The gold standard method for assessing ketosis is a laboratory analysis on BHB and AcAc from blood samples, but in many cases only BHB is measured since AcAc is unstable and make little contribution to total ketones (BHB+AcAc) at increasing ketosis (142). Biomarker levels may differ between arterial compared to venous blood (143), but when we planned our trial, we had not identified any comparisons on handheld ketone meters applied to capillary finger pricks versus venous blood samples. In our study, the cost of the laboratory method was >10-fold higher than for the handheld meter, which provides a rationale for validating its performance.

Although ketones can be detected by analyses on AcAc in urine (144) or acetone in exhaled air (145), such methods are not validated for scientific purposes. (N=1 analyses on myself indicated that those methods have low potential to be a proxy for BHB in blood, data not shown).

2.3.5 Evidence on Cognitive Health Outcomes

No ketogenic diet interventions targeting cognitive health among healthy older adults have been identified, with the exception of a 6+6 weeks cross-over study in MCI (n=11) and cognitively normal older adults (n=6), reporting potential benefits on the gut microbiome on a Modified Mediterranean Ketogenic Diet (MMKD), compared to an American Heart Association Diet (AHAD) (95). A 3-month single-arm KD intervention, also including MCT-supplements, in 15 AD patients where 10 of them completed the study, reported feasibility, safety, and cognitive improvement (146). Preliminary results

from a 12-week RCT in MCI/AD (N=14), comparing KD (Modified Atkins diet) vs. National Institute on Aging (NIA) recommended diet for seniors, reported trends of enhanced episodic memory, possibly more related to carbohydrate restriction than ketosis, and possibly enhanced effects in ApoE4-carriers vs. non-carriers (147). Feasibility was challenging, and the study was underpowered for conclusions on causality. A 6-week study among MCI patients (N=23), reported improved verbal memory for KD compared to a high carbohydrate diet (148), and a crossover-study (6+6 weeks, N=23, SCI/MCI) found improved cerebrospinal fluid biomarkers related to AD (Aβ42 and tau), and some improved metabolic markers on MMKD, compared to AHAD. Cognitive outcomes improved on both diets, and compliance was high (8). For all studies within this paragraph, ketosis cannot be singled out as the mechanistic driver since the interventions included substantial changes macronutritional composition, and for one study (148) also substantial caloric restriction compared to the control group.

Ketogenic supplements in the form of ketogenic medium-chain triglycerides (kMCT) (114) and exogenous ketones (79) provide a strategy to investigate the impact of ketosis disentangled from macronutritional changes. At the planning stage of this project, significant improvements on cognition (ADAS-Cog) had been reported from a 3-month trial in mild/moderate AD (149) but that was not replicated in a larger 6-month trial (150). Reviews on *kMCT➔cognition* among MCI/AD (151, 152) and cognitively normal older adults (153) include several studies reporting significant improvement on selected tests, but results on *one prespecified primary outcome* (e.g., a composite score on a cognitive test battery) are scarce. Inconsistent selection of reported cognitive outcomes in nutrition studies has been acknowledged (154), and that may complicate a synthesis of accumulating evidence.

2.4 Diet as a Preventive Factor

Leaving the role of individual micronutrients and foods aside, some general compositional aspects of diet may be of interest:

2.4.1 Following the Official Dietary Guidelines

Whether higher adherence to official dietary guidelines reduces the risk for dementia or cognitive decline might depend on the comparator diet. Typically, official guidelines like NNR (155), Dietary Guidelines for Americans (DGA) (156), or WHO (72), recommend food choices that may partly overlap with dietary patterns like MeDi, DASH, or MIND (72, 155, 157). Results as well as methodology from RCT (n=10) and observational studies (n=83) were inconsistent for DASH, MeDi and MIND in relation to cognitive health outcomes in a 2023 review (158). Fig. 6 show approximate CFr ranges compatible with various guidelines and dietary patterns. DASH has an eCarb:eFat target at 55:27 (159), but reduced blood pressure equally well compared to a control diet when modified to 43:40 (E%), and additionally lowered triglycerides compared to the standard DASH (160). When MeDi and DASH scores were analyzed in relation cognitive function in the

same cohort (n=6647, age 55-75 y, overweight and metabolic syndrome), point estimates were positive for all nine cognitive outcomes for MeDi while being predominantly negative for DASH (161). Results for MIND, which was also studied, appeared to fall in-between MeDi and DASH.

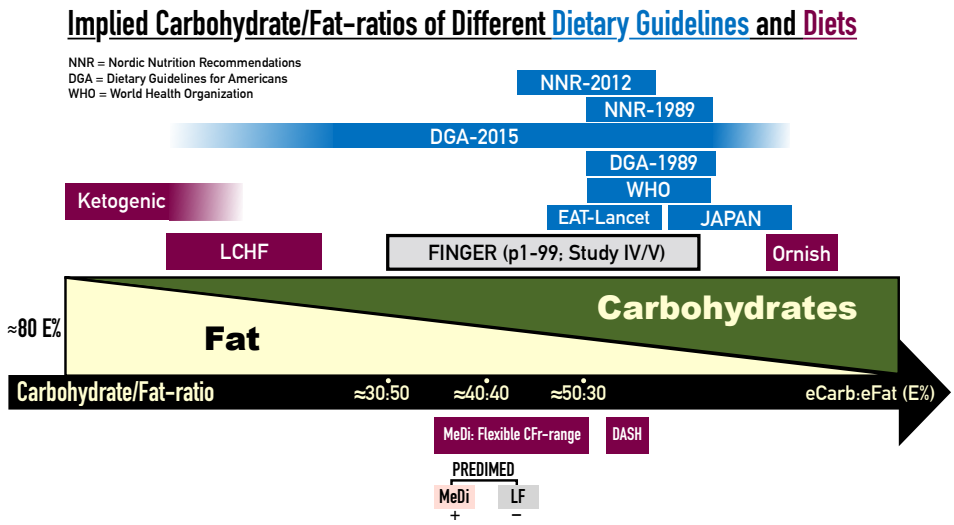


Fig. 6: Illustration of the large modifiable range of the carbohydrate/fat-ratio (CFr). Approximate ranges compatible with various dietary guidelines and diets are indicated. Distribution of CFr in the FINGER sample analyzed in Study IV/V is shown for percentile (p) 1 to 99. PREDIMED means (not distributions) indicated; +/– indicate favorable RCT results for cognitive decline. MeDi-range as defined by Martinez-Gonzales et al. (162). LF: low-fat diet (notably, that label should only be interpreted *relative* to the intervention groups of PREDIMED).

In addition to recommending MeDi (moderate evidence), the cognitive WHO risk reduction guidelines states: "A healthy, balanced diet should be recommended to all adults based on WHO recommendations on healthy diet", with quality of evidence ranging from low to high depending on dietary component (72). The recommendation is *conditional*, meaning that individual decisions should be made. Components include eFat <30 E%, SFA <10 E%, and free sugars <10 E% (but preferably <5 E%).

A recent population-based study in Malmö, Sweden (N=28025, 61% women, age 58 y at baseline, median follow-up 20-years) found that neither adherence to conventional dietary recommendations nor MeDi reduced the risk for developing all-cause dementia, AD, vascular dementia, or AD-pathology in CSF (163). Those results are consistent with two other studies: No association between quality of midlife diet (The Alternate Healthy Eating Index, AHEI) and subsequent risk for dementia was found among 8225 participants (mean age 50 y) with a median follow-up of 25 years (164), and no different risk of 20-year change in cognitive function or incident dementia was found for a "western/unhealthy" versus a "prudent/healthy" diet (n=13588, mean age 55 y at baseline) (165). In contrast to those null findings, the highest vs. lowest quintile on AHEI (n=27860, mean age 66 y, high CVD risk) had lower risk for cognitive decline (MMSE)

during 56 months of follow-up. And comparisons of categories defined by *low* (9%), *intermediate* (70%) and *high* (21%) adherence to dietary guidelines indicated that *low* compared to *high* had increased risk for non-AD dementia but not AD dementia in the Copenhagen General Population Study (n=94184, mean age 58 y, median follow-up 9 y) (166). Those inconsistent findings provide a rationale for research on alternative diets or dietary parameters.

2.4.2 The Mediterranean Diet

Reviews covering RCT (167) and combined RCT/observational (168, 169) evidence among older adults suggest an advantageous effect of Medi on cognitive function or outcomes of MCI/AD/dementia. An important follow-up question may be asked: *Compared to what?* In observational studies, adherence to MeDi is typically measured by high/low intake (defined by median split within that sample) of various food components (162), which suggest a relatively weakly defined target trial where MeDi is compared to the *average* effect of any other diet ("usual diet"). It cannot be excluded that alternative dietary patterns with low prevalence are superior to MeDi. MeDi has not been compared with vegan/vegetarian diets that do not include alcohol, or LCHF/KD on cognitive health outcomes. Moreover, MeDi may be applied with a flexible CFr range where the target for eFat is 30-45 E% (162), with a reciprocal difference in eCarb since variation in protein intake is little. I have not identified any study on whether the effect on cognitive health is different between a low-CFr MeDi versus a high-CFr MeDi. The most robust RCT evidence that MeDi promotes cognitive function may come from a sub-study of the PREDIMED trial (n=447, mean age 67 y, median follow-up 4.1 y), which applied a *low-CFr* MeDi (>40 E% eFat) (170). The control group (advice to reduce total fat intake) had a higher CFr, although their fat intake did not decrease substantially (it rather increased in the intervention groups). Intake of saturated fat and protein did not differ between the groups. In an extended follow-up on the full PREDIMED sample (n=7038), the highest versus the lowest quintile of *total fat* intake had a significantly lower hazard ratio for CVD and all-cause mortality (171). The authors acknowledged that this may provide an alternative interpretation (beyond MeDi itself) of what was driving the previously reported (172) [and problematized (173, 174)] intervention effect on CVD events. The same ambiguity may apply to interpretations of the cognitive sub-study, i.e., it cannot be excluded that a lower CFr was driving the effect, rather than "Mediterranean" diet components or the provided nut or olive oil supplements.

A higher adherence score for MeDi was associated with larger cortical thickness in several brain regions among 672 cognitively normal individuals (mean age 80 y, 53% men) examined by MRI (175). In the same study, higher intake of fish and legumes were also associated with larger cortical thickness, while higher carbohydrate intake was associated with lower entorhinal cortical thickness. Both fruit intake and refined carbohydrates had this negative association with cortical thickness, which led the

authors to raise the question whether high glycemic load—regardless of food source—may contribute to neuronal loss and cognitive dysfunction via disrupted insulin signaling and worsened glucose metabolism. Those findings may strengthen the case for hypothesizing MeDi (and other dietary patterns) to be more effective if applied with relatively lower CFr targets.

2.4.3 The Proportional Intake of Carbohydrates and Fat

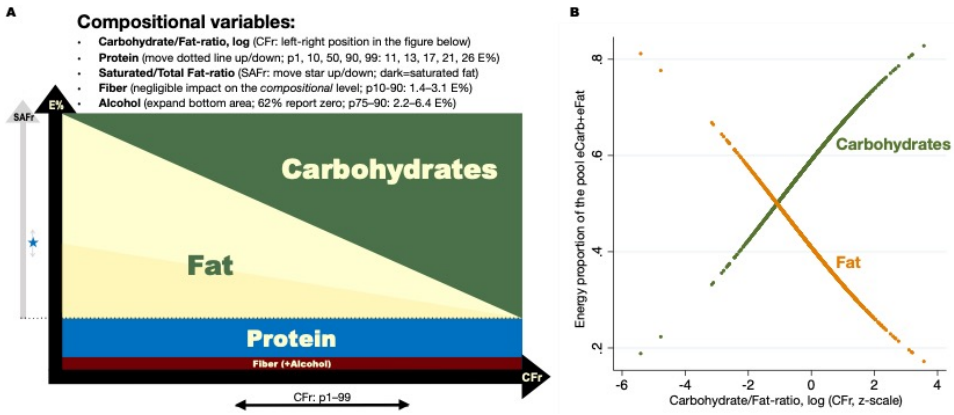


Fig. 7: Visualization of macronutrient parameters (A) The reciprocal relation between carbohydrates and fat and the distribution of their ratio (CFr) are illustrated for FINGER data. The substantially lower E% ranges of the other macronutrients at baseline are described. The sum of protein+fiber+alcohol is approximated to 20 E% in the figure. The actual distribution for p10, 25, 50, 75, 90 was 16, 18, 20, 23, 27 E%. **(B)** Visual validation of approximately interpreting CFr as "iso-caloric exchange between carbohydrates and fat within their internal pool". p: percentile [This figure was prepared for the thesis and published in Study IV, *Current Developments in Nutrition* (116)]

The primary dietary parameter of interest within this thesis is *the proportional intake of carbohydrates and fat*, which according to theories for compositional data analysis (176) is quantitatively captured by their log-ratio CFr. By applying the concept *target trial (110)*, the research question would in an RCT context correspond to iso-caloric exchange between eCarb and eFat—either between subjects (groups) in a parallel design or within subjects in a cross-over design—while ideally keeping other diet parameters constant. As demonstrated in Fig. 7B, CFr represents a close approximation of iso-caloric substitution of eCarb versus eFat, particularly within their internal pool. Since the internal pool of eCarb + eFat has a narrow distribution around 80 E%, CFr is a close representation of iso-caloric exchange between eCarb and eFat overall. Alternatively, a "substitution model" (leave-one-out model) (66) could address the research question.

I could not identify any observational study that had investigated CFr explicitly or reported a substitution model for eCarb–eFat in relation to a cognitive health outcome, beyond a substitution analysis in younger adults suggesting better reaction time with lower eCarb (177). RCT evidence related to the research question is limited to some pilot

studies in the ketogenic range of CFr (8, 148, 178). There is no evidence base that can directly guide recommendations on a specific CFr range to promote cognitive health. Can we get guidance from studies reporting intake of carbohydrates or fat as separate variables?

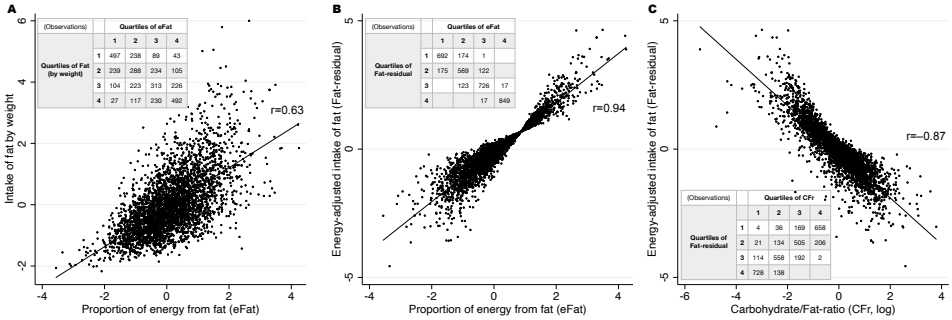


Fig. 8: Correlations between different diet variables in FINGER data. A and **B** suggest that studies combined in the same meta-analyses on fat intake have not investigated the same dietary parameter. Number of observations per quartiles are shown for comparison of the different conceptualizations of fat intake. **C** indicates that energy-adjusted fat intake (the two-step residual method) may have an interpretation as a proxy for CFr. (z-scales).

Systematic reviews and meta-analyses on **total fat** intake in relation to cognitive health (179-181) have included a mix of studies which use different conceptualizations of fat intake: 1. By weight; 2. Energy-adjusted intake by weight (a two-step method (81)); 3. By E%. As illustrated by FINGER data (Fig. 8) and as pointed out by others (182), those methods do not represent the same research question. Fig. 8C imply that eFat is highly reciprocal to eCarb. The conclusions from those three reviews covering observational studies on fat intake (179-181), were that *total fat intake was not associated with cognitive health outcomes*, including cognitive decline, MCI, AD, and dementia. Relevant RCT evidence is scanty (67) but results from a sub-set (n=1606) of the Women's Health Initiative (WHI)—where a low-fat diet (combined with increased intake of fruits and vegetables) was compared to a *usual diet* with a comparably lower CFr—indicated that the intervention group had a lower hazard ratio for "possible cognitive impairment", in screening tests by a modified MMSE (183). Subsequent analyses on MCI and possible dementia did however not indicate a significant difference between the groups. WHI had an eFat target at 20 E% but it was not reached (161).

Fewer studies have reported analyses of the proportional intake of **carbohydrates** in relation to cognitive health. In a U.S. sample (n=937, median age 79.5 y, cognitively normal, follow-up 3.7 y [median]) both *higher eCarb* and *lower eFat* increased the risk for incident MCI or dementia (184). Notably, that study ends up in the evidence lines both for eCarb and for eFat. Contrasting results were reported from a cross-sectional Chinese study (n=661, age <65 y) which found an increased risk for cognitive decline in association with *lower eCarb* and *higher eFat* (185). In analyses on the U.K. Biobank

(n=120963, mean age 56 y, mean follow-up 11 y), no association with dementia was found for eCarb, eFat, or a low-carbohydrate-high-fat cluster (186). That study however reported increased risk of all-cause mortality for the highest compared to the lowest tertile of eCarb.

In summary, it is not possible to define an optimal proportion of eCarb versus eFat (CFr) for the promotion of cognitive health, based on the current evidence base. It would however be premature to conclude that this dietary parameter does not matter, for two reasons: First, analyses on eCarb and eFat alone only address the research question by proxy and may be more confounded by outliers in protein and alcohol compared to explicit analyses on CFr (see (116) and 5.4.2. below); the log-ratio CFr should always represent the internal proportions of eCarb and eFat. Second, there is a substantial literature suggesting that APOE4 carriers might be specifically sensitive to variations in CFr, as described below, and since those roughly represent 25% of the population but 75% of total AD cases in the Nordic countries, any specific dietary needs for those may imply a large prevention potential if adequately addressed.

Observational data on LCHF and KD is lacking due to their low prevalence in the general population. Leading researchers, with otherwise diverging views, have agreed that both high-CFr and low-CFr diets may be compatible with good health and low chronic disease risk, and the optimal macronutrient composition may differ between sub-groups (187).

2.4.4 The Sub-Macro Level: Types of Fats and Carbohydrates

In the 1960's it was established that a higher ratio of SFA versus PUFA predicted higher blood cholesterol levels, although with a heterogenous response for different SFA (188). During the 1970-90's, official recommendations emphasized reduction of *total* fat intake (mirrored by higher eCarb) but in a 2001 paper (189) it was stated: *"It is now increasingly recognized that the low-fat campaign has been based on little scientific evidence and may have caused unintended health consequences. It is also increasingly appreciated that different types of fats have different health effects."* This was followed by liberalized limits for total fat in NNR and DGA (but not WHO) guidelines, in combination with an increased emphasis on (already present) recommendations to reduce SFA (155, 190). However, more recent analyses have led to the conclusion that the assumed health benefits of reducing SFA are not supported by available evidence, and those authors emphasized more focus on individual food sources and specific fatty acids (191). It may be noted that Japanese recommendations and intake levels for total fat (mean 25 E%) and SFA are lower compared to western countries (192).

One meta-analysis (age ≥55 y) found an increased risk for cognitive impairment with higher intake of SFA, but no association for MUFA or PUFA (179). However, another meta-analysis—investigating MCI, AD, and dementia as the outcomes in prospective cohort studies (age ≥40 y)—found no significant association with intake of neither SFA, MUFA,

nor PUFA (181). Consistent with that, a recent review commissioned by the Committee for updating the NNR guidelines found "no robust association between intake of any fatty acids type and the development of dementia" among adults aged ≥50 years (193). Not only results, but also the research questions were inconsistent between the various studies included in those reviews, since fatty acids were either investigated by grams, E%, or as ratios between various fat sub-types. That means that they represent different target trials and are not really suited for compilation in the same meta-analysis.

The primary research question of interest regarding fat sub-types within this thesis (and *Study IV/V*) is how the proportion of SFA as a ratio of *total fat* intake (SAFr) impacts cognitive health. [In FINGER data, SAFr correlates r=0.99 with the log-ratio SFA/(MUFA+PUFA) which should have a synonymous interpretation.] This should represent a well-defined compositional research question. Moreover, by combining CFr and SAFr within the same analysis framework, the collinearity problem that applies to compositional data analysis (176) can be circumvented (as intuitively illustrated in Fig. 7). Analogously, sub-macro ratios within the other "parent-macros", i.e., carbohydrates and protein, might optionally be added to the framework, e.g., ratios of sugar/starch or animal/plant-based food sources. A somewhat related methodological approach based on ratios on different hierarchical levels has been proposed by Kelly et al., using the terminology *intra-* and *inter-macronutrient ratios* (194).

Sub-analyses on carbohydrates may target sugar/starch proportions or glycemic load. Elevated brain amyloid in cognitively normal older adults was associated with a high-glycemic-load pattern, sugar intake, and carbohydrate intake in a cross-sectional study using PET scans (195). A systematic review on the influence of glycemic index (GI) on cognitive function found inconclusive results, including positive, negative, and neutral results (196). A later longitudinal study (n=1252) found no association between diet GI at age 53 y and cognitive performance at age 69 y (197). A narrative review by Muth & Park concluded that fiber intake is positively associated with cognitive performance whereas the opposite applies to sugar and simple carbohydrates (121). As mentioned previously, fiber may be considered a parent-macro of its own, due its fundamentally different metabolization, but nevertheless it might be an indicator of preferred food sources.

How fiber, and its various sub-types, may be mechanistically linked to cognitive function has been reviewed by Berding et al. (102), but the authors conclude that more research is needed before causal effects on brain health from fiber can be clearly established. Notably, in the previously cited study reporting higher risk for MCI/dementia with higher intake of carbohydrates, also higher fiber intake (highest vs. lowest quartile) was associated with increased risk (184). I have not identified any direct evidence that the sub-division of carbohydrates or the intake of fiber impacts the risk for AD, or dementia. Since I did not have access to data on sub-types of carbohydrates and fiber, those research questions were not addressed in *Study IV/V*.

Bayer-Carter et al. compared a HIGH diet which in relation to its comparator (LOW) had lower CFr, higher SAFr, and higher glycemic index in a 4-week RCT (198). The HIGH diet had an advantageous effect on CSF Aβ42 among amnesic MCI (n=29), while the opposite effect was seen for healthy adults (mean age 69 y). When similar diets were compared in a single meal cross-over challenge, the effects on cognitive function and plasma Aβ42 were modulated by APOE, distinctly for cognitively normal and MCI (199). The authors found the results to be in line with other reports, suggesting a "western" (HIGH) diet might be more negative for non-APOE4 than APOE4. It may however be difficult to distinguish whether results were driven by differences in CFr, or the sub-macro changes in that study design.

2.4.5 Protein and the Plant/Animal-based Dimension of Diet

While NNR proposes protein intake in the range 10-20 E% in general, the range 15-20 E% applies to individuals of age ≥65 years. The ratio between animal- versus plant-based protein sources is roughly 2:1 in the U.S. and the Finnish populations (200, 201). From a sustainability and health perspective, The EAT-Lancet diet—with a protein target at 10 E% and an emphasis on plant-based food—has been proposed as a target for reformation of global food systems (202), but notably no analysis on brain health was included in that paper. In fact, a recent review concluded that the effect on cognitive health of such a food transformation cannot be evaluated based on the current evidence base (203). This may be one of the most urgent knowledge gaps to fill within nutritional epidemiology.

This thesis does not have the plant/animal-dimension of food as an explicit research target, but it is not possible to fully separate macronutritional composition from that dimension. Carbohydrates and fiber emanate exclusively from plant-based sources, with the rare exception that some glycogen from hunted animals may be ingested, e.g., in Inuit diet. A Danish vegan diet may be lower in SFA, MUFA, and protein, while being higher in PUFA and fiber (204). Similarly, when diets differing in the plant:animal ratio for protein (30:70, 50:50, 70:30) were compared in a Finnish intervention study, a higher plant-based proportion of protein corresponded to a higher PUFA/SFA-ratio and fiber intake, and lower intake of total protein (205). If the plant/animal-dimension affects health by pathways independent from macronutritional composition, it may confound macronutrient studies.

There are very few studies on vegetarian diets in relation to cognitive health, and the potential impact of a vegan diet on AD risk has only been theoretically discussed (206). The hazard ratio (0.67, $p<0.05$) for developing dementia was lower among vegetarians (defined as excluding meat, fish, and poultry) compared to non-vegetarians among 5710 participants (mean age 58 y, females: 74% of vegetarians and 57% of non-vegetarians, mean follow-up 9.2 y) (207). All participants were exposed to the recommendation to

become vegetarians as a part of a training program for Buddhists, and the authors acknowledge that there may be a selection bias for those who followed that recommendation. Inconclusive results for the dementia risk among vegetarians have previously been reported from the Adventist Health Study (208).

2.4.6 Precision Nutrition: Is the Optimal Diet Different Between Sub-Populations?

The rationale for translating scientific evidence into general recommendations to the population rely on the assumption that the response is acceptably homogenous between individuals and sub-populations. Concepts like *personalized nutrition* or *precision nutrition* refer to a perspective when inter-individual differences in response to diet are considered; such differences may depend on factors like genetics, metabolism, and microbiota (209). For APOE genotype, there may be a rationale for stratified analyses for any life-style factor of interest just because it is the most important genetic risk factor for AD and appear to be sensitive to life-style factors (62), but it may be particularly relevant in studies of macronutritional composition considering the key role of the ApoE protein in lipid metabolism.

A substantial body of hypotheses and empirical data has accumulated during the last decades, suggesting that APOE may be related to food adaptation, and hypotheses are commonly based on an evolutionary perspective. The chronological order of the isoforms turned out to be opposite to their already given sequential number: APOE4 is the ancestral isoform while APOE3 (≈200-300 kya) is of similar age as modern humans and APOE2 (≈80 kya) appears at a time of increased global migration (210). There are some misconceptions in the literature that APOE4 would be equal to Chimp-APOE, but APOE4 is a new isoform emerging after Chimp-split (6-7 Mya) but before Denisovan (1 Mya) and possibly in relation to climate change, increased meat eating, and brain expansion 2-3 Mya (210). In fact, the iso-electric point of the Chimp-ApoE protein falls in between human ApoE3 and Apo2 (211) and Chimp-ApoE might be functionally more closely related to them than to APOE4 (210). Both humans and chimp are defined as omnivores, but chimp has a substantially more plant-based diet and a much higher capacity to extract energy from fiber (possibly ≈30 E%) by a different anatomy of intestines and colon (212, 213). Opposing hypotheses have suggested that APOE4, compared to APOE3, would be either less (214) or more (210, 215) customized for a dietary pattern lower in CFr and higher in meat (and fat) intake. To my understanding, both perspectives assume that APOE3 added *metabolic flexibility,* but they diverge in their assumptions regarding the preceding APOE4. While assuming "*All direct human ancestors are believed to have been largely herbivorous.",* Finch & Stanford (2004) hypothesized that increased meat eating in the recent millions years may have increased the risk for chronic disease in aging, and suggested that APOE3 might be a "meat-adaptive" gene that mitigated such harm, e.g., by more preferential blood levels of LDL and HDL cholesterol compared to APOE4 (214). In contrast, Huebbe & Rimbach

(2017) suggested that *APOE4* may have provided adaptation to meat-eating by improved digestion of dietary lipids and better defense against parasites and pathogens (210). They argued that APOE3 may have provided improved detoxification of *plant-based* food components by a previously described (216) mechanism related to the transcription factor Nrf2.

The latter view has an interesting compatibility with a hypothesis (not related to APOE) presented by Ben-Dor et al. (2021), which imply that the shift towards a lower plant/animal-based food ratio in the evolution from chimp to humans was not a linear decrease. They rather suggest a U-shaped pattern, where a hyper-carnivorous period (defined as less than 30% of food from plant-based sources) developed a few million years ago, followed by a return towards a more balanced omnivorous pattern with start a few hundred thousand years ago (217). Taken together with what we know about the timing of the APOE4-3-2 mutations, that hypothesis is compatible with APOE4 occurring in relation to a low plant/animal-based ratio (lower CFr and fiber, and likely higher protein intake), while APOE3 and even further APOE2 would correspond in time with an increasingly higher plant/animal-based ratio of food sources. Henderson (2004) suggested that APOE4 might be less adapted to a high carbohydrate diet, with one argument being its low prevalence in agricultural populations—implying a selection against APOE4 in such contexts (215). There is indeed an established north-south gradient in Europe with APOE4 prevalence at 20-30% in the Nordic countries compared to 5-10% around the Mediterranean, and a similar gradient has been shown for China (218, 219). Those references further indicate that APOE4 is over-represented in tropical areas.

Compelling evidence in support of the hypothesis that higher intake of unprocessed read meat would be protective particularly for APOE4 carriers can be found in *results* (including supplementary material) from analyses on the U.K. Biobank (n=493888, mean age at baseline 57 y, mean follow-up 8 y) (220). The hazard ratio (HR) for all-cause dementia per 50 g/day increment of unprocessed read meat intake was 0.81 ($p=0.011$) in the full sample, but 0.64 ($p<0.001$) among APOE4. For non-APOE4, HR was 0.93 ($p=0.59$), and the interaction effect by APOE was $p=0.019$ in a model adjusted for age, gender, ethnicity, education, and socioeconomic status. In a "fully adjusted" model—which among >10 other covariates included *family history of dementia*—the interaction was $p=0.095$, and this was commented in the conclusions as "unprocessed red meat intake may be associated with lower risks, independent of *APOE* ε4 carriage", while the authors focused on the significantly higher dementia risk from *processed* meat that was also indicated by the analyses. There was also a trend ($p=0.054$) of APOE interaction for *total* meat intake in the same study and $p<0.05$ is after all an arbitrarily chosen cut-off (221). It should be acknowledged that the U.K. Biobank is a self-selected sample which

may lead to biased results (222), but it appears unlikely that the gene-diet interactions discussed here would be substantially biased by the selection of participants.

There is a rich body of evidence from human and animal studies indicating that APOE modulates the associations between diet, metabolic biomarkers, and cognitive function, as reviewed by Farmer et al. (223) and Egert et al. (224). Lane-Donovan & Herz found that APOE3 and APOE4 targeted replacement mice had different responses to high-fat diets and that the response was distinct for ketogenic (3% eCarb) and non-ketogenic (20 E% eCarb) diets (225). However, I have not identified any human studies that have disentangled the impact of the dietary parameters of interest within thesis: CFr, fiber, SAFr, and protein. Over-all, there is a robust rationale for selecting APOE as the primary effect-modifier of interest for the *diet➔cognition* analyses in *Study V* of this thesis. (Due to the high methodological focus of *Study IV*, stratified analyses needed to be addressed in a separate paper).

Insulin status was our second stratification variable of interest for several reasons. First, it has a key function in the metabolization of carbohydrates and fat and LCHF interventions have shown promising results among individuals with diabetes mellitus, type 2 (T2D) (5). Second, insulin resistance is a risk factor for AD, by possible mechanisms reviewed by Neth & Craft (128). Third, *low* insulin has emerged as a risk factor for dementia, suggesting a U-shaped pattern for the relation between insulin and dementia (226, 227). Among 1622 women living in Göteborg, Sweden, measured at age 38-60 y and followed over 34 y, the lowest (HR: 2.3 [CI 1.5–3.6]) but not the highest (1.3 [0.8–2.0]) tertile had a significantly higher risk for dementia compared to the mid tertile, while the highest tertile had increased risk for developing T2D (226). The authors argue that low insulin in this context cannot be interpreted as preclinical T2D, but rather represents a distinct hypo-insulinemic risk phenotype, which notably was over-represented by APOE4. Morris et al. have given similar suggestions on distinct metabolic AD risk types (228). I have not identified data or hypotheses on any specific response to diet by such a phenotype, which makes it a relevant target for *Study V*.

2.5 Multi-Domain Interventions: Additive or Synergistic Effects

By combining preventive strategies from several domains, e.g., diet, exercise, cognitive training, and monitoring of vascular risk factors—like in the FINGER trial (117)—an additive effect may be achieved. Potentially, synergistic effects—i.e., "the whole is more than the sum of the components"—may be detected by combining studies on single and multiple domains; such synergistic effects have been reported for, e.g., MeDi and physical activity (229).

The FINGER trial was the first multi-domain intervention to show an effect on cognitive function in older adults, which were selected by risk the CAIDE risk score and cognitive screening to achieve a sample of increased risk for dementia but without any

substantial cognitive decline (230). A Cochrane review including nine multi-domain interventions with cognitive function, MCI, or dementia as the outcome found high-certainty evidence for a small positive effect on cognitive function, with an interaction effect showing a larger effect among APOE4-carriers (231). Those authors found no clear evidence that the effect was modulated by cognitive status at baseline or the CAIDE risk score, and no effect on incident dementia was found based on the two studies reporting that as an outcome. Many multi-domain interventions are currently in the planning or performance phase globally within the World-Wide FINGERS network (WW-FINGERS) (232).

The advantages of multi-domain approaches come with the price of uncertainty regarding the contribution of each intervention domain. This may provide a rationale for accompanying multi-domain approaches with single-domain studies to shape the knowledge for that specific component.

3 Research Aims

3.1 The Overall Aim

An overall aim of this thesis was to increase the knowledge base for guiding strategic decisions on future RCT designs within the field. Just because a diet is labelled *ketogenic* it cannot automatically be assumed that *ketosis* is the primary mediator of potential effects; high ketone levels might be a marker for macronutritional changes which act by other pathways. An overall hypothesis of this thesis was that even moderate macronutritional changes may influence cognitive health, and that it is feasible to consider ketogenic supplements and time-restricted carbohydrate intake (TRC) as potential complementary strategies. Taken together with the accumulating evidence base within the field, the studies within this thesis were expected to help researchers to prioritize between investigating moderate diet changes (LCHF) versus more dramatic shifts (VLCHF), and whether to consider TRC or supplementation with coconut oil or C8 as complementary strategies.

3.2 Study I/II/III

One aim of this thesis was to investigate *strategies for inducing transient ketosis* (*Study I*). We compared coconut oil against sunflower oil (LCFA, known to be negligibly ketogenic) and C8 (100% MCFA, known to be substantially ketogenic) and hypothesized that coconut oil would have a ketogenic effect closer to C8 than to sunflower oil in older healthy adults. In addition, we tested the hypothesis that a 16-hour period without carbohydrate intake would make a substantial contribution to raised blood ketone levels, by comparing intake of the test oils *with* and *without* accompanying intake of glucose. Assessment of tolerance and satiety were exploratory research aims.

BDNF levels—as an exploratory cognitive health outcome—was investigated in relation to ketosis (*Study II*), but the initially planned cognitive testing was left out of this trial due to logistic limitations (and saved for a possible follow-up study). For the BDNF analyses we did not have a prespecified hypothesis regarding differential responses for mBDNF versus proBDNF or whether any response would be linear, delayed, or transient. We stated an open research question: Is there any BDNF response to mild/moderate ketosis within a timeframe of 4 hours?

With the aim to strengthen the internal validity of *Study I/II*, agreement was compared between a handheld ketone meter (used as our primary BHB metric) and the gold standard laboratory method for measuring blood ketone levels (*Study III*).

3.3 Study IV/V

The other main aim of this thesis was to investigate the impact of macronutritional composition (in the non-ketogenic range) on cognitive performance in older adults with risk factors for dementia. Since the field has not established a consistent methodology for selecting predictor variables, a sub-goal of this thesis (and specifically *Study IV*) became to propose a structured variable selection for describing a macronutritional pattern. With CFr as a pre-specified predictor of special interest, the aim of *Study IV* thus was to apply this methodological approach in panel analyses on the FINGER sample, exploring an expectation that reporting of eCarb and eFat would provide little information in addition to CFr.

The aim of *Study V* was to further investigate how the effect of diet on cognitive performance was modulated by genetic (APOE) and metabolic (insulin) factors. We were particularly interested in whether our data would be compatible with any of the contradicting hypotheses (210, 214, 215) regarding optimal proportions of carbohydrates versus fat for APOE4 compared to APOE3. Secondary diet parameters of interest were protein, fiber, and the ratio saturated/total fat (SAFr). Exploratory biomarker analyses were included in the research plan for a broader understanding of mechanistic relations, potential safety considerations, and a holistic analysis of the impact of diet on health.


Karolinska Institutet

Kaffe & grädde: Studiedeltagare sökes

Är du 65-75 år, vid god hälsa och intresserad av att delta i en forskningsstudie?
Vi söker dig som är daglig kaffedrickare och inte har något emot att ha lite grädde i ditt kaffe.

Studien kommer att genomföras vid Klinisk Farmakologisk Prövningsenhet (KFP) vid Karolinska Universitetssjukhuset i Huddinge under v. 33-40, med introduktionsmöten från och med 2/8. Det är en fysiologisk studie som syftar till att vi bättre ska förstå hur olika näringsämnen tas upp i kroppen.

Varje deltagare förväntas komma till KFP vid sex tillfällen med ungefär en veckas mellanrum. Du kommer att få dricka en kopp kaffe som innehåller näringsämnen i varierande proportioner. Under fyra timmar kommer vi sedan att genomföra regelbundna blodprov för att analysera hur näringsämnena påverkar olika markörer i blodet. Du kommer även att få svara på frågor och bjudas på lunch. Totalt tar varje besök ca 5-6 timmar.

Ekonomisk kompensation utgår med 5400 kr (skattepliktigt) för fullt genomförd studie. Studien är granskad och godkänd av Etikprövningsnämnden i Stockholm. Ansvarig forskare är professor Miia Kivipelto

Mejla din intresseanmälan så skickar vi mer information!
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Fig 9: Recruitment advertisement for the *Coffee & Cream* trial

4 Materials and Methods

4.1 Study I/II/III: The Clinical Trial "Coffee & Cream"

4.1.1 Study Design

A cross-over RCT design was applied. The participants were randomized to one of 4 different sequences of the treatments: A (421653), B (216435), C (164253) or D (642135). The two treatments including glucose intake (3 & 5) was consequently put in the end to avoid interference with perceived satiety or tolerance in the comparisons of the other four treatments. A wash-out period of typically 7 days—and never less than 3 days—between the exposures was assumed to eliminate any carry-over effects.

In the *Study I* article, the term "arm" is used to describe the six different *treatments*, but it may be more correct terminology to refer to the four *sequences* as arms (233). The treatments are described in Table 3.

Tab. 3: Description of treatments in the *Coffee & Cream* trial.

Treatment	Test oils	Glucose intake	Calories (≈)	MCFA
1	Sunflower oil (30 g)		300 kcal	0%
2	C8 (20 g) + Sunflower oil (10 g)		300 kcal	67% (C8)
3	C8 (20 g) + Sunflower oil (10 g)	X	500 kcal	67% (C8)
4	Coconut oil (30 g)		300 kcal	62% (mainly C12)
5	Coconut oil (30 g)	X	500 kcal	62% (mainly C12)
6	C8 (20 g) + Coconut oil (30 g)		500 kcal	78% (C8/C12)

Note: 2.5 dl coffee with 15 g full-fat cream was used as the vehicle. Glucose intake: 50 g resolved in water, 15 minutes prior to intake of test oils. MCFA: medium-chain fatty acids; C8: caprylic acid; C12: lauric acid

4.1.2 Participants

Fifteen healthy older adults (age 65–73 y, 53% females, BMI 24 ±4 kg/m^2) were recruited by advertising in a daily newspaper (Fig. 9). Exclusion criteria included diabetes, fasting during the study or the preceding month, and high-intensity physical activity more than 3 times per week.

4.1.3 Experimental Procedure

Participants were instructed to stay on their habitual diet and not make any other life-style changes during the 6-week study period. They were tested once a week by study nurses at the Clinical Pharmacology Trial Unit (CPTU) at the Karolinska University Hospital. Participants arrived at 7:30 AM to the CPTU after an overnight fast. Instructions were to avoid intake of any food or drink beyond water after 8 PM the preceding evening, and any self-reported deviation from that was registered by the study nurses. After baseline blood draw, participants were served the test oils in a covered cup around 8 AM. Coffee with 15 g full-fat cream was used as the vehicle, and glucose was ingested dissolved in water 15 minutes prior to coffee intake when applicable (exposure 5-6 only). For the subsequent 90 minutes, blood draw was performed every 15 minutes and thereafter every 30 minutes until the end of the 4-hour study period. Before leaving the CPTU, participants answered a questionnaire on satiety and tolerance, including registration of any adverse events. Beyond the BHB measures with the handheld meter, and BDNF which was analyzed by members of our group (45), all biomarkers were sent to two different hospital laboratories for analyses.

4.1.4 Statistical Analyses

4.1.4.1 Study I

Levels of the ketone BHB (AUC) in the different treatments were compared by ANOVA for repeated measures. For comparisons of concentrations between different timepoints (as categorical variables since levels did not change linearly), a mixed regression model was used. A significance level of $p<0.05$ was applied.

4.1.4.2 Study II

In line with *Study I*, ANOVA for repeated measures was used for comparing AUC measures between the treatments and mixed regression was used for comparing levels between timepoints. In contrast to *Study I* where *absolute* AUC levels of BHB was our interest, here we analyzed AUC for BDNF in relation to their baseline level.

Repeatability (intra-individual stability of the repeated baseline measures) was assessed by calculating intra-class correlation coefficients (ICC) (234).

4.1.4.3 Study III

We expected graphical analyses to be informative and used that as a starting point for analyses. Agreement, i.e., comparisons of values from different measurement methods in *absolute* values, was quantified by *Lin's concordance correlation coefficient of absolute agreement* which has a value in the range –1 to 1. It can never be larger (in absolute values) than the correlation coefficient Pearson's *r,* which we used for comparing methods in *relative* terms.

After excluding very low BHB values (≤0.2 mmol/L, considered as absence of ketosis) the methods were compared by linear regression. An exploratory non-parametric Passing-Bablok regression was added in the comparison of capillary and venous measures with the handheld meter since we identified it to be a frequently reported analysis in the methods comparison field.

4.1.5 Ethical Considerations

This study investigated "fat coffee" which was already widely applied in the population at that time after being popularized in social media and the health literature. An initial consideration was that the research community may have a responsibility to allocate resources for increased understanding of health effects of such spontaneously implemented trends.

We did not expect the participants to be exposed to any risk within this study, beyond potential transient mild/moderate gastro-intestinal symptoms or inconvenience related to blood draw. Since participants were tested by experienced study nurses at a university hospital, any adverse effect was expected to be rapidly and adequately handled. The benefits of increased knowledge generated by the study was expected to clearly outweigh any risks; hence, we considered the study ethically defendable. Ethical approval was achieved by the Regional Ethical Review Board in Stockholm. Written informed consent was given by all participants, and they were informed that they could withdraw participation at any time.

4.2 Study IV/V: Panel Analyses on FINGER Data

4.2.1 Development of Analysis Framework and Study Design

After concluding that variability in diet was substantial both *within* and *between* subjects (shown by ICC in Fig. 10), a study design incorporating both those dimensions was chosen. Since I did not identify any previous nutrition study with a similar design—which allows disentangled analyses on within-effects and between-effects—references from other fields, e.g., biology (235) and epidemiology (236), were used for guidance. The approach relies on that both the independent (X) and dependent (Y) variables are repeatedly measured, and that Y is not an irreversible outcome, e.g., disease conversion. Many nutrition studies use data from a single time-point (➔no within-subjects variability in X) and/or an irreversible Y, and that may explain why this methodology is rarely seen in the nutrition field.

Although it would be possible to perform three cross-sectional analyses with this data structure, that is not a typical approach for panel data (and only included as an additional sensitivity analysis in *Study IV*). Rather, two different strategies can be used, either alone or combined (235): In the pure between-subjects analysis of X➔Y, intra-

individual mean levels of X and Y are compared between subjects, meaning that each subject contributes with one data point (X, Y) which is a collapsed mean of its repeated measures. To assess the within-subjects effect for X➔Y, each longitudinal data point within a subject is analyzed in relation that subjects' own intra-individual mean of X and Y, thereby generating a within-slope for each subject, which then can be summed for all subjects and "averaged" to an over-all within-subjects effect. Analogous with a cross-over trial, the within-slope is not confounded by variables that is consistent within subjects over time, even unmeasured traits like genetics or "health-awareness" (236). A mixed effects model can be used to integrate the between- and within-components in the same estimate.

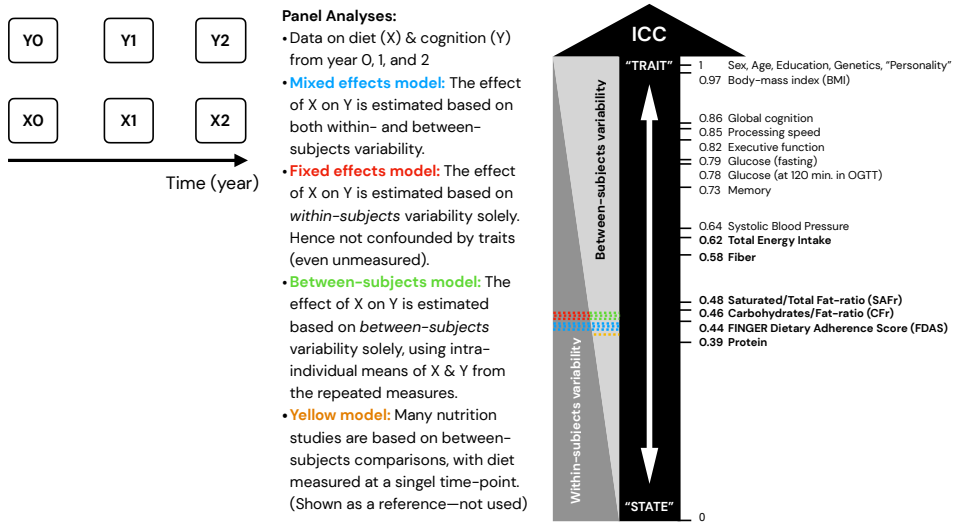


Fig. 10: Description of data structure and analysis of between- versus within-subjects variability.
Color coding illustrate how various analytical approaches take advantage of different components of the information within the data set. Each dotted colored line represents data from one year. Intra-class correlation coefficients (ICC) indicated for variables in the FINGER dataset (n=1251; year 0, 1, 2)
OGTT: Oral glucose tolerance test

The key difference between observational and RCT science is that X (the potentially causal agent of interest) is *passively observed* versus *actively manipulated*. For FINGER, only the multi-domain intervention *as a package* has an RCT interpretation. In this case, X (some diet parameters) were indeed manipulated by *given recommendations*—with different intensity for the randomization groups—and we needed to understand whether that had substantial impact on the variability in X. However, only 1-2% of the variability in all diet variables (as indicated by R^2 in a linear regression) could be explained by *time*, *time* x *randomization group*, and *age* combined. Even when including multiple relevant covariates, R^2 rarely exceeded 5%. This suggests that variability in diet within our data

primarily reflect *spontaneous* (unexplained) levels and changes, which should justify an observational approach. The weak impact of *age* (interpreted as a proxy for preclinical disease progression) would suggest that reverse causality may not be a serious issue here. In the preparatory work, I further concluded that intra-individual range between the highest and lowest value of X was substantial (median ≈1 SD) and that the order of high-mid-low values appeared unsystematic ("random") within subjects (as demonstrated in supplementary material of *Study IV*).

Within-subjects variability may have two primary sources: 1. Measurement error, and day-to-day fluctuations where a 3-day food record may not capture habitual diet with exact precision. If this was thought to be the primary source of variability, the between-subjects approach (based on collapsed mean level of X) might be the most valid. 2. True changes in habitual diet between the years. If this was thought to be the primary source of variability, the within-subjects approach may be more relevant. By assuming that variability represents a mix of 1 & 2, a mixed effects approach was considered most appropriate and chosen a priori as the primary estimate. However, additional reporting of the separate sub-components (within/between) was expected to facilitate interpretations: The between-effect was conceptually assumed to represent a long-term level of diet over several years, while the within-effect may indicate effects of dietary *changes* within the study period in relation to corresponding changes in the outcome. For clarification, the within-effect is not a linear trajectory over *time,* but a representation of "change versus change" in X and Y. Compared to analyzing "change versus change" in terms of ΔX➔ΔY (Δ=difference between year 0 and 2) in a between-subjects comparison, the current approach has important advantages: 1. It incorporates the data from year 1, which is valuable as we now know that X does not change *linearly* over time. 2. It is not confounded by "traits" (time-invariant variables) of the subjects. The within-effect is synonymously referred to as a *fixed effect* (236).

The methodology and study design are extensively described in *Study IV*, which then serves a methods reference for *Study V*.

4.2.2 Participants

The data was collected within The Finnish Geriatric Intervention Study to Prevent Cognitive Impairment and Disability (FINGER), a multi-center (n=6) RCT in older adults (N=1259, age 60–77 years at baseline, 47% females). The participants had no substantial cognitive impairment (MMSE=26.7 ± 2.0), but an inclusion criteria was to have ≥6 points on the CAIDE risk-score (Tab. 5.) to indicate increased predicted risk for developing dementia within 20 years (50, 51). They were further screened by cognitive testing with the Consortium to Establish a Registry for Alzheimer's Disease (CERAD) neuro-psychological battery, to include only individuals with cognitive performance at the mean level or slightly lower than expected for age. An inclusion criterium was to fulfill at

least one of the following: (1) Word List Memory Task (10 words x 3) ≤19 words; (2) Word List Recall ≤75%; or (3) MMSE ≤26 points.

Tab. 5: Description of the CAIDE risk score

Risk Factor	Category	Score
Age (years)	< 47	0
	47–53	3
	> 53	4
Sex	Female	0
	Male	1
Education (years)	≥ 10	0
	7–9	2
	< 7	3
Hypertension (Systolic Blood Pressure)	≤ 140 mmHg	0
	> 140 mmHg	2
Total Cholesterol	≤ 6.5 mmol/L	0
	> 6.5 mmol/L	2
Obesity (Body-Mass Index)	< 30	0
	≥ 30	2
Physical Inactivity	No	0
	Yes	1

Exclusion criteria were malignant diseases, major depression, dementia, MMSE <20, symptomatic cardiovascular disease, revascularization within 1 year, severe loss of vision/hearing/communicative ability, conditions preventing co-operation as judged by the study physician, and co-incident participation in any other intervention trial.

From a global perspective, a few points may be noticed regarding this Finnish sample. After experiencing exceptionally high CVD prevalence in the middle of the last century, pioneering public health campaigns for risk reduction were implemented in Finland at a time when this sample was in their young adulthood (237), potentially implying relatively high health-awareness in the sample. One component of those campaigns was a reduction in total fat intake towards ≈30 E% from levels at ≈40 E% in the 1960's (238).

For unknown reasons, Finland has the highest mortality rate from dementia in the world [≈70% higher than other Nordic countries (239)] and a 2017 paper hypothesized that toxic factors in the environment might contribute to that (240). Access to diagnostics and care, and changes in practices to record death might also play a role. Additionally, the relatively high APOE4 prevalence in the Nordic countries [20–30% (219)] should be a contributing factor. In summary, the FINGER trial may be seen as a component of a long tradition of public health work in Finland.

4.2.3 Database

A STATA data file was provided by the FINGER study team after sending a data application form.

4.2.4 Statistical Analysis

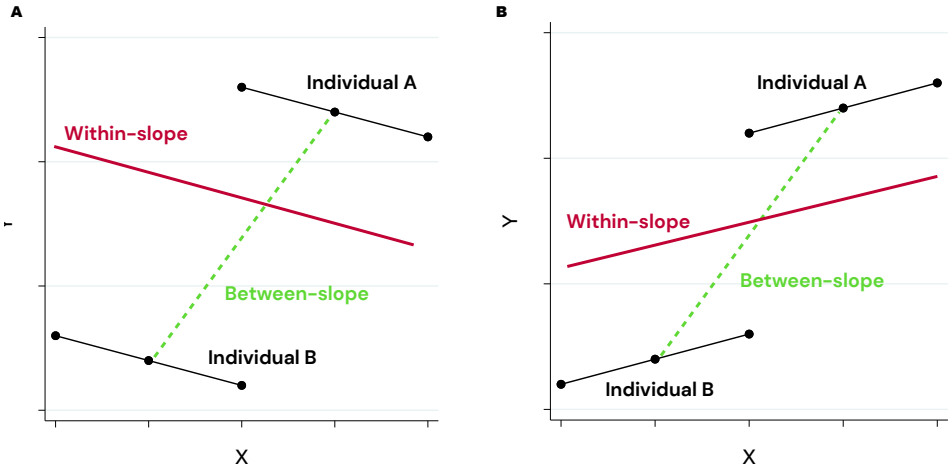


Fig. 11: Hypothetical patterns of disentangled between- and within-subjects effects. Data points represent repeated measures (in no specific order) for individuals A and B. Panel **A** illustrates Simpson's paradox, which could arise from the possible scenario when intake of food X—with a truly detrimental effect on Y—is higher among individuals with higher levels of the trait "health-awareness". Such individuals may have higher levels of Y *for other reasons*, which confounds the between-slope if that trait is not measured and adjusted for. In contrast, the within-slope (fixed effects) is not confounded by traits of the subjects and therefore detects the negative causal effect of X on Y. Panel **B** illustrate a coherent pattern with the same direction for both slopes, which may indicate a lower risk for unmeasured confounding. The differing magnitudes might arise from remaining confounding but may alternatively reflect that the between-slope likely captures a cumulative effect of a longer timeframe. For the between-slope, each individual contributes with one data-point: its collapsed mean levels of X and Y.

Linear regression models with X and Y as continuous variables were applied, unless non-linearity was identified in graphical linearity checks performed on all X. A mixed regression model—with *study site* and *individual* as random factors to account for clustering—was defined a priori as our primary estimate. This was based on the assumption that it most extensively incorporates the information within the dataset, by

integrating within- and between-effects (235). We additionally report fixed-effects [STATA: xtreg Y X, fe] and between-effects [xtreg Y X, be] to disentangle within- and between-effects, as motivated in 4.2.1. Moreover, separated slopes give us an opportunity to identify the potential phenomenon known as Simpson's paradox (241), illustrated simplified with just two hypothetical subjects in Fig. 11. Such opposing directions of the within- and between-slopes should raise questions on unmeasured confounding, as exemplified in the figure legend.

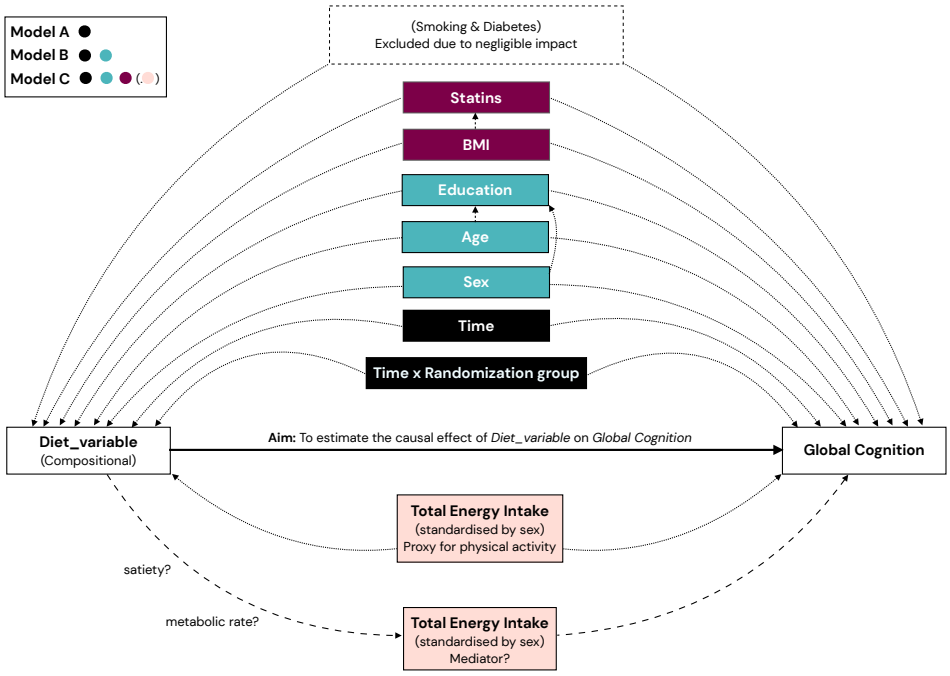


Fig. 12: Directed Acyclic Graph showing the assumed causal structure for Study IV. Striped lines indicate an alternative interpretation for total energy intake (TE). Some main pathways are illustrated but TE may have additional incoming arrows from some covariates above, e.g., age. Model labels refer to Study IV. *Statins* refers to any cholesterol lowering drug.

To optimize the likelihood that our estimates may have a causal interpretation, covariate selection was made according to causal inference theory (103), as illustrated by the DAG in Fig. 12. Adjustment for *time*, and *time* x *group*, aimed at mitigating bias from the intervention effect of the trial, aging, potential learning effects on the cognitive tests, and potential social trends in food composition. For total energy intake (TE), we considered alternative interpretations. Our primary assumption was that it may be a confounder as a proxy for physical activity, since that is one of the most important factors for inter-individual differences in TE (66). An alternative interpretation might be that it is a mediator, since the composition of macronutrients could potentially affect the magnitude of ad-libitum energy intake (242) or total energy expenditure (TEE) (243). *BMI* and use of *statins (or other cholesterol lowering drug)* was considered as

confounders, assuming they represent a selection bias with impact on diet as well as cognitive performance. From a longer time-perspective, it may be possible to consider a reversed direction of causality, i.e., compositional aspects of diet may impact *BMI* and *statins*, making them possible mediators rather than confounders. We did however consider the other interpretation more likely within the timeframe of this data collection. We defined Model C + TE our primary model a priori but reported multiple models in *Study IV* to explore the impact of various covariates. In *Study V*, *APOE* and *APOE* x *time* was added, since cognitive progression over time was confirmed to be modulated by APOE, as we expected.

4.2.5 Ethical Considerations

These analyses were performed on already collected data and did not expose the participants to any risk. The data collection was ethically approved by Finnish authorities.

5 Results

5.1 Study I: Ketogenic Strategies

Caprylic acid (C8) raised ketone levels (BHB) significantly more than coconut oil and sunflower oil, which did not differ significantly from each other (in area under the curve [AUC] for the 4-hour study period). C8 intake was followed by substantially lower BHB levels *with* (compared to *without*) glucose intake, and those levels were not significantly higher than for coconut oil *without* glucose intake. The ketogenic contribution from a 16-hour non-carbohydrate window (12-h overnight fast + no glucose intake) appeared to be in the same range as the effect of 20 g C8 intake. The ketogenic response was very similar whether 20 g C8 was combined with 10 g sunflower oil or 30 g coconut oil, further indicating negligible ketogenic properties for coconut oil over sunflower oil. Some individuals experienced transient BHB peaks in the range 1–1.5 mmol/L, while the mean BHB level (AUC/time) was 0.45 mmol/L during the 4-hour study period after intake of 20 g C8 without glucose (regardless whether combined with 10 g sunflower oil or 30 g coconut oil). C8 with glucose (0.28), coconut oil (0.22), sunflower oil (0.18), and coconut oil + glucose (0.08) were all significantly lower than the two non-glucose C8 treatments.

Mean blood glucose decreased similarly (≈0.3-0.5 mmol/L) during the first hour for all non-glucose treatments, and the magnitude of the decrease correlated with increase in BHB. Blood glucose remained stable during the rest of the study period. After glucose intake, blood glucose increased as expected.

Substantial hunger at the end of the study period was only reported by 7-20% of the participants, and lower caloric content (≈300 vs. 500 kcal) was not (descriptively) associated with more hunger. An anecdotal observation was that the participant (of normal weight) who consequently reported substantial hunger, had a remarkably low ketogenic response. Another unreported anecdote was that a metabolic outlier (BMI>30 kg/m^2, fasting insulin trending towards prediabetes) had a substantially delayed (by hours) ketogenic response.

Tolerance, assessed by self-reported inconvenience, appeared mostly good. In the lower-caloric treatments without glucose intake, most participants reported "no inconvenience": sunflower oil (93%), C8 (87%), coconut oil (80%). Otherwise, reported inconvenience in those arms was primarily *minor*, beyond one *moderate* (coconut oil). For treatments with glucose intake, ≥80% reported *no/minor* inconvenience while 2 subjects reported *moderate* and 1 reported *major* inconvenience in the combination with coconut oil. One participant experienced severe diarrhea in the evening following the first study visit (when this individual ingested coconut oil). Connection to the treatment was considered possible/probable and that participant was excluded from the study for safety reasons and replaced by another participant from the waiting list.

5.2 Study II: Ketosis ➔ BDNF

Mature BDNF (mBDNF) was positively associated with BHB at baseline but had no association with change in BHB after intake of the test oils. The highest increase in mBDNF was after treatment with sunflower oil (where BHB increased the least), but it should be noted that mean baseline levels of mBDNF were remarkably low for that treatment.

proBDNF—the precursor and functional antagonist to mBDNF—increased significantly more after intake of *C8* (which was concluded to be the ketogenic agent) *+ coconut oil* compared to *sunflower oil* or *coconut oil* alone. We also found a delayed association between BHB (increase hour 0–2) and proBDNF (hour 0–4).

The results reported here is a selection from a larger pool of analyses which had no prespecified internal priority. With all $p \leq 0.007$ (except baseline mBDNF: $p=0.02$) we considered statistical significance acceptable even in the context of multiple comparisons.

5.3 Study III: Methods Comparison for Ketone Measures

A main finding was that all methods for measuring ketones correlated well ($r \geq 0.88$) with each other. However, agreement in absolute values with the gold standard laboratory method—measured by Lin's concordance correlation coefficient of absolute agreement—was higher when the handheld meter was applied to venous (0.91) compared to capillary (0.73) blood. In a linear regression, BHB in capillary blood was 27% higher than in venous blood. However, the two handheld measures had similar correlation with the laboratory test ($r=0.91$–0.92).

To get a similar proportion of observations classified as *ketosis* for venous (handheld or lab) and capillary measures, we showed that adjusted cut-off points at either 0.3 together with 0.5, or 0.5 with 0.8 mmol/L would be suitable.

In the laboratory method, AcAc added negligible information to *total ketones* (AcAc+BHB), since BHB alone correlated almost perfectly ($r=0.99$) with total ketones. Interestingly, BHB in the capillary fingerpricks had good agreement (0.91) with *total* ketones.

5.4 Study IV: Diet ➔ Global Cognition

5.4.1 Published Main Results

A *lower CFr* was associated with *better global cognition* in our primary model ($\beta=-0.022$, CI: −0.039, −0.005; $p=0.011$), with the largest magnitude in the *memory* domain ($\beta=-0.028$, $p=0.005$). The results changed very little when we exploratorily added or subtracted various covariates. TE (which was adjusted for in the estimates above) was

the most influential covariate and reduced the magnitude of the unadjusted estimate by ≈20%. *Protein* had no substantial trend of association with cognition. *SAFr* appeared to have an inversely U-shaped association with global cognition in the graphical linearity check, and that was confirmed by a cross-sectional baseline analysis by quintiles, indicating significantly lower cognition in the extreme quintiles compared to the mid-quintile. *Fiber* was not associated with global cognition.

5.4.2 Methodological Results (unpublished)

In the article we provide estimations suggesting that dividing the β-coefficient for CFr (log-transformed and standardized) with 6.3 would correspond to 1 E% exchange between eCarb and eFat. Here we add a comparison with a conventional substitution-model ("leave-one-out" (66)) for eCarb vs. eFat and conclude that both approaches give similar results: The difference in β was 1-4%–depending on whether we adjusted our CFr-model for eProt, eAlc, eFib or not—but our CFr-estimate had a narrower CI (estimates not shown).

Tab. 5: Comparison of magnitudes of within/between/mixed effects.

Estimated effect on global cognition (z) of replacing 15 E% eCarb with eFat. (CFr-β/6.3 x 15)				
Sample	**Mixed effects**	**Between-effects**	**Within-effects**	**B/W-ratio**
All	0.060	0.25	0.038	6.5
"Compliant"	–	0.38	0.079	4.8

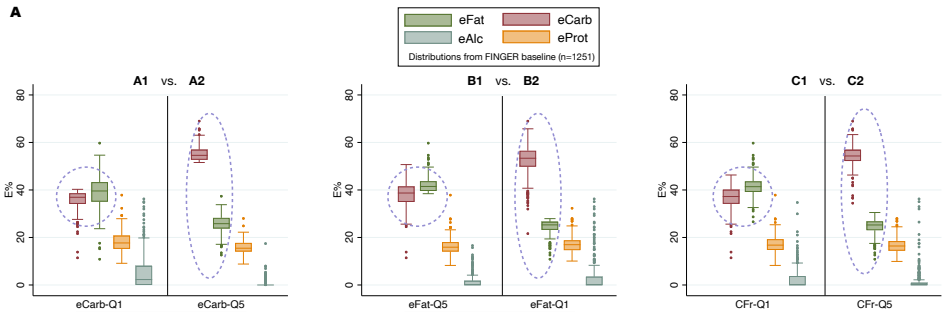
Note: n=947 with complete data from all timepoints. "Compliant" defined by median-split of intra-individual CFr-range, as clarified in text below this table, meaning that an individual is either compliant for the within- or between-analysis but never for both. Adjusted for age, sex, education, BMI, statins, total energy intake, time, time x group, and study site (time-invariant variables automatically excluded for within-models). B/W-ratio: between-subject effect divided by within-subject effect; CFr: carbohydrate/fat-ratio.

A comparison of the effect sizes of between-, within- (fixed), and mixed effects is shown in Tab. 5, including a "per-protocol" analysis (111) with only those who were considered "compliant" to a hypothetical target trial. Those were defined by median split of their intra-individual range of CFr as either *CFr-stable* (compliant to the long-term parallel-design, assumed to underly the between-slope) or *CFr-unstable* (by larger contrast in CFr between the timepoints, more compliant to the cross-over design assumed to underly the within-slope). The cut-off was ≈7 E% exchange eCarb versus eFat between their highest and lowest measure of CFr. Notably, the mixed effect is much closer to the within-effect than the between-effect. Assuming the within-effect captures diet changes made (on average) half-way since the last visit, i.e., 6 month, a

potential cumulative effect over 2-years would be ≈4 times larger. That would come quite close to the between-effect, as indicated by the table above.

A comparison of CFr, eCarb, and eFat as predictor variables is performed in Fig. 13, providing evidence that—despite their different labelling—those three variables represent the same dietary parameter within this dataset.

What dietary parameter are we primarily investigating when we compare quintiles A1 vs. A2, B1 vs. B2, and C1 vs. C2 respectively on health outcome Y?



Conclusion: All (A/B/C) are comparisons between a lower and a higher CFr!

- A & B do *not* exclusively represent eCarb & eFat respectively just because we put that label on them.
- eCarb and eFat confound each other so substantially that it is impossible to disentangle their impact.
- The only parameter that can be investigated is CFr, for which eCarb & eFat are proxies.
- Confounding by eAlc/eProt-outliers in A and (reversely) B, but less in C; may explain differing estimates.

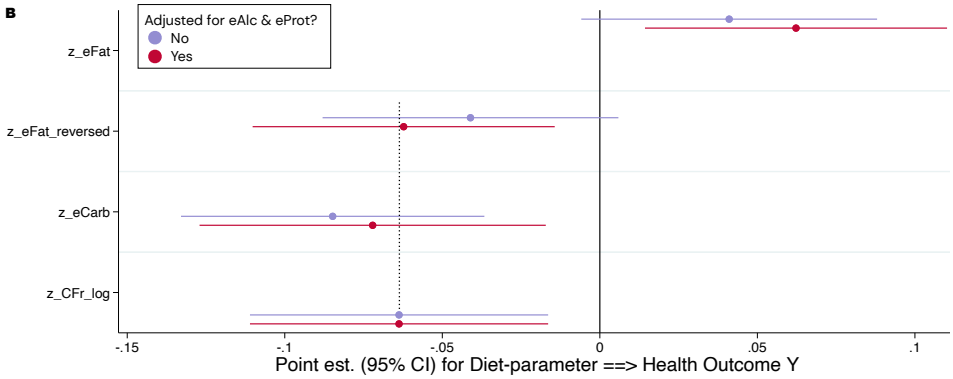


Fig. 13: Methodological analysis of the variables CFr, eCarb, and eFat. Panel **A** uses extreme quintiles based on eCarb, eFat, and CFr respectively to illustrate the reciprocal relation between eCarb and eFat. Panel **B** uses the same variables as continuous predictors to show how eAlc/eProt confound the results (in opposite directions) for eCarb and eFat, but not for CFr which have a more even distribution of eAlc/eProt. Y is a masked health outcome in FINGER data estimated by mixed linear regression. Q: quintile; eCarb, eFat, eFib, eAlc: carbohydrates, total fat, fiber, and alcohol as percentages of total energy intake (E%)

The conclusion is not limited to extreme quantiles but represent a linear reciprocal relation between eCarb and eFat, as shown in Fig. 14 which plots the corresponding distributions of macronutrients for all observations. Estimates in regression models are generated from *observations* which may be organized according to increasing level of,

e.g., eFat, eCarb, or CFr, as shown. The figure illustrates that in a shift towards an observation with a higher level of eCarb, that observation is inevitably predicted to have a lower level of eFat, of an approximately similar magnitude. Based on these analyses—which were in line with my expectations—I felt confident to exclude eCarb and eFat as predictor variables in Study V.

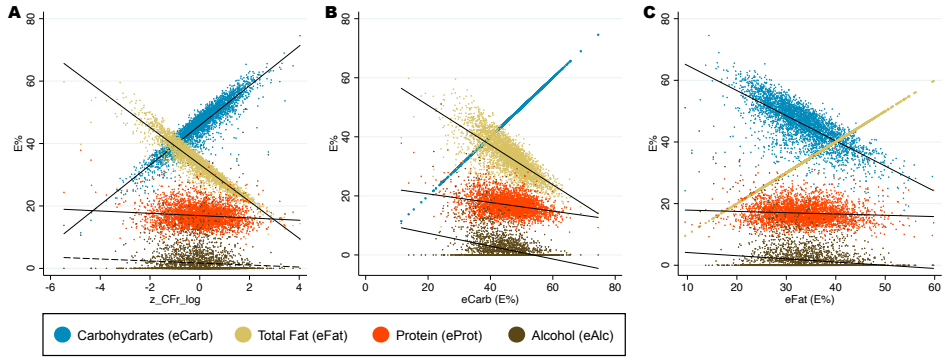


Fig. 14: Interpretative analysis of the variables CFr, eCarb, and eFat. Each observation within the FINGER dataset (n=3753) is plotted by four vertically aligned points indicating the levels of eCarb, eFat, eProt, and eAlc respectively. Fitted regression lines are shown (significant correlation unless striped).

To confirm my assumption that the validity of interpreting a macronutrient-ratio as iso-caloric replacement (between the numerator and the denominator) is unique for eCarb and eFat, a corresponding analysis was made for eCarb versus eProt. A crude regression on macronutrient levels with the standardized log-ratio eCarb/eProt (CPr) as the predictor suggested that CPr represents 5.2 E% increase in eCarb, 2.7 E% decrease in eProt, and 2.3 E% decrease in eFat. Corresponding analysis for eProt/eFat (PFr) suggested that PFr represents +2.2 E% eProt, −4.7 E% eFat, and +2.2 E% eCarb (the residuals may represent eAlc and eFib). A difference of 1 SD in CFr predicts a 0.3 E% change in eProt, and that comparably small effect gives CFr the interpretation of *≈6.3 E% exchange between eCarb and eFat* within this sample.

5.5 Study V: Diet ➔ Global Cognition (by APOE & Insulin)

5.5.1 Main Results Submitted for Publication

As the article is not yet peer-reviewed and published, numerical reporting is minimized here.

The estimates *diet ➔ cognition* were significantly modified by APOE-genotype for all prespecified diet parameters (CFr, protein, fiber, SAFr) and for a composite score (*comp* = mean z-scores of CFr, fiber, and inversely protein) defined a posteriori. Beyond interactions in the complete stratification (APOE23/24/33/34/44), a continuous APOE-gradient [coded −1 (23), −0.5 (24), 0 (33), 1 (34), 2 (44)] was a significant effect modifier

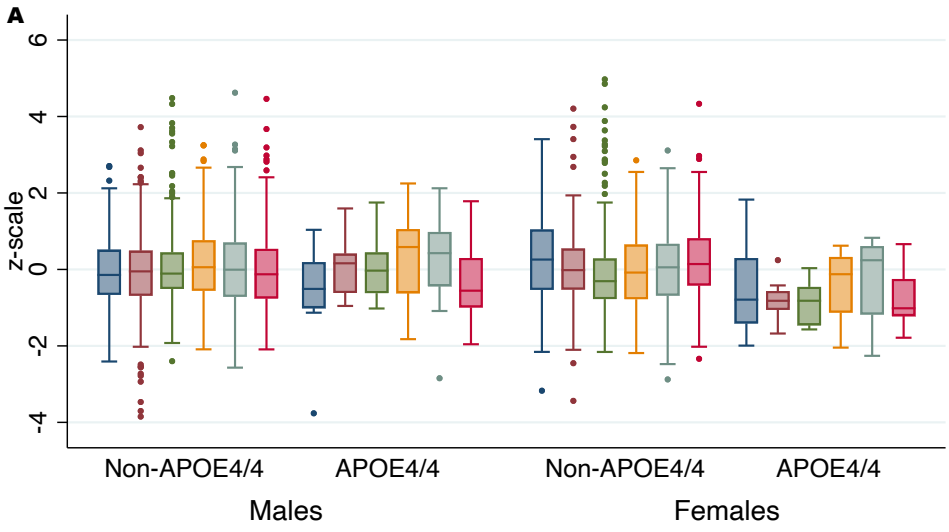
for *CFr*, *protein*, and *comp* in relation to *global cognition*. The gradient was implied by the categorical analyses and validated by excluding each stratum at a time. The results suggested that it was valid to dichotomize APOE [34/44 (APOE+) vs. 23/24/33 (APOE−)] for increased power in subsequent analyses stratified by insulin status.

At increasing levels of the APOE-gradient (23-24-33-34-44), the slopes for *diet➔cognition* became less favorable for *CFr*, *fiber*, and *comp*, and more favorable for *protein*. For the stratum in the middle of the gradient—APOE33, the most common genotype—there was no trend of association with *global cognition* for any of the investigated diet parameters (all p>0.24), and the point estimates were relatively close to zero. APOE23 and APOE44 had antagonistic directions of the point estimates for all diet variables in relation to cognition.

The insulin stratification was initially performed by tertiles—with special interest of the lower tertile, which had increased risk for dementia in a previous study (226)—but after dose-dependent associations were implied by sensitivity analyses by quartiles, median-split, and quintiles, we decided to use a graphical model combining those multiple stratifications. For *CFr*, *fiber*, and *comp*, *insulin* modified the slopes *diet➔cognition* primarily for APOE+, in a quadratic-like manner with increasing (negative) magnitudes towards both ends of the insulin spectrum. For protein, (positive) effect modification was only seen at increasing insulin levels in the APOE+ stratum, and for *SAFr* no effect modification by insulin was observed.

A sensitivity analysis by sex for CFr revealed an anomaly to the over-all pattern: female APOE44 (n=11) had a positive association *CFr➔cognition* with a significant interaction against male APOE44. Since this is the stratum at highest risk for AD, we found it motivated to exploratory report more stratified analyses for those. The sex interaction was however not significant for any other diet variable, although some trends in the same direction could be seen. Female APOE44 stood out from the sample for several descriptive baseline parameters as shown in Fig. 15 (and further by supplementary material in *Study V*).

The results of the mixed model appeared more influenced by within- than between-subject effects, and several results were significant also in the exploratory fixed effects (within) model. Based on estimations from the composite score (*Study V*, Tab. 2), the magnitude of the within-estimate for APOE34 and 44 was substantial in a hypothetical shift from diet A (eCarb:eFat:eProt:eFib) with the distribution 35:41:21:1.4 (E%) to diet B (58:25:13:3.1). Global cognition was estimated to be about *half a standard deviation lower* with diet B compared to A for APOE44. For APOE34 the magnitude was ≈50% compared to APOE44.



Baseline distributions (n=676). Boxes indicate percentiles 25, 50, 75. Log-transformed variables, except Systolic Blood Pressure.

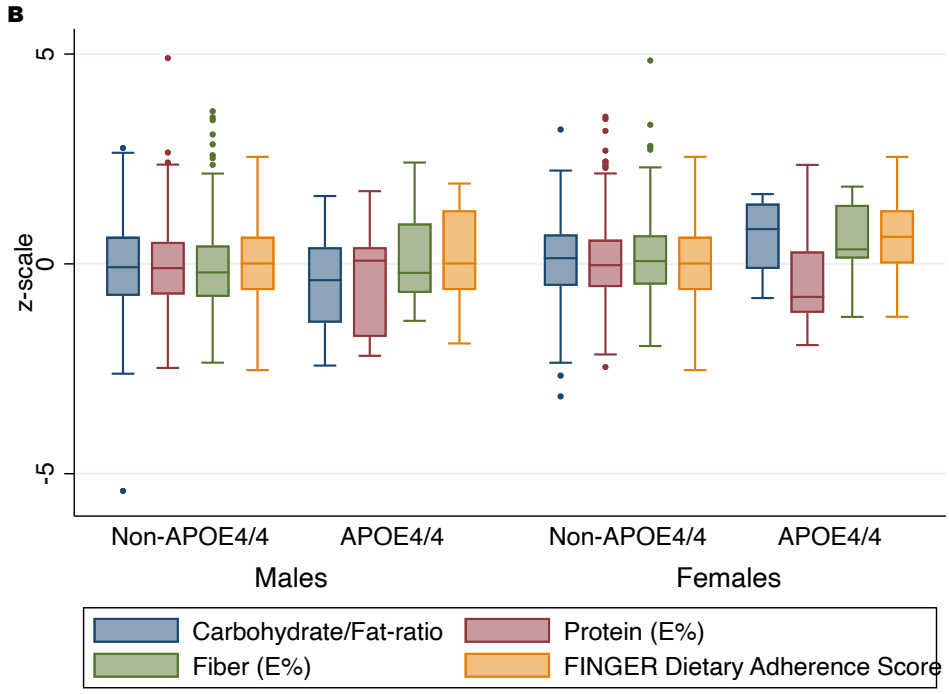


Fig. 15: Descriptive biomarker (A) and diet (B) distributions at baseline for APOE44 by sex. Boxplots indicate percentile 25, 50, and 75. hsCRP: high-sensitivity C-reactive protein. A few outliers excluded for enhanced scaling, but none of those were APOE44. See supplementary material to *Study V* for complete APOE-stratification and more variables.

5.5.2 Complementary Biomarker Analyses (unpublished)

In the research plan of this project, several exploratory biomarker analyses where specified, aiming to give a more holistic *diet* ➔ *health* interpretation beyond the cognitive analyses, and possibly add mechanistic understanding. Some key analyses that could not be included in the article are reported below.

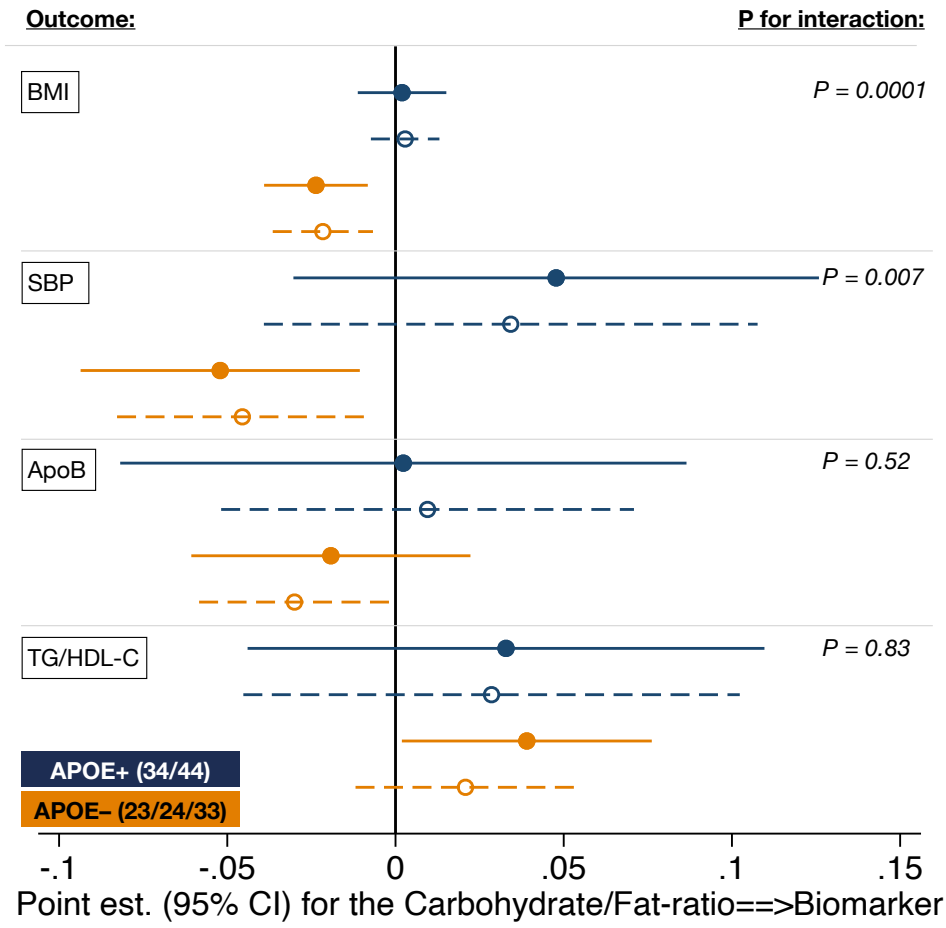


Fig. 16: Associations between the carbohydrate/fat-ratio (CFr) and biomarkers by APOE. Mixed linear regression with study site and subject as random factors. Diet and outcomes measured at year 0, 1, and 2 (n=676, 207/469 APOE+/–; all non-diabetic). Striped/hollow adjusted for *time*, and *time* x *randomization group*. Solid additionally adjusted for *age*, *sex*, *education*, *BMI*, *cholesterol-lowering drugs*, and *total energy intake standardized by sex*. P-values for interaction by APOE refer to the fully adjusted model. BMI: body-mass index; SPB: systolic blood pressure; ApoB: apolipoprotein B; TG/HDL-C: ratio triglycerides/HDL-cholesterol. All outcomes except SPB were log-transformed. Standardized coefficients.

The aim was to use the binary stratification APOE34/44 (APOE+) versus APOE23/24/33 (APOE–) for all analyses, as shown for *BMI*, *systolic blood pressure*, *ApoB*, and the *triglycerides/HDL-cholesterol ratio* in Fig. 16, but after checking validity of the

dichotomization in sensitivity analyses with complete stratification (23/24/33/34/44), it turned out that *glucose* and *insulin* called for complete stratification due to outstanding estimates for APOE44 (Fig. 17-18). The APOE interaction for *CFr➔ BMI* was followed up by a quantitative estimation of *CFr➔weight* for APOE–. That suggested that replacing 10 E% eFat with eCarb corresponded to ≈0.4 kg lower weight among APOE– (p=0.004), with no effect (0.0, p=0.72) for APOE+, and an interaction effect at p<0.0001. The APOE– estimate was similar in a fixed-effects model (p=0.030).

Since an interaction had been found between male and female APOE44 for *CFr➔Cognition*, exploratory sex stratifications were performed. However, interaction was not significant for *fasting glucose* (p=0.18), *OGTT* (p=0.86), or *insulin* (p=0.73). Nevertheless, there was a trend that females were driving the 44-results for fasting glucose; the estimate *CFr➔Fasting glucose* was β=–0.28, p=0.005 for female APOE44. Since *insulin* was only measured at baseline (fasting), it was only possible to perform a cross-sectional analysis on that variable and the sample size was slightly reduced (n=676➔659) due to missing covariate data. Fiber was added as a predictor since it is expected to influence insulin status.

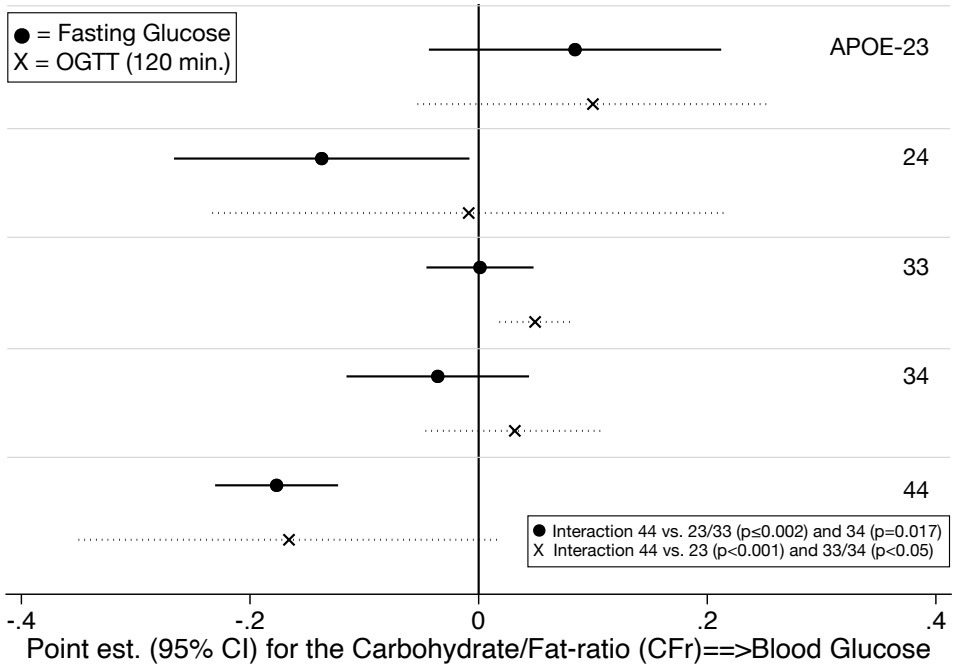


Fig. 17: Associations between the carbohydrate/fat-ratio (CFr) and blood glucose. Mixed linear regression with study site and subject as random factors, adjusted for *age*, *sex*, *education*, *BMI*, *cholesterol-lowering drugs*, and *total energy intake standardized by sex, APOE, time*, *time* x *randomization group*. Diet and outcomes measured at year 0, 1, and 2 (n=676/577 for fasting /OGTT, n= 61/17/391/183/24 for APOE23/24/33/34/44; all non-diabetic). Glucose and CFr were log-transformed. Standardized coefficients. OGTT: oral glucose tolerance test

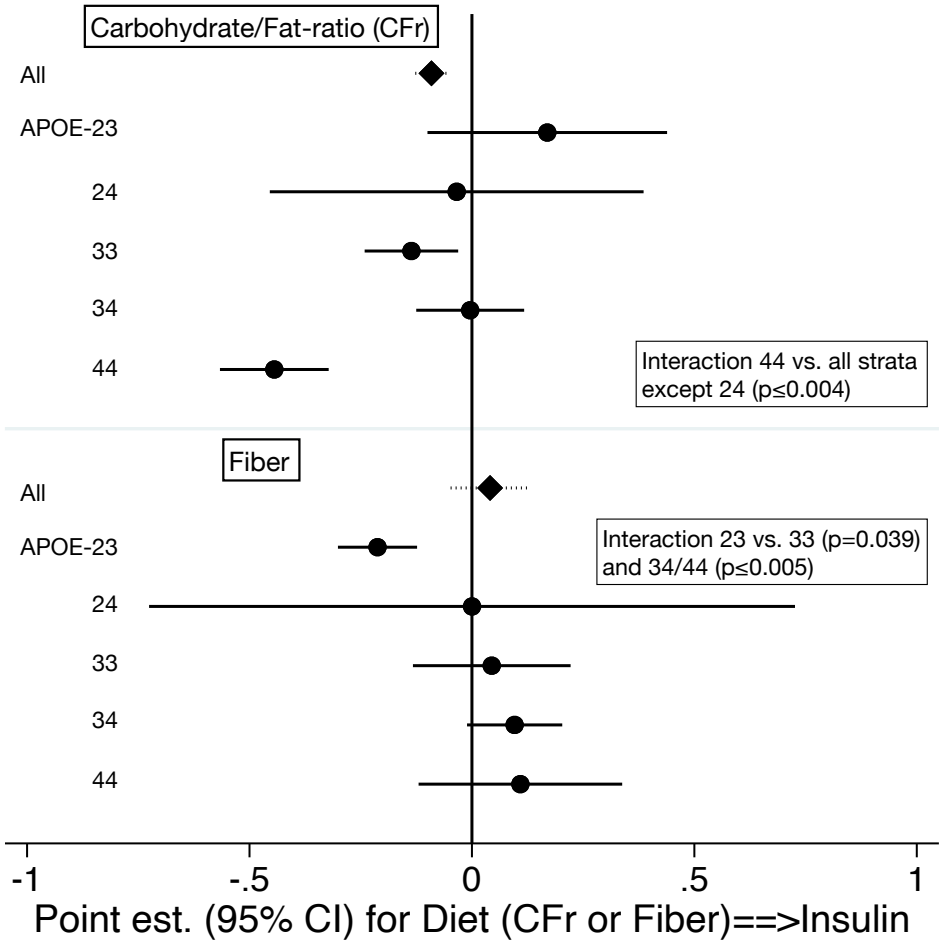


Fig. 18: Associations between the carbohydrate/fat-ratio (or fiber) and insulin. Mixed linear regression with study site as random factor. Cross-sectional analysis at baseline (n=659 [57/17/384/177/24 for APOE 23/24/33/34/44]; all non-diabetic). Stratified analyses on APOE-genotype, except diamond which indicate full sample. Adjusted for *age, sex, education, BMI, cholesterol-lowering drugs, total energy intake standardized by sex,* and *APOE*. CFr and fiber also mutually adjusted for each other (but this did not substantially affect the results). Insulin and CFr were log-transformed. Standardized coefficients.

6 Discussion

6.1 The Clinical Trial "Coffee & Cream"

6.1.1 Study I: Ketogenic Strategies

Some important conclusions on ketogenic strategies among older adults following their usual diet may be drawn from the *Coffee & Cream* trial: 1. Contrary to our hypothesis when preparing the project plan it turned out that blood levels of the ketone BHB differed negligibly after intake of coconut oil versus sunflower oil. 2. In line with our hypothesis, extending the overnight non-carbohydrate window until noon contributed with roughly the same magnitude of ketosis (AUC, hours 0-4) as intake of 20 g C8.

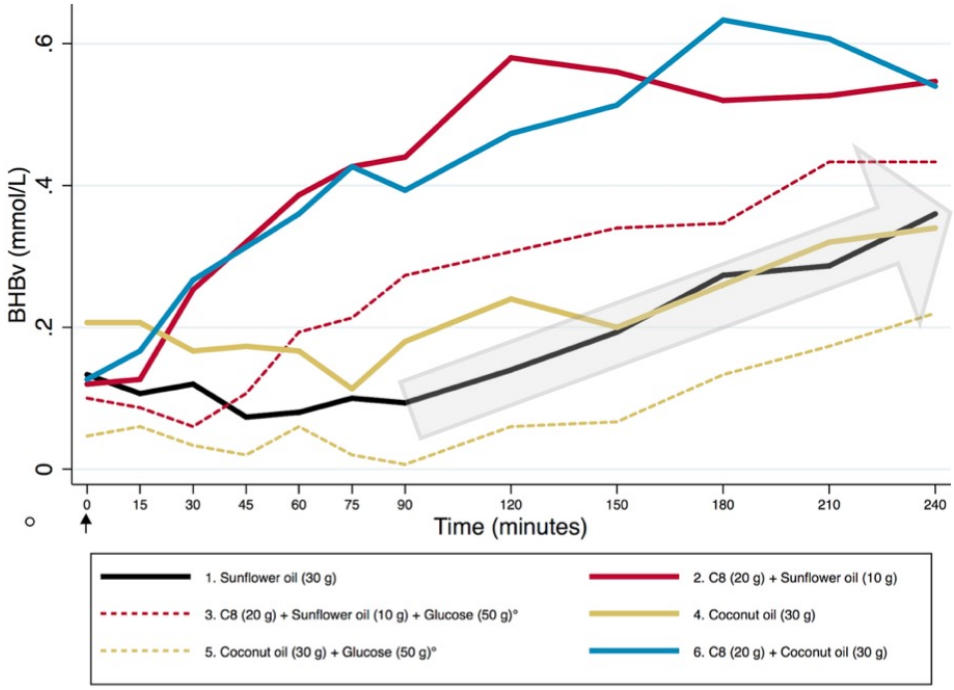


Fig. 19: Mean BHB levels in the various treatments over time. BHB: The ketone β-hydroxybuyrate measured in venous blood with handheld meter. Arrow indicates intake of coffee with test oils; ring indicate glucose intake 15 min. before that. The image is an adaptation of our figure published in Frontiers in Nutrition (114).

Intermittent fasting is a well-established ketogenic strategy (126), but here we applied it on carbohydrates (and protein) specifically while allowing intake of fat. When we refer to a *16-hour non-carbohydrate window* —a form of time-restricted feeding regarding carbohydrates (TRC)—as a ketogenic agent, it should be acknowledged that we cannot tell whether TRC per se was driving the mild ketosis or whether its combination with intake of any fat and/or coffee was a prerequisite. Ideally, the trial should have included one treatment with *coffee alone* and one with *water alone* to determine that. As

illustrated by the grey arrow in Fig. 19, all treatments have a fairly uniform increase in BHB between minute 90 and 240. This corresponds to an expected increase in BHB after 12-16 hours fasting (10), and it is therefore not unlikely that a similar pattern would have been observed even without intake of fat and coffee. In fact, a similar pattern was found when MCT was ingested with water instead of coffee as the vehicle after a 12-hour fast (244). Since we observed the increase also after glucose intake, that would imply that 50 g of glucose was not sufficient to suppress ketogenesis beyond 90 minutes. An alternative interpretation would be that coconut oil *and* sunflower oil exerted a ketogenic effect, although substantially milder (or slower) than C8. In line with other ketogenic studies (245), sunflower oil was chosen as the control condition since it contains only LCFA and therefore expected to be negligibly ketogenic. However, the ketogenic properties of LCFA may be higher than zero (138). Although caffeine has been shown to increase ketone levels (246, 247), the relatively low magnitudes reported from those studies suggest that caffeine should not be a major driver of ketosis in our study.

St-Pierre et al. (12) found that BHB increased less when C8 was ingested with a carbohydrate rich breakfast compared to intake (4 hours later) without an accompanying meal, but plasma levels of octanoate (the free fatty acid derived from the C8 supplement) were not different. My interpretation would be that the relatively lower ketosis after intake of carbohydrates in that study—as well as in ours—is a result of suppression of basal ketogenesis (assumingly via insulin release in response to increased blood glucose) rather than slower uptake of the ingested C8. In that study it was also shown that C10 and C12 were not more ketogenic than the control condition (vehicle only). Our results replicate the conclusions for C12 (as the main constituent of coconut oil) with two extensions: 1. We used an iso-caloric LCFA-oil as control; 2. Our sample was older adults.

A previous publication (141) from the trial cited above (12), reporting a differential selection of arms, showed that coconut oil was not significantly more ketogenic than the control drink. Since that study was published after the preparation of the project plan—but before the detailed planning of our trial was finalized—it decreased our confidence in our initial hypothesis regarding potential ketogenic properties of coconut oil. We concluded that before those results had been replicated—and extended with consideration of an iso-caloric control condition, potential accompanying carbohydrate intake, and age of the sample—it was not meaningful to address a cognitive health outcome as the primary endpoint of a trial comparing these components. Taken together with that study, and a recent study in seven young female adults (246), our study has contributed to corroborate that the capacity to substantially raise blood ketones after a single dose intake appears exclusive to the fatty acid C8. It is *not* a general property of MCT, regardless of whether referring to the group C8-C10-C12 or the narrower definition C8-C10 commonly used in "MCT-oils". Since coconut oil is

constituted by only ≈7% C8 (and ≈50% C12) it cannot be expected to be substantially ketogenic. This does not exclude that coconut oil may have beneficial effects on cognitive health by other mechanisms, as discussed by Fernando et al. (248), and the various MCFA may all have broad physiological impact, as reviewed by Watanabe (249). Interestingly, a recent study in mice and cell cultures demonstrated that exposure to C10, C12, and C14 increased Aβ degradation and influenced the activity of insulin degrading enzyme (IDE), which acts on both insulin and Aβ and is implicated in AD (250). In summary, it may be premature to exclude coconut oil from further research on cognitive health outcomes.

For long-term use of coconut oil, potential effects on risk factors for CVD (251) may be considered in a risk/benefit analysis and accompanying intake of MUFA or PUFA might be motivated to balance the level of SAFr.

6.1.2 Study II: Ketosis ➔ BDNF

This was to our knowledge the first study reporting an association between BHB and proBDNF in humans, which supports the hypothesis that BHB has a signaling role in human BDNF function. It was also the first reported analyses of potential associations between BHB and mBDNF in older adults, which did not indicate any connection under the investigated treatment conditions. Interpretations are however complicated because compared to Study I, this article has a substantial weakness: While sunflower oil is established as a *non-ketogenic* control condition (245), it cannot automatically be assumed to be neutral to BDNF. Potentially, it may influence BDNF by pathways independent from ketosis and therefore comparisons between treatments can only be interpreted in *relative* terms. Indeed, mBDNF increased substantially the first hour after intake of sunflower oil, and significantly more than the other treatments. Although we did not have a specified hypothesis, those mBDNF results were unexpected and some interpretations are discussed below.

An important conclusion from this study may be that without a prespecified hypothesis on the dynamic response to the exposure, and/or a cognitive outcome to relate the blood concentrations to, it is difficult to draw any conclusions on whether any of the treatments would have an advantageous or disadvantageous effect on brain function. We stated an open question whether there would be any detectable BDNF signal within a timeframe of 4 hours in response to mild/moderate ketosis, and the proBDNF results indeed give some support that BHB may have acted as a signal molecule, as previously reported in non-human studies (49, 50, 252). Nevertheless, the absence of an mBDNF response to increased ketosis may be a bit discouraging. Possibly, BDNF function should be understood as being *in the right place at the right time*, and for our participants who were resting without a cognitive challenge, there may not have been any demand for increased mBDNF, e.g., for solving a memory task. That might be consistent with a mice

study where BHB induced activity particularly on a *bdnf* promoter which is activity-dependent (50).

While our results may not have an immediate interpretation on whether exposure to any macronutrient or to ketosis appears beneficial for cognitive health, they may be interesting from a basic science perspective. Replication of the rapid mBDNF increase after intake of sunflower oil could imply that linoleic acid might have such properties. (A failure to replicate that finding would strengthen the case for interpreting our results in terms *of regression to the mean* after the remarkably low baseline levels in the sunflower treatment, i.e., a chance finding.) It would also be interesting to replicate our proBDNF findings in response to C8 (rather alone than combined with coconut oil), followed over a longer timeframe than 4 hours. To conclude whether any replicated effect is driven by ketosis independent of C8, proBDNF should be measured in other ketogenic conditions, e.g., fasting.

Optimal brain plasticity requires strengthening of valuable connections (mBDNF) but also getting rid of "spam connections" by disconnecting those (proBDNF), with a simplified analogy. Since proBDNF is both a precursor to mBDNF and a functional agent of its own, interpretation of its concentrations becomes complicated. Potentially, increased understanding in further studies could be achieved by measuring tissue plasminogen activator (tPA) which promotes the conversion from proBDNF to mBDNF (253).

A serendipitous finding, which may be important, was the trait-like stability of proBDNF within subjects (ICC=0.96) when measured in the fasting state at three repeated study days within a month. This may encourage further studies on proBDNF as a potential marker for brain function or progression of neurocognitive/neurological diseases.

An over-all rationale for analyzing BDNF was that it exemplifies one possible *signal* pathway by which BHB may affect brain health by its function as an HDAC inhibitor (18), which may modify gene expression. This provides a distinct function for BHB beyond being an energy carrier. However, some studies have suggested that BHB, compared to the structurally similar SCFA *butyrate* (C4), have relatively weak signaling properties (254).

Whether exposure to BHB at various levels impacts human cognitive health positively—and by a clinically meaningful magnitude—may still be uncertain. Intake of kMCT may improve cognition among MCI/AD according to some reviews (151, 152), where the most recent concluded that the strongest evidence of an effect on MCI may come from a 6-month trial (n=122, 2 x 15 g/day C8/C10) which reported significant results on selected sub-tests, in correlations with BHB levels. A trial reporting effects on *one prespecified* cognitive outcome would strengthen the case for the ketone hypothesis, and before such results have been presented any clinically meaningful effect remains unclear. The

largest kMCT intervention to date in mild/moderate AD, N=413, 26 weeks, 20 g/day C8) found no effect on the primary outcome ADAS-Cog11, and notably, MMSE decreased significantly *more* on kMCT compared to placebo. (150). This was despite excluding APOE4 carriers, which had not responded to some previous (149, 255) kMCT interventions with significant results among non-APOE4. The authors concluded that BHB levels may have been too low to achieve an effect on cognitive performance. Exogenous ketones have emerged as tool to raise BHB substantially more than C8, up to 4 mmol/L (256), and that may lead to more powerful trials which can test whether BHB substantially may improve cognitive function and prevent MCI/dementia.

Confounding by food composition is unavoidable in KD trials and separate trials on ketogenic supplements may be the most effective way to test the ketone hypothesis, while the effects of macronutritional changes are investigated separately in other trials. Potential additive or synergistic effects of ketosis and macronutritional changes may be investigated in a second step, when potential effects of those separate components are better understood.

6.1.3 Study III: Methods Comparisons for BHB

The results from this study showed that the BHB outcomes in *Study I* and *II*—measured with the handheld meter—have high validity and correspond well with the gold standard laboratory method. This has large implications for further research because the handheld meter is substantially more economic (>10-fold cheaper). Considering our results, it may be preferential for future studies use to the handheld since it allows more frequent measuring which should give a better estimate of AUC, despite values are only reported with one decimal.

The difference in capillary and venous levels was not previously reported in the methods comparison literature and our results may contribute to more valid comparisons between studies and raised awareness of the potential need to adjust cut-offs for ketosis. The results should be replicated, and it would be interesting to see if capillary BHB are similarly higher than venous BHB also at higher levels of ketosis, and at sustained stable ketosis.

6.2 Study IV/V: FINGER

6.2.1 Evaluation of the Methodology

The analysis framework includes several novel aspects for the *diet➔cognitive health* field—and to some degree for nutritional epidemiology in wider terms, according to my literature search. But it should not be excluded that my search has failed to capture important references. The novel aspects target variable selection; the ambiguous role of

TE as a covariate; separate analyses on within- and between-subjects effects; and treating APOE-genotype as a linear continuous gradient.

6.2.1.1 Should CFr replace separate reporting of eCarb and eFat?

Analyses above (5.4.2) demonstrated that eCarb and eFat represents two sides of the same coin, and that their individual impact impossibly could be disentangled within this data set. Both represent the carbohydrate/fat-ratio but are more prone to confounding by outliers in eProt and eAlc compared to the explicit CFr variable. The unadjusted slopes in Fig. 13B may erroneously imply that "*the effect appears to be driven by eCarb while eFat is not significant*", but the adjusted slopes demonstrate that differentiating confounding may explain the diverging β-coefficients. Adjustment for eAlc/eProt may not always lead to similar slopes for eCarb and eFat, but we cannot disentangle their individual effects just by comparing their slopes.

From a biological perspective, it may be possible to hypothesize that, e.g., an increasing carbohydrate proportion affects a causal pathway, but whether the corresponding decrease in eFat simultaneously is acting by another pathway cannot be known just based on observations of the data. It might even be more relevant to think of the ratio itself (CFr) as a one-dimensional causal agent rather than an additive effect from one macronutrient combined with a subtractive effect from the other. Ludwig et al. state that "*even discrete changes in diet (such as the ratio of dietary fat to carbohydrate) will directly affect numerous hormones and metabolic pathways involving many organ systems*" (88). Likewise, Kelly et al. (84) argue that focusing on *ratios* between nutrients aligns with a biological way of thinking and they exemplify how ratios of electrolytes, sex hormones, and glucose/insulin may provide more information on human metabolism than the separate components. In contrast, Tomova et al. (182) argue from what they label a "causal inference perspective" that ratios may have an obscure interpretation and prohibits an estimation of the individual causal effects of the components. That argument may miss two important points: First, the biologically causal agent might be *the ratio itself* rather than additive/subtractive effects of the separate components. Second, for eCarb and eFat the distribution may be so strongly reciprocal that it is not even possible to disentangle their individual effect in observational analyses on a typical population distribution. It appears likely that these conclusions from the FINGER dataset would be generalizable to most population-based datasets (at least in a modern western context), since it should be a consequence of the relatively low and narrow distribution of eProt—consistently observed within and between populations (90, 257) and in populations over time (200). However, that assumption should be empirically investigated in various population-based datasets to see how much variation there may be in the macronutrient distributions.

Corrêa Leite (258) has acknowledged that conventional theories for compositional data analysis have rarely been applied in the nutrition field and demonstrate a method based on isometric log-ratios according to principles introduced by Aitchison 1982 (82). Kelly et al. have also applied ratios in macronutrient analyses (194). In comparison with those analyses, our framework adds a hierarchical approach where the parent-macro level (eCarb+eFat+eProt+eAlc+eFib=100 E%) is treated separated from the sub-macro level, i.e., types of fat etc. This may be motivated by: **1.** eCarb and eFat together almost exclusively harbor the collinearity on the parent-macro level and once they are replaced by CFr, collinearity may become negligible on that level. Since eCarb+eFat sum up at ≈80 E%, it should be fairly valid to treat CFr, eProt, eAlc, eFib, and any sub-macro ratio as unrestricted data in relation to each other within a typical population distribution. This may allow a simplified method where not every dietary parameter of interest needs to be represented by a ratio, and where isometric log-transformation may not be necessary. Indeed, Corrêa Leite states that "isometry is not a requirement when using compositions as explanatory variables" (176). **2.** Despite some differences in metabolism exist between sub-types of a macronutrient, the main differences take place *between* the parent-macro categories (78); a distinct analysis on that higher level should be biologically motivated before looking at the sub-types. CFr may be an important diet dimension in relation to health (88) which we may want to disentangle from the effect of sub-macro composition. **3.** CFr and SAFr represent distinct, well-defined compositional research questions compatible with a target trial approach: "What's the impact of the proportions of eCarb vs. eFat?" and "What's the impact of the proportions of saturated vs. unsaturated fats?". Those questions may correspond to two relatively *independent* real-life choices in food composition, as supported by the relatively low correlation (r=−0.09) between CFr and SAFr within FINGER data. In contrast, eSFA (by E%) is highly correlated with eCarb, eFat, and CFr (all |r|=0.68-0.76).

The discussion above has implications for the interpretation of "substitution models" (66) for replacing eSFA with eCarb: Accepting that eCarb is a reciprocal proxy for eFat, does it really make sense to analyze replacement of a *sub-type of fat* against (the reciprocal of) *total fat,* which the sub-type itself is a component of? This should add an argument for establishing *sub-macro ratios* over *fat-subtypes by E%* as the standard way of reporting macronutritional composition, unless motivated by a specific hypothesis.

Protein intake (eProt) was shown to be a relatively independent dimension since 1 SD increase in CFr estimated the following shifts: +6.3 eCarb, −6.3 eFat, and only −0.3 E% eProt. This might be interpreted in terms of CFr, eProt, (and SAFr) being "natural" (independent) dimensions in spontaneous diet composition here, and probably in other epidemiological datasets as well. In contrast, eProt vs. eCarb or eFat did *not* appear as natural dimensions, as shown in 5.4.2. That makes sense if we accept that both those

analyses actually represent eProt vs. "CFr by proxy". In an RCT it is of course possible to keep eFat fixed and investigate replacement of eCarb vs. eProt. However, in that case you do not keep CFr fixed. Can we tell whether modulation of eCarb←→eProt is a more important causal agent than the simultaneous change in CFr? Ultimately, biological knowledge or assumptions must be considered in such interpretations. In contrast to eCarb and eFat, protein has a more emphasized *functional* impact over being "raw fuel" (78). Further, it appears that variability in eProt takes place against the balanced pool of eCarb+eFat in our data (*Study IV*, Fig. 3). Therefore, I would conclude that eProt may be relevant to analyze as a stand-alone variable ("against anything") in combination with CFr. In contexts of substantial weight change, caloric restriction, or malnutrition there might be a rationale for analyzing protein by weight, but at least for the cognitive field I think eProt (E%) may be preferred since it is a compositional concept.

It may be acknowledged that although CFr is conceptualized as eCarb/eFat here, it represents the same ratio as carbohydrates/fat by gram (beyond a constant factor given by the higher energy content by weight for fat) since TE is equaled out in the calculation. This further demonstrates that CFr is a *compositional* measure separated from the quantitative (TE) measure.

6.2.1.2 Disentangled effects of dietary composition and quantity

Corrêa Leite argue that methods based on ratios have several advantages over "substitution" models, including the separation of effects from the compositional parameter and the quantitative parameter (TE). While TE has typically been discussed in terms of confounding, see (81, 182) and references therein, this may be problematized from a causal inference perspective: If the compositional aspect of diet (e.g., CFr) *affects* ad libitum TE by modulating factors like satiety, cravings, or even basal metabolic rate, then it may have a role as a *mediator*, particularly if the outcome is weight. There is indeed support for such a direction of causality in some contexts (242). Furthermore, a recent publication showed that during the recent decades, basal metabolic rate—but not activity level—has decreased in the population (259), which may generate hypotheses on potential impact from compositional aspects of diet. Whether adjustment for TE is more likely to decrease or increase bias for a compositional diet parameter in cognitive studies may be discussed, but having the option to analyze both alternative models should be preferred. Particularly in the absence of a measure of *physical activity lev*el, adjustment for TE might decrease confounding by capturing that by proxy. This was the primary rationale for adjusting for TE (standardized separately by sex) in *Study IV/V*. Reporting of both TE-adjusted and unadjusted models was a not novel thing, but we added a discussion on their ambiguous interpretations from a causal inference perspective.

A discussion may be initiated in the nutrition field whether the two-step residual method and "substitution" models may have better alternatives and should be phased out. If our research question is compositional, analyzing a nutrient by E% should be preferred over the two-step method since E% is a universal compositional concept directly measured in every observation, while the other is a sample specific estimate. While it might be reasonable to conceptualize the health effects of protein, alcohol, and fiber, by E%, i.e., against any other compositional part, I have provided arguments why eCarb/eFat and sub-types of those are better analyzed as ratios. Moreover, *Study V* demonstrates how this variable selection facilitates analyses on effect-modification, which would be more complicated in a leave-one-out model.

If the field could agree on a "standard panel" for analyzing the causal effect of macronutritional composition on health, more effective meta-analyses may be performed—which leads to better decision making. A panel including CFr, eProt, and eFib (and optionally eAlc) may effectively capture composition on what I hierarchically refer to as the parent-macro level. Those variables may be combined with *ratios* on the sub-macro level, e.g., SAFr, sugar/starch, or plant/animal-based sources. This variable selection should be compatible with a target trial approach, making it more clear how both observational and RCT research may contribute with evidence to the same well-defined research question. The target trial concept is a key component of causal inference theory applied to epidemiology (111), and was encouraged in an editorial in the American Journal of Clinical Nutrition (110). That terminology was only retrospectively applied to *Study IV/V* and a more formal *emulation of a target trial* (111) may be applied in future research.

The need for *field specific subject matter knowledge*, beyond statistical competence, has been acknowledged as a difference between research aiming at causal inference versus prediction (99). While this may typically be motivated by a need to identify the most likely causal structure between variables for adequate covariate selection, the argumentation above may have illustrated an additional reason in the context of macronutrient research: It is necessary to understand the very non-random distribution of macronutrient proportions in population-based data and how variables may be highly correlated in a well-expected pattern. This may help to identify those dietary parameters that are biologically meaningful and *possible* to make causal inference on.

6.2.1.3 Between- and within-subjects effects were disentangled and compared with the mixed effects model.

When a mixed model from a panel analysis is reported alone, it may be unclear whether the estimate primarily represents within- or between-subject effects (235). This has implications for the likelihood of confounding, since between-effects are more prone to that while within-effects avoids confounding by factors that are consistent within

individuals, regardless of whether those are measured or not (236). Since our mixed effects model—defined as the primary estimate a priori—appeared closer to the within-effect, that might indicate lower confounding. In fact, supplementary analyses in *Study IV* showed that adjustment for *age* (at baseline), *sex*, and *education* (all time-invariant) had very little impact on the estimate for *CFr➔global cognition*. Considering the participants were exposed to multiple life-style recommendations, "health-awareness" or healthy user bias (260) might be an unmeasured confounding factor. As a hypothetical example, the tendency to follow the recommendation to reduce SAFr might correlate with the tendency to increase physical activity, which potentially could be the true causal driver of an effect mirrored by SAFr. From that perspective, it should be a strength of *Study V* that significant within-effects were shown, which should have a low risk for bias by "health-awareness" or other traits.

In *Study IV*, within-effects were not significant in the full sample. However, when including only participants with larger intra-individual contrast between their repeated measures (approximately >7 E% exchange eCarb–eFat), it was significant. This sensitivity analysis was intuitively initiated but appeared retrospectively compatible with a hypothetical "per-protocol" analysis as described within the target trial framework (111). Since no previous diet study reporting separated between/within-analyses was identified, discussions from other fields (235, 261) were considered for interpreting how they may reflect different angles of the research question. As discussed in the methods of *Study IV*, the between-slope was assumed to capture an effect over longer term, while the within slope was assumed to capture effects of changes within the study period. This interpretation is compatible with the estimates in Tab. 5, indicating that the between-effect was 4.8–6.5 times larger than the within-effect. It appears plausible that the difference could be due to a cumulative effect over longer time, and not necessarily due to more confounding of the between-effect, although that might also contribute to the differing magnitude.

The fact that some significant within-effects were detected has important implications: 1. It supports the use of diet interventions to promote cognitive health, by showing that dietary *changes* at old age matters. 2. It suggests that time-invariant factors can be excluded as confounders for those analyses, e.g., baseline Aβ-status, genetics, "health-awareness", or pre-study habits. 3. It might strengthen the case for using cross-over trials in RCT contexts, since power to detect effect modifications appear to have been stronger for within-effects than for between-effects. If the response to diet changes is expected to be heterogenous, it may be important to understand how different sub-samples respond.

6.2.1.4 Analyzing APOE-genotype as a gradient

The most common way of analyzing APOE may be APOE4-*carriers* (24/34/44) vs. *non-carriers* (22/23/33) but more complete stratification has been encouraged and the categorization of APOE24 may be ambiguous (62). We applied complete stratification as a start, but after a gradient emerged between the point estimates in several graphical analyses, a continuous APOE-gradient (ε-23-24-33-34-44, coded as -1, -0.5, 0, 1, 2) was systematically investigated in *Study V*. The gradient was a significant effect modifier for *diet*➔*cognition*, *diet*➔*biomarkers*, and *biomarkers*➔*cognition*, implying that it is a biologically meaningful dimension in metabolic and cognitive research.

The continuous APOE-gradient may increase statistical power for future studies on effect modification by APOE. The mean value of the gradient in a study sample could potentially be used as an *APOE-index* for a study sample, which could be used to adjust for potential confounding by differing APOE-distributions between studies in meta-analyses. In the FINGER sample that index was 0.24 (for both *Study IV* and *V*). It is possible that our unstratified CFr results (significant negative slope to cognition in *Study IV*) would not be replicated in a sample with a lower APOE-index.

Only one APOE22 was present in the FINGER sample and that individual was therefore excluded in our analyses. Coding value for APOE22 may be suggested as –2. The value of APOE24 (–0.5) may be considered preliminary because it was a small stratum with large confidence intervals in our analyses. The position was motivated by higher expected protein levels of ApoE2 compared to ApoE4 (62). The continuous APOE-gradient may be further investigated as an effect modifier of associations between *diet* and *cognitive health outcomes*. For biomarker analyses, the biological validity of using the gradient must be considered, e.g., it may be relevant for LDL and HDL cholesterol but not for triglycerides were levels typically are APOE2>APOE3<APOE4 (62).

6.2.2 The Impact of Macronutritional Composition on Global Cognition

In the full sample, only the CFr dimension had a significant (negative) linear association with global cognition, but not protein, SAFr or fiber. That does not support that *type* of fat would be more important than *total* fat for cognitive function. Our results suggest that the proportional composition of total fat and carbohydrates should be a prioritized dietary parameter to further investigate in clinical trials and observational research. Stratified analyses showed that protein was indeed associated with global cognition, but antagonistic estimates for APOE23 (negative) vs. APOE34/44 (positive) cancelled each other out in the full sample.

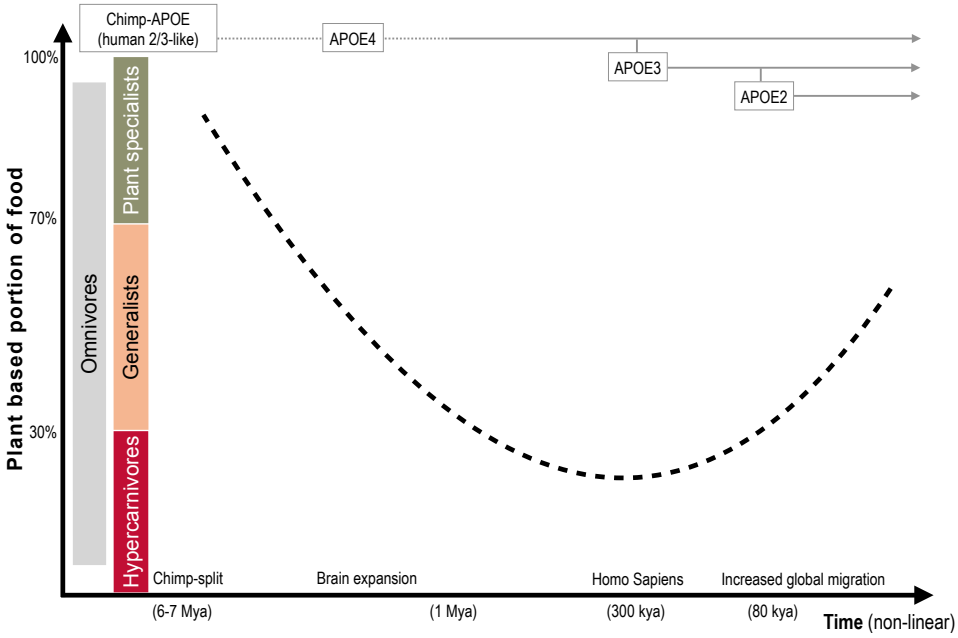


Fig 20: Possible correspondence in time between APOE-mutations and food patterns.
U-shaped plant/animal-based diet development roughly adapted from hypothesis by Ben-Dor, Sirtoli, & Bakai (217); levels describe an ordinal pattern rather than exact proportions here. Categories *plant specialists, generalists, hypercarnivores* defined by the same reference. Superimposed approximate timeline of APOE-mutations based on Huebbe & Rimbach (210), with the dotted interval representing uncertainty of time for APOE4. Chimp-APOE described as *functionally* human 2/3-like based on iso-electric point in-between APOE2 & APOE3 (211) and assumptions by others (210). A higher plant-based proportion of food may correspond with a higher carbohydrate/fat-ratio, higher fiber intake, and possibly lower protein intake.

Suggestions that APOE3 provides metabolic flexibility (210) were supported by our results: Primary estimates for all investigated diet variables were relatively close to zero and non-significant for APOE33 (although a significant negative *between*-slope for CFr was found in that sub-analysis, possibly indicating some impact in longer term). Our results were more compatible with the hypothesis that APOE4 carriers would be *less,* rather than *more*, adapted to a high CFr in diet. While that has been frequently hypothesized (210, 215, 262), our results gave rise to a new hypothesis which we have not seen stated before: APOE23 typically had antagonistic estimates to APOE34/44 which suggested that *APOE2 might provide lower adaptation to a low CFr/more animal-based diet compared to APOE3*. Interestingly, estimated timing of the APOE2 mutation corresponds to increased global migration, and to the FADS mutation (≈85 kya)—which facilitated elongation of fatty acids and decreased the need for animal based VLCFA sources (263). That would imply two complementary adaptations to less dependence on animal-based food sources. It has in fact been hypothesized that human diet had shifted towards an increasing plant/animal-based ratio at that time, after a temporary hyper-carnivorous period (217) which would have corresponded in time with the

ancestral APOE4. As shown in Fig. 20, APOE3 may have appeared close in time to the turning-point, potentially related to the diet shift as a cause or an effect. Our data appear compatible with the hypothesis presented by Ben Dor, Sirtoli & Barkai (217).

Insulin was an additional effect modifier of our analyses *CFr/fiber/protein➔global cognition*, but only in the APOE34/44 stratum. Our results support the hypothesis (262) that individuals with the combination of APOE4 and insulin resistance would be a target for precision nutrition, with focus on low-carbohydrate diets. But our findings add that also individuals with *low* insulin compared to the mean (and a biomarker profile contradicting preclinical T2D) might benefit from similar diet changes. This is particularly interesting considering suggestions (226, 228) that hypoinsulinemia may represent a distinct dementia risk type and has implications for decisions on inclusion criteria in future diet and multi-domain interventions: If we aim to capture the metabolic syndrome-like phenotype, do we accidently filter away an important target group?

While the CFr results were primarily in line with our hypothesis, the negative association *fiber➔cognition* for APOE4 (with modulation by insulin) was unexpected. Even among researchers who disagree on the optimal CFr in diet, fiber intake is typically thought to be beneficial to health (187). This shifted focus on the interpretations from macronutrients per se to a possible plant/animal-based dimension (which we could not measure by explicit data). By creating a composite score (comp) from the averaged z-scores of CFr, fiber, and inversely protein, we aimed at possibly capture that dimension by proxy. I make no claim that *comp* is a strong proxy, but there is some support that a shift in the plant/animal protein ratio would correspond to that macronutrient profile (205). As it is a post-hoc analysis, a stricter p-value threshold might be considered for *comp* (25). We did not make any such systematic adjustments in the analyses *comp➔global cognition* but concluded that with $p<0.001$ for the negative slope in the APOE34/44 stratum, as well as for the interaction against APOE23/24/33, it appears unlikely that this dietary dimension would not be particularly important for APOE34/44. One suggested mechanism—beyond glucose/insulin pathways—that could explain the results is related to detoxification of plant versus animal based food components. While APOE4 may provide better protection against parasitic infections, APOE3 may improve the defense against plant-based toxins, via functions of the transcription factor Nrf2 (216), as suggested by Huebbe & Rimbach (210).

APOE3 may be protective against recurrent pregnancy loss (264), and highest fertility has been found in either APOE3 or APOE4 carriers depending on population/ethnicity while APOE2 typically had the lowest fertility (265). Fertility was higher for APOE4 Ghanaian women exposed to higher compared to lower pathogen levels (266), in line with suggested gene–environment interactions on reproductive efficiency by APOE (267). How global APOE distributions may be related to functional and dysfunctional APOE-modulated biological pathways, interacting along the lifespan, has been reviewed

by Abondio et al. (218). Based on the implied *APOE x diet* interactions in our results, a speculative hypothesis would be that APOE, by reciprocal effects on fertility among APOE4 vs. APOE2 depending on diet, has been functional for "calibration" of human populations to the food (and infectious) environments during global migration. Fertility among APOE33 might be more insensitive to such regulation, in line with assumptions of "metabolic flexibility". While differing APOE distributions in the source population may be one explanation to the heterogenous regional prevalence of the APOE isoforms, this would add an "APOE2/APOE4 filtering mechanism" on migration pathways as they pass through environments where the CFr/plant-based food component is either very low or high respectively. The extremely low prevalence of APOE2 among Inuit, Siberians, and Native Americans (219) might be compatible with that. A testable hypothesis would be that CFr (and/or the plant/animal-ratio) is relatively higher for APOE4 and lower for APOE2 carriers—compared to APOE33—among clients at infertility clinics.

6.2.3 Interpretations of an Anomaly: Female APOE44

A sensitivity analysis revealed one anomaly in *Study V*: Female APOE44 (n=11) deviated from the general pattern by having a positive slope for *CFr ➔global cognition* with a significant interaction effect against male APOE44 (n=13). It would not be unlikely that an anomalous finding in one small stratum occurred by chance, but some interpretations from the perspective of a possibly meaningful finding will be discussed here:
By conventional definitions, female APOE44 appears as the metabolically healthiest stratum within the sample, including outstandingly low levels of insulin and HOMA-IR, as shown in Fig. 15. If this profile is not a true mirror of the population, a selection bias may explain our results. This stratum—which is already at the highest risk for AD—might be subject to survival bias: At age 60–77 years, having the metabolic syndrome as an additional risk factor to being female and homozygous APOE4 might result in such a high likelihood of already having developed MCI/AD that such individuals rarely passed the inclusion criteria for FINGER. On the other hand, our female APOE44 stratum resembles the hypoinsulinemic at-risk phenotype for dementia observed in women already at age 38-60 years (226). If the profile of our female APOE44 stratum is indeed representative of the population, a second question is whether that also applies to their outstanding baseline diet (Fig. 15). Could it be a cause/effect relation in any direction between their diet and biomarker profiles?

A speculative interpretation on a scenario where there would be a true difference in food adaptation by sex among APOE44 could add an evolutionary perspective: Beyond humans and whales, female survival beyond reproductive age is very rare among mammals (268). This has given rise to *the grandmother hypothesis*, i.e., that older females facilitate transferring of knowledge over generations and provide alternative social structuring beyond the nuclear family by supporting their daughters with things like foraging (269). To my understanding, it is not fully established whether the

emergence of *grandmothering* played a substantial role already among homo erectus (≈2.5 Mya) or is a more recent phenomenon that emerged during the last 100 kya (269-271). Could plant-based food adaptation specific for (postmenopausal?) females (prior to the APOE3-mutation)—in a hypothesized hyper-carnivorous context (217)—have facilitated the emergence of *grandmothering*?

6.2.4 Exploratory Biomarker Analyses

APOE was an effect modifier of associations *CFr➔biomarkers*, and for *biomarkers➔cognition*. Results above (5.5.2) are particularly interesting since they imply that APOE (34/44 [+] vs. 23/24/33 [–]) does not only modulate the association between CFr and cognitive function but also for CFr and health in broader terms, in a coherent pattern. Significant interaction by APOE was seen for both BMI and systolic blood pressure: A lower CFr appeared neutral to BMI and trended to be advantageous on blood pressure for APOE+, while significantly higher levels for both BMI and blood pressure were seen for APOE– in relation to a lower CFr. A lower (advantageous) ratio triglycerides/HDL-cholesterol was seen in both strata in relation to lower CFr, but it was only significant for APOE–. ApoB appeared neutral in relation to CFr (beyond the unadjusted APOE– slope). In conclusion for APOE34/44, a lower CFr implied an advantageous association to global cognition without any disadvantageous associations with lipid markers, BMI, or blood pressure. For APOE–, a lower CFr was neutral to cognition with mixed biomarker associations.

A notable finding in the supplementary material to *Study V* was that blood glucose levels were (negatively) associated with cognition only among APOE+. This applied both to fasting levels and OGTT, and interestingly the slopes were relatively similar between insulin strata. This means that even in the lowest insulin tertile, with HOMA-IR in the normal/low range, glucose at 120 minutes was negatively associated with cognition. We discussed whether that level might be interpreted as a marker for lower uptake rate of glucose in the brain, which is expected to be APOE dependent (272). The double roles of insulin degrading enzyme (IDE), by also being a degrader for Aβ, has given rise to hypotheses on competition or interference between those tasks (273), but pathophysiological pathways leading to AD differ by APOE (274). Associations *CFr➔glucose/insulin* were here (5.5.2) modulated by APOE. Our summarized results strongly support focusing on CFr–insulin–glucose pathways in further cognitive research but imply that those pathways may primarily be important for APOE4 carriers.

6.2.5 Applying the Bradford Hill Viewpoints on Study IV/V

One possible interpretation of the results in *Study V*, under the assumption that the estimates describe a causal effect, is that a diet high in CFr, and fiber, and low in protein, has a disadvantageous effect on cognitive function for APOE+ (34/44). This could be an effect of macronutritional composition per se, but potentially also be related to a higher

plant/animal-based ratio in food sources, which we tried to capture by our composite score. The Bradford-Hill viewpoints (108, 109) will here be used for discussing the likelihood that our observed associations represent causation. He formulated his viewpoints in a context where accumulating observational results suggested that smoking had detrimental effects on health, and since using an RCT to answer that question was unrealistic, he aimed at taking advantage of available data to optimize decision making.

6.2.5.1 Strength

As a reference from the FINGER sample, a descriptive baseline comparison of the high vs. low group by median-split for age (mean 65 vs. 73y) and education respectively, gives a mean difference for global cognition at ≈0.6 SD for both variables (in my exploratory calculations). When applied to *change* in cognitive performance, the cumulative effect by time must be considered in the interpretation. The 2-year intervention effect in FINGER (Cohen's *d*=0.13) could—under assumptions of a cumulative increase in effect size when behavior changes are kept beyond that timespan—make a substantial difference from a life-time perspective (230).

By using a conversion factor on CFr-β, the difference in *Global Cognition* from replacing 15% eCarb with eFat was estimated (5.4.2). This corresponds to a shift from a WHO-compatible MeDi to the PREDIMED-version of MeDi (72, 170). Among hypothetically "protocol compliant" subjects, the within-effect was 0.079 and the between-effect was 0.38. A possible interpretation could be that the first value estimates a <1-year effect (≈6 month) while the other (4.8 times larger) represents a cumulative effect over >2 years. If we speculate that the effect can be extrapolated to eCarb:eFat at 20:60 E%, the magnitude would be doubled, and we know that the effect was even larger among APOE34/44. That is not small effect size and would suggest that a substantial part of the potential effect of shifting to LCHF/KD could be explained by factors independent of ketosis.

6.2.5.2 Consistency

There are very few studies which have explicitly investigated the CFr-dimension in relation to cognitive health, but ,*e.g.*, results from Roberts et al. (184) on MCI/dementia in relation to eCarb and eFat are consistent with the direction of our results. APOE as an effect modifier of associations *macronutrients ➔ cognition/biomarkers* has frequently been reported (223, 225).

6.2.5.3 Specifity

Our exploratory analyses suggested that CFr was not negatively associated with BMI, blood pressure, ApoB, or the triglyceride/HDL-cholesterol ratio among APOE+. This

would be compatible with hypotheses that APOE4 carriers may have a relative benefit for health in broader terms from a lower CFr, but particularly for cognitive health.

6.2.5.4 Temporality

Nutrient studies in free-living individuals rely on assumptions that diet measured at one time-point is a proxy for habitual intake (retrospectively and/or prospectively). Many diet studies only measure diet at a single timepoint, and regardless of whether the analysis is cross-sectional or longitudinal over 20 years, the comparison can only be *between* subjects. A strength of *Study IV/V* is the additional inclusion of within-subjects slopes which represent a longitudinal analysis and are less prone to confounding.

6.2.5.5 Biological Gradient

Graphical linearity checks indicated that associations *diet➔cognition* was linear (for a continuous predictor and outcome), except for SAFr. We demonstrated effect modification for *diet➔cognition* by a linear APOE-gradient which suggested that higher CFr (and composite score) appeared less favorable per step from APOE23 to 24-33-34-44, with the opposite pattern for protein. This is compatible with the suggestion (210) that APOE3 added plant-based adaptation over APOE4.

Additionally, insulin modulated *diet➔cognition* in a dose dependent way, but only for APOE+. Interestingly, effect modification increased both at gradually higher and lower insulin levels. The latter is interesting considering the suggestion that a hypoinsulinemic phenotype may be at increased risk for dementia (226).

6.2.5.6 Plausibility

This refers to biological plausibility and there are a few factors that makes APOE a plausible effect modifier of a potentially causal effect of CFr (or the plant/animal-ratio) on health: the ApoE-protein has a key role in lipid metabolism and the iso-forms have differentiating binding preferences to HDL vs. VLDL (58), APOE may affect plant-based detoxification (210, 216), and HDL- and LDL-cholesterol levels vary by the APOE-gradient (62). Notably, based on the higher levels of LDL-cholesterol for APOE4, some (214) have suggested that those should *avoid* meat and fat, but our data are more compatible with the opposite (215) hypothesis. Furthermore, there is an evolutionary grounded plausibility as described above.

6.2.5.7 Coherence

The AD risk ratio for APOE44 vs. APOE 33 (RR4433) in western populations is ≈12; in contrast, in East Asian samples RR4433 may be ≈40, while CFr is typically substantially higher (62, 275). The highest fat intake in the world (51 E%) was found in a Nigerian sample (276), and RR4433≈1 has been reported in another Nigerian sample (277). Although such regional/ethnic differences in part may be explained by genes

surrounding APOE (278), the differences are compatible with a hypothesis that RR4433 is modifiable by changing CFr in a population. That can be systematically investigated.

6.2.5.8 Experiment

Hill states that this may be the criteria giving the strongest support for causation, but it does not exclusively refer to randomized experiments. He suggests that even effects on the outcome from spontaneously induced changes in the exposure may give important information. In *Study IV/V*, the within-subjects effect might be seen as a "quasi-experimental" analysis, in the meaning that we investigate how dietary changes—which we conclude are mainly unsystematic and "spontaneous" on the individual level—are related to variability in cognition. If dietary changes had been primarily linear, we could have suspected some systematic bias, e.g., aging-related changes (reverse causality) or a bias from the on-going trial. Changes however took place in both directions with very little difference between the randomization groups; variability explained by *time*, *time* x *group*, and *age* combined was small (R^2=0.01-0.02 for all diet variables). There were indeed some statistically significant differences in group means over time, modulated by group for some variables (279), but to exemplify a typical magnitude, eCarb and eFat changed by ≤1 E% per year. FDAS means–reflecting adherence to official dietary guidelines–were 5.0-5.4-5.2 vs. 5.0-4.9-5.0 for the intervention and control group respectively. By adjusting for *time*, and *time* x *group*, confounding from those changes should have been mitigated.

By retrospectively applying target trial terminology (111), the within-effects could be viewed as a cross-over trial comparing exposure to a (relative) low/mid/high level of the diet parameter in a "quasi-random" order. Both magnitude and statistical significance increased in a "per-protocol" analysis for *CFr➔global cognition*, were only those individuals who had a relatively large contrast (by median-split) in CFr level between the years were included. In line with a cross-over trial, the within-effects are not confounded by time-invariant factors. Could there be time-variant confounders? *BMI* and *TE* could potentially be such factors and be markers for changes in physical activity, but those were adjusted for without any substantial changes in the results.

To our knowledge, no previous study has reported within-effects for *diet➔cognition*. Since we demonstrated that the mixed model—defined as our primary estimate—seems to mainly reflect the within-effect, that should be a strength of Study IV/V, .i.e., the risk for unmeasured confounding is smaller than in a between-subjects analysis (236).

6.2.5.9 Analogy

While our research question targets macronutritional composition, we acknowledged that such analyses may be confounded by the plant/animal-dimension of diet since high CFr, and fiber levels may imply a high plant-based component. Therefore, studies

on APOE-modulated associations *meat intake➔health* may be relevant to acknowledge. Results from the U.K. Biobank (n=493888, mean age 57 y) are not compatible with the hypothesis that avoiding meat intake would promote cognitive health among APOE4 carriers, but rather suggest a protective role for unprocessed red meat: Per 50 g/day intake, a hazard ratio of 0.64 ($p<0.001$), i.e., a 36% lower risk, for developing dementia within a mean follow-up time at 8 years was reported for the dichotomized APOE4 carrier stratum. Among non-APOE4, unprocessed meat appeared neutral (HR: 0.93, p=0.59), and interaction was p=0.019/0.095 depending on model. Those results are compatible with ours and suggest that macronutrient analyses should ideally include information on plant/animal-based origin. Animal based protein has comparably high density and bioavailability of essential amino acids that are important for growth and maintenance of health (280).

A Chinese study on *meat/fish intake➔all-cause mortality* (mean age 82 y, mean follow-up 5 y) points in the same direction, suggesting a relative advantage of meat and fish intake among APOE4 compared to non-APOE4. Ylilayri et al. did however not find APOE interaction for the association *meat intake➔cognitive performance* (281).

6.2.5.10 Concluding Analysis: Need to Take Action?

In line with the aims of Bradford Hill, any philosophical discussion on the definition of causality is not the intention here. The question is: *What interpretation is most likely?*
I suggest the most likely interpretation is the following: *Certain dietary parameters may have a causal impact on cognitive performance among older adults, and with a substantially larger magnitude for APOE34/44 than for APOE33. We cannot exclude that this extends to increased AD risk in longer term if the diet is disadvantageous*.

Based on our results and the literature, none of the following can be excluded:
• *Lowering* CFr might be disadvantageous for certain APOE strata. • *Increasing* CFr might be disadvantageous for certain APOE strata. • *Lowering* SAFr (particularly SFA/PUFA) might be disadvantageous for certain APOE strata. • *Increasing* SAFr might be disadvantageous for certain APOE strata.

Sufficient RCT evidence for guidance on the optimal CFr for the promotion of cognitive (and general) health will not be available within several years. Long-term RCT with hard disease outcomes (MCI/AD) might never be practically or ethically feasible. Do we need to act on available evidence to adjust current dietary guidelines? One immediate step could be to keep guidelines neutral regarding CFr, i.e., refrain from specifying targets for eCarb and eFat. That would not be very dramatic because that was actually the decision that was made for DGA2015 (190). The WHO guidelines conclude that evidence strength for each component in their definition of a "healthy diet" varies between *low* and *high* (72) and the eFat limit at 30 E% may indeed have low evidence overall (187), and may

not be considered evidence based for cognitive health (as reviewed above). The Swedish National Board of Health and Welfare (Socialstyrelsen) states:

"*Evidence-based work has its basis in ethical positions—that it is unethical to intervene in people's lives if there is no support that the interventions are at least not harmful.!*"

In contrast to *additive/subtractive* life-style recommendations (take a daily walk; stop smoking; check your blood pressure), diet recommendations may be considered *invasive*. Each person already has a habitual diet with the CFr, and plant/animal parameters set. If shifting those parameters in one direction is beneficial, the other direction is per definition unbeneficial. When we are not confident about the optimal direction, we may consider refraining from giving advice on that parameter.

A main conclusion would be that we rapidly should allocate more resources to high-quality research on *diet➔health*, which focus on gene-diet interactions and cognitive health. Finally, effects on brain health must be incorporated in risk/benefit considerations when transformations of global food systems towards a more plant-based diet (202) are discussed. It is a major global challenge to reach climate goals and the highly plant-based EAT-Lancet diet has been proposed as a strategy to reach those (202). The role of animal-based food in diet tends be discussed with a mix of scientific and political aspects (280), and it may be important to refine knowledge on the human health effects to guide risk/benefit-evaluations when prioritizing between different strategies to reach climate goals. Our results (*Study V*) and others (220, 282) suggest that a possible negative effect on health—specific to APOE4 carriers— cannot be excluded if diets *universally* shift towards a higher proportion of carbohydrates and plant-based food sources. More research on this aspect is urgent and may preferably be performed in APOE-enriched populations: the Nordic countries, tropical areas, Americans with African over European ancestry, and individuals with AD, including the preclinical or prodromal state (34, 219).

6.3 Summarizing Discussion

The title of this thesis implies that macronutritional composition was investigated "from normal aging to Alzheimer's disease", which may give rise to the question: *Where are the AD data?* As clarified in the introduction, AD is a biologically defined brain disease with a preclinical phase which may last for decades before cognitive symptoms and dementia manifest (30). Brain amyloid PET scans in a subsample (n=41) of participants in *Study V* indicated that 12% of non-APOE4 and 56% of APOE4 carriers were Aβ positive (283). This gives a rough estimation of the number of participants with possible preclinical AD in the whole sample, indicating that the intentions behind the FINGER inclusion criteria were successful: This may indeed be a sample at increased risk for dementia, i.e., an optimal target group for prevention. By excluding individuals with high cognitive performance at baseline, the cognitive test battery may have become more sensitive by

avoiding ceiling effects. This do limit generalizability of the results to the general population, particularly to countries with low APOE4 prevalence, but on the other hand they may be particularly relevant for "at risk" samples. It should also be noted that components of the inclusion criteria may have biased relations between each other, e.g., if you collected points on the CAIDE risk score by being *male* and of older *age*, you may have been accepted with lower metabolic risk factors compared to a younger women. This may relate to a form of collider bias (222), and may motivate careful considerations of pros and cons of using risk scores as inclusion criteria. We however considered it unlikely that the diet analyses would be substantially biased by that.

Could Aβ status be an unmeasured confounder? Is it possible that "benefits" on cognitive function of a lower CFr act via Aβ clearance and is only detectable for those with pathological levels? If so, our stratified analyses might not represent APOE-genotype per se. However, the relative "advantage" of a lower CFr for APOE34/44 was not specific to cognitive function but extended to BMI and blood pressure, which may contradict Aβ as a necessary mediator. Moreover, since Aβ pathology can be predicted by increasing age (284), we would then expect lower CFr to be increasingly "beneficial" by age, but a post-hoc analysis indicated no such trend (β=0.01, p=0.35 for interaction *CFr x age➔cognition*).

There was significant effect modification of associations *CFr➔blood glucose/insulin*, particularly for APOE44. Those may be difficult to interpret in terms of "good/bad" since insulin seems to have a U-shaped relation to cognitive health (226). But such results may strengthen the case for considering AD a metabolic disease related to insulin and glucose function via diet (under assumptions that cognitive function within this sample to some extent represents preclinical AD). But interestingly, our results suggested that this conclusion primarily applied to APOE4 carriers. Is AD among APOE23/33 less related to diet? Our results were consistent with suggestions that APOE33 may have metabolic flexibility, and that macronutritional composition is weakly related to cognitive health for those. If such flexibility extends to dietary factors beyond macronutrients, that could be one explanation to the inconsistent results (161, 163, 166) for associations between dietary patterns (or adherence to official guidelines) and cognitive health outcomes in the literature.

A key follow-up question when interpreting the *APOE x diet* interactions in our data is whether they (if causal) are age specific or represent how a protective diet would be constituted over lifetime. Could APOE-tailored dietary guidelines given at younger age promote cognitive health and even protect against AD? Would health beyond cognition be affected? Such tailored advice would require APOE-screening at younger age, which might be discussed if future research would support that APOE-tailored advice may reduce the risk for chronic disease.

A holistic safety perspective must be included, and results on CFr-related effects on all-cause mortality are inconsistent. A higher adherence to a "low-carbohydrate diet" by quintiles (defined by a point system including several macronutrients) was associated with higher mortality in a U.S. study (n=165698, age 50-71 y, median follow-up 23.5 y) (285). In contrast, the previously cited U.K. study (n=120963) found higher mortality in the highest compared to the lowest carbohydrate tertile (186). If those studies had recognized the reciprocal relation between eCarb and eFat and reported explicit analyses for CFr, they had been easier to compare and they had represented a more well-defined research question. Anyway, they illustrate inconsistency in the results. It is well recognized that *some*, but far from all, individuals experience elevated LDL-cholesterol after adopting LCHF/KD; it is important to identify such *hyper-responders* which appear to be more common among individuals with relatively normal/low BMI and otherwise preferential health markers (56). In the cognitive field, it cannot be excluded that a diet that improves memory has some unwanted consequences on other health markers. If so, a well-informed risk/benefit-analysis must guide decisions. Such analyses may differ at age 35 versus 75 years since the risk of disadvantageous markers may act cumulatively over time. Safety analyses on LCHF/KD (286, 287) indicate that more long-term analyses are warranted and that individualized decisions are recommended.

Results of this thesis, taken together with the cited literature, suggest that it may be more important to better understand the *heterogenous* responses on diet between individuals—and particularly APOE-genotypes—than analyzing average population effects of diet on health. The results are compatible with a mismatch-hypothesis where conventional diet guidelines are primarily health-promoting for non-APOE4 (which by their numerical dominance color the population estimates); meanwhile, the optimal diet for APOE4 carriers may be different, based on evolutionary grounded biology. Particularly for the cognitive field, it may be motivated to allocate most research resources to understand those potentially APOE4 specific needs. Since those are numerically dominating among AD patients (≈75%), the largest prevention potential in number of individuals would be to optimize precision nutrition for APOE4 carriers, as already has been conceptually proposed by others (262).

7 Conclusions

• A lower dietary carbohydrate/fat-ratio (CFr) was associated with better global cognition among older adults with risk factors for dementia. The magnitude was larger for APOE34/44 carriers (dichotomized against 23/24/33), and for those a lower CFr was associated with better global cognition without any disadvantageous association with BMI, blood pressure, or blood lipid profile. The results are consistent with some hypotheses on a role for APOE in food adaptation at different stages of human evolution.

• The results provide a possible alternative/complementary explanation to *ketosis* as the driver in the interpretation of *ketogenic di*et (KD) interventions. The cognitive health field may consider performing RCT on liberal LCHF over strict KD for increased understanding of potential non-ketogenic pathways, and likely higher feasibility. The emergence of exogenous ketones—with a substantially higher ketogenic potential than kMCT—may facilitate distinct studies on the impact of ketosis in separate trials.

• A precision nutrition approach may be prescribed in research to promote cognitive health. Targeted recommendations to specific sub-samples may have a larger prevention potential than universal guidelines.

• It cannot be excluded that long-term exposure to a high-carbohydrate-low-fat diet may have adverse effects on cognitive health among APOE34/44. As a precautionary principle, dietary guidelines may avoid specifying ranges for the proportions of total fat and carbohydrates until this has been further studied. A possible additional role of the plant/animal-based dimension of food should be investigated.

• CFr—as a one-dimensional variable—may be preferred over *carbohydrates* and *fat* (by E%) as separate predictor variables in nutritional epidemiology.

• Avoidance of carbohydrate intake for 16 hours induced mild ketosis which was roughly doubled by intake of caprylic acid (C8) but not by coconut oil in older adults following their usual diet. Both treatments were well tolerated for further studies on hypothesized ketogenic and non-ketogenic pathways that might promote cognitive health, but current evidence of any substantial effects on cognitive performance is weak.

• Serum levels of mBDNF and proBDNF changed distinctly within a 4-hour window after intake of *C8 + coconut oil*, *sunflower oil*, and *coconut oil alone*, but only proBDNF changes (positive) were related to induced ketosis.

• A handheld ketone meter may be used as a reliable, economic, and flexible tool in research on nutritional ketosis, but we found systematically higher BHB levels in capillary compared to venous blood, which may be considered in comparisons between studies.

8 Points of Perspective

It is easy to find anecdotal reports on individuals who report substantial health benefits after shifting to LCHF/KD, but the same may be experienced by others who switch to a high-carbohydrate vegan diet. Results within this thesis add to a growing evidence base which suggests that such individual responses may have a biological rationale, grounded in genetic and metabolic factors. APOE appears as a key candidate for further research on gene–diet interactions in relation to health. For the cognitive field this has implications for selection of target groups for future diet interventions: By focusing on individuals with prodromal or preclinical AD (biological Alzheimer pathology) over MCI, SCD, or cognitively normal, APOE4-enrichment can be expected in the sample (288).

Changing one factor of interest while keeping everything else equal promotes the understanding of the effect of that factor. Changing several factors as a package—if expected to work in the same direction—may increase the effect but at the cost of lower explanatory precision on each factor. In considerations on RCT designs, this trade-off applies to combining carbohydrate restriction + ketosis; combining several diet aspects to a dietary pattern; or combining life-style interventions from several domains. Certainty about *direction* of effect for each component is preferred; otherwise, cancelling effects may hide important information. Results within this thesis indicated that cancelling effects may also arise from antagonistic responses between genetic sub-groups. A quantitative *parametric* approach—rather than a qualitative *patterns* approach—may facilitate more rigorous and reproducible research in the nutrition field, in line with suggestions by Ludwig et al. (88). Why would we choose point scales based on sample specific quantiles over universal, continuous, compositional parameters as our causal agents of interest? After all, dose dependent relations are expected in the natural sciences and any potential thresholds may be absolute rather than sample specific. By replacing potentially biased terms like “quality”, “healthy/unhealthy”, and “western” with purely descriptive terms in scientific reporting, researchers may indicate that they work with an open mind, not excluding that our definition of healthy/unhealthy may change over time and may differ for sub-groups. A *parametric* over a *patterns* approach should be more compatible with the emergence of precision nutrition and facilitate a synthesis of accumulating evidence. Moreover, a parametric approach would be compatible with the target trial framework (98, 103) which—in combination with insights from the theoretical framework for compositional data analysis (176)—may provide more formalized studies in nutritional epidemiology, where observational and RCT research accompany each other for better decision making on dietary choices.

Our results indicate that knowledge gaps in the cognitive field may be particularly important to fill for the following parameters: the carbohydrate/fat-ratio, the proportion of protein, and the plant/animal-ratio of food sources. Further, our results add to a large

body of evidence (220, 223-225) suggesting that basically all diet studies in the cognitive field need to be designed and interpreted with consideration of APOE.

Regarding cognitive effects of kMCT supplementation among MCI/AD, the trend from RCT evidence seems to be that promising effects on cognitive function has primarily been shown for non-APOE4 (152), although significant interaction effects by APOE has not been reported (to my knowledge). Taken together with our results in *Study V*, it may be hypothesized that APOE4 carriers (vs. non-carriers) would have a comparably larger improvement in cognitive performance by shifting from a high-carbohydrate-low-fat diet (HCLF) to LCHF, while any additional benefit for those from a stricter ketogenic diet or ketogenic supplements appears uncertain. An overall research aim of this thesis was to guide decisions on future RCT designs in the cognitive field. A conclusion may be that a study comparing liberal LCHF with HCLF in prodromal AD would address an important knowledge gap. By *not* including ketogenic supplementation, the explanatory value of the study may increase and potential dropouts due to intolerance can be avoided. Expected heterogeneity in the response might encourage a cross-over design.

Happily, our research group received resources to perform a pilot study in line with those suggestions. Alongside this doctoral project, I was proud to have been given the responsibility to have a leading role in the design and planning of that upcoming clinical trial: *COGNIFOOD—Investigating the therapeutic potential of changing the dietary carbohydrate/fat-ratio to prevent cognitive decline and Alzheimer pathology: A pilot study*. The trial has been ethically approved and will launch later this year.

The gene-diet interactions among older adults reported within this thesis raise questions on whether such interactions by APOE may be found also among younger adults and children, and possibly in other areas of brain health. In fact, our exploratory biomarker analyses strengthen the case for analyzing APOE as an effect modifier in metabolic research overall. Further, this thesis has included some gene-diet speculations in relation to evolutionary research, which might be followed up by experts within that field.

The FINGER trial has played a groundbreaking role for highlighting lifestyle factors in relation to cognitive health. This thesis has showed how the collected FINGER database has an additional value for observational research and hopefully the methods for panel analyses applied here may give inspiration for others. Our results are compatible with the assumption that diet changes in older age matters. The challenge may be to address the right dietary parameter, in the right direction, in the right individual.

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10 References

1. Cunnane SC, Trushina E, Morland C, Prigione A, Casadesus G, Andrews ZB, et al. Brain energy rescue: an emerging therapeutic concept for neurodegenerative disorders of ageing. Nat Rev Drug Discov. 2020.

2. A DB. The Ketogenic Diet in Epilepsy. Can Med Assoc J. 1931;24(1):106-7.

3. Barzegar M, Afghan M, Tarmahi V, Behtari M, Rahimi Khamaneh S, Raeisi S. Ketogenic diet: overview, types, and possible anti-seizure mechanisms. Nutr Neurosci. 2021;24(4):307-16.

4. Danan A, Westman EC, Saslow LR, Ede G. The Ketogenic Diet for Refractory Mental Illness: A Retrospective Analysis of 31 Inpatients. Front Psychiatry. 2022;13:951376.

5. Hallberg SJ, McKenzie AL, Williams PT, Bhanpuri NH, Peters AL, Campbell WW, et al. Effectiveness and Safety of a Novel Care Model for the Management of Type 2 Diabetes at 1 Year: An Open-Label, Non-Randomized, Controlled Study. Diabetes Ther. 2018;9(2):583-612.

6. Ludwig DS. The Ketogenic Diet: Evidence for Optimism but High-Quality Research Needed. The Journal of nutrition. 2020;150(6):1354-9.

7. Lennerz BS, Mey JT, Henn OH, Ludwig DS. Behavioral Characteristics and Self-Reported Health Status among 2029 Adults Consuming a "Carnivore Diet". Curr Dev Nutr. 2021;5(12):nzab133.

8. Neth BJ, Mintz A, Whitlow C, Jung Y, Solingapuram Sai K, Register TC, et al. Modified ketogenic diet is associated with improved cerebrospinal fluid biomarker profile, cerebral perfusion, and cerebral ketone body uptake in older adults at risk for Alzheimer's disease: a pilot study. Neurobiology of aging. 2020;86:54-63.

9. Laffel L. Ketone bodies: a review of physiology, pathophysiology and application of monitoring to diabetes. Diabetes/metabolism research and reviews. 1999;15(6):412-26.

10. Cahill GF, Jr. Fuel metabolism in starvation. Annu Rev Nutr. 2006;26:1-22.

11. Dayrit FM. The Properties of Lauric Acid and Their Significance in Coconut Oil. Journal of the American Oil Chemists' Society. 2014;92(1):1-15.

12. St-Pierre V, Vandenberghe C, Lowry CM, Fortier M, Castellano CA, Wagner R, et al. Plasma Ketone and Medium Chain Fatty Acid Response in Humans Consuming Different Medium Chain Triglycerides During a Metabolic Study Day. Frontiers in Nutrition. 2019;6.

13. Poff AM, Koutnik AP, Egan B. Nutritional Ketosis with Ketogenic Diets or Exogenous Ketones: Features, Convergence, and Divergence. Current sports medicine reports. 2020;19(7):251-9.

14. Westman EC, Feinman RD, Mavropoulos JC, Vernon MC, Volek JS, Wortman JA, et al. Low-carbohydrate nutrition and metabolism. American Journal of Clinical Nutrition. 2007;86(2):276-84.

15. Zilberter T, Zilberter Y. Ketogenic Ratio Determines Metabolic Effects of Macronutrients and Prevents Interpretive Bias. Frontiers in Nutrition. 2018;5.

16. Miller VJ, Villamena FA, Volek JS. Nutritional Ketosis and Mitohormesis: Potential Implications for Mitochondrial Function and Human Health. Journal of nutrition and metabolism. 2018;2018:5157645.

17. Zhu H, Bi D, Zhang Y, Kong C, Du J, Wu X, et al. Ketogenic diet for human diseases: the underlying mechanisms and potential for clinical implementations. Signal Transduct Target Ther. 2022;7(1):11.

18. Newman JC, Verdin E. Ketone bodies as signaling metabolites. Trends in Endocrinology and Metabolism. 2014;25(1):42-52.

19. Harvey PD. Domains of cognition and their assessment. Dialogues Clin Neurosci. 2019;21(3):227-37.

20. Pase MP, Stough C. An evidence-based method for examining and reporting cognitive processes in nutrition research. Nutr Res Rev. 2014;27(2):232-41.

21. Gomez-Pilar J, Martínez-Cagigal V, García-Azorín D, Gómez C, Guerrero Á, Hornero R. Headache-related circuits and high frequencies evaluated by EEG, MRI, PET as potential biomarkers to differentiate chronic and episodic migraine: Evidence from a systematic review. J Headache Pain. 2022;23(1):95.

22. Harrison JE, Rentz DM, Brashear HR, Arrighi HM, Ropacki MT, Liu E. Psychometric Evaluation of the Neuropsychological Test Battery in Individuals with Normal Cognition, Mild Cognitive Impairment, or Mild to Moderate Alzheimer's Disease: Results from a Longitudinal Study. J Prev Alzheimers Dis. 2018;5(4):236-44.

23. Lachat C, Hawwash D, Ocké MC, Berg C, Forsum E, Hörnell A, et al. Strengthening the Reporting of Observational Studies in Epidemiology-Nutritional Epidemiology (STROBE-nut): An Extension of the STROBE Statement. PLoS Med. 2016;13(6):e1002036.

24. Moher D, Hopewell S, Schulz KF, Montori V, Gotzsche PC, Devereaux PJ, et al. CONSORT 2010 explanation and elaboration: updated guidelines for reporting parallel group randomised trials. BMJ (Clinical research ed). 2010;340:c869.

25. Sorkin JD, Manary M, Smeets PAM, MacFarlane AJ, Astrup A, Prigeon RL, et al. A guide for authors and readers of the American Society for Nutrition Journals on the proper use of P values and strategies that promote transparency and improve research reproducibility. The American journal of clinical nutrition. 2021.

26. Mella N, Fagot D, Renaud O, Kliegel M, De Ribaupierre A. Individual Differences in Developmental Change: Quantifying the Amplitude and Heterogeneity in Cognitive Change across Old Age. J Intell. 2018;6(1).

27. Hall A, Mattila J, Koikkalainen J, Lötjonen J, Wolz R, Scheltens P, et al. Predicting progression from cognitive impairment to Alzheimer's disease with the Disease State Index. Curr Alzheimer Res. 2015;12(1):69-79.

28. Molinuevo JL, Rabin LA, Amariglio R, Buckley R, Dubois B, Ellis KA, et al. Implementation of subjective cognitive decline criteria in research studies. Alzheimer's & dementia : the journal of the Alzheimer's Association. 2017;13(3):296-311.

29. Sachs-Ericsson N, Blazer DG. The new DSM-5 diagnosis of mild neurocognitive disorder and its relation to research in mild cognitive impairment. Aging Ment Health. 2015;19(1):2-12.

30. Dubois B, Hampel H, Feldman HH, Scheltens P, Aisen P, Andrieu S, et al. Preclinical Alzheimer's disease: Definition, natural history, and diagnostic criteria. Alzheimer's & dementia : the journal of the Alzheimer's Association. 2016;12(3):292-323.

31. Jack CR, Knopman DS, Jagust WJ, Petersen RC, Weiner MW, Aisen PS, et al. Tracking pathophysiological processes in Alzheimer's disease: an updated hypothetical model of dynamic biomarkers. The Lancet Neurology. 2013;12(2):207-16.

32. Winblad B, Palmer K, Kivipelto M, Jelic V, Fratiglioni L, Wahlund LO, et al. Mild cognitive impairment--beyond controversies, towards a consensus: report of the International Working Group on Mild Cognitive Impairment. J Intern Med. 2004;256(3):240-6.

33. Dubois B, Villain N, Frisoni GB, Rabinovici GD, Sabbagh M, Cappa S, et al. Clinical diagnosis of Alzheimer's disease: recommendations of the International Working Group. Lancet Neurology. 2021;20(6):484-96.

34. Gustavsson A, Norton N, Fast T, Frolich L, Georges J, Holzapfel D, et al. Global estimates on the number of persons across the Alzheimer's disease continuum. Alzheimer's & dementia : the journal of the Alzheimer's Association. 2022.

35. Wojtowicz A, Larner AJ. Diagnostic test accuracy of cognitive screeners in older people. Progress in Neurology and Psychiatry. 2017;21(1):17-21.

36. 2022 Alzheimer's disease facts and figures. Alzheimer's & dementia : the journal of the Alzheimer's Association. 2022;18(4):700-89.

37. Livingston G, Huntley J, Sommerlad A, Ames D, Ballard C, Banerjee S, et al. Dementia prevention, intervention, and care: 2020 report of the Lancet Commission. Lancet (London, England). 2020;396(10248):413-46.

38. Barber RC. The genetics of Alzheimer's disease. Scientifica (Cairo). 2012;2012:246210.

39. Scheltens P, De Strooper B, Kivipelto M, Holstege H, Chételat G, Teunissen CE, et al. Alzheimer's disease. Lancet (London, England). 2021;397(10284):1577-90.

40. Andersson E, Janelidze S, Lampinen B, Nilsson M, Leuzy A, Stomrud E, et al. Blood and cerebrospinal fluid neurofilament light differentially detect neurodegeneration in early Alzheimer's disease. Neurobiology of aging. 2020;95:143-53.

41. Ashton NJ, Hye A, Rajkumar AP, Leuzy A, Snowden S, Suárez-Calvet M, et al. An update on blood-based biomarkers for non-Alzheimer neurodegenerative disorders. Nat Rev Neurol. 2020;16(5):265-84.

42. Pereira JB, Janelidze S, Smith R, Mattsson-Carlgren N, Palmqvist S, Teunissen CE, et al. Plasma GFAP is an early marker of amyloid-β but not tau pathology in Alzheimer's disease. Brain. 2021;144(11):3505-16.

43. Teunissen CE, Verberk IMW, Thijssen EH, Vermunt L, Hansson O, Zetterberg H, et al. Blood-based biomarkers for Alzheimer's disease: towards clinical implementation. The Lancet Neurology. 2022;21(1):66-77.

44. Woo NH, Teng HK, Siao CJ, Chiaruttini C, Pang PT, Milner TA, et al. Activation of p75NTR by proBDNF facilitates hippocampal long-term depression. Nat Neurosci. 2005;8(8):1069-77.

45. Norgren J, Daniilidou M, Kåreholt I, Sindi S, Akenine U, Nordin K, et al. Serum proBDNF Is Associated With Changes in the Ketone Body β-Hydroxybutyrate and Shows Superior Repeatability Over Mature BDNF: Secondary Outcomes From a Cross-Over Trial in Healthy Older Adults. Frontiers in Aging Neuroscience. 2021;13.

46. Balietti M, Giuli C, Conti F. Peripheral Blood Brain-Derived Neurotrophic Factor as a Biomarker of Alzheimer's Disease: Are There Methodological Biases? Mol Neurobiol. 2018;55(8):6661-72.

47. Lima Giacobbo B, Doorduin J, Klein HC, Dierckx R, Bromberg E, de Vries EFJ. Brain-Derived Neurotrophic Factor in Brain Disorders: Focus on Neuroinflammation. Mol Neurobiol. 2019;56(5):3295-312.

48. Hu E, Du H, Shang S, Zhang Y, Lu X. Beta-Hydroxybutyrate Enhances BDNF Expression by Increasing H3K4me3 and Decreasing H2AK119ub in Hippocampal Neurons. Frontiers in Neuroscience. 2020;14.

49. Marosi K, Kim SW, Moehl K, Scheibye-Knudsen M, Cheng AW, Cutler R, et al. 3-Hydroxybutyrate regulates energy metabolism and induces BDNF expression in cerebral cortical neurons. Journal of neurochemistry. 2016;139(5):769-81.

50. Sleiman SF, Henry J, Al-Haddad R, El Hayek L, Abou Haidar E, Stringer T, et al. Exercise promotes the expression of brain derived neurotrophic factor (BDNF) through the action of the ketone body beta-hydroxybutyrate. Elife. 2016;5.

51. Mohorko N, Cernelic-Bizjak M, Poklar-Vatovec T, Grom G, Kenig S, Petelin A, et al. Weight loss, improved physical performance, cognitive function, eating behavior, and metabolic profile in a 12-week ketogenic diet in obese adults. Nutrition Research. 2019;62:64-77.

52. Walsh JJ, Myette-Cote E, Little JP. The Effect of Exogenous Ketone Monoester Ingestion on Plasma BDNF During an Oral Glucose Tolerance Test. Frontiers in physiology. 2020;11:1094.

53. Kapogiannis D, Boxer A, Schwartz JB, Abner EL, Biragyn A, Masharani U, et al. Dysfunctionally phosphorylated type 1 insulin receptor substrate in neural-derived blood exosomes of preclinical Alzheimer's disease. FASEB J. 2015;29(2):589-96.

54. Björkhem I, Meaney S. Brain cholesterol: long secret life behind a barrier. Arterioscler Thromb Vasc Biol. 2004;24(5):806-15.

55. Kivipelto M, Ngandu T, Laatikainen T, Winblad B, Soininen H, Tuomilehto J. Risk score for the prediction of dementia risk in 20 years among middle aged people: a longitudinal, population-based study. The Lancet Neurology. 2006;5(9):735-41.

56. Norwitz NG, Feldman D, Soto-Mota A, Kalayjian T, Ludwig DS. Elevated LDL Cholesterol with a Carbohydrate-Restricted Diet: Evidence for a "Lean Mass Hyper-Responder" Phenotype. Curr Dev Nutr. 2022;6(1):nzab144.

57. Sniderman AD, Thanassoulis G, Glavinovic T, Navar AM, Pencina M, Catapano A, et al. Apolipoprotein B Particles and Cardiovascular Disease: A Narrative Review. JAMA Cardiol. 2019;4(12):1287-95.

58. Ferrier DR. Lippincott's illustrated reviews Biochemistry. 6. ed. . Philadelphia: Lippincott Williams and Wilkins 2014.

59. Blesso CN, Fernandez ML. Dietary Cholesterol, Serum Lipids, and Heart Disease: Are Eggs Working for or Against You? Nutrients. 2018;10(4).

60. Lu Y, Feskens EJ, Boer JM, Imholz S, Verschuren WM, Wijmenga C, et al. Exploring genetic determinants of plasma total cholesterol levels and their predictive value in a longitudinal study. Atherosclerosis. 2010;213(1):200-5.

61. Sniderman AD, Navar AM, Thanassoulis G. Apolipoprotein B vs Low-Density Lipoprotein Cholesterol and Non-High-Density Lipoprotein Cholesterol as the Primary Measure of Apolipoprotein B Lipoprotein-Related Risk: The Debate Is Over. JAMA Cardiol. 2021.

62. Belloy ME, Napolioni V, Greicius MD. A Quarter Century of APOE and Alzheimer's Disease: Progress to Date and the Path Forward. Neuron. 2019;101(5):820-38.

63. Rutjes AW, Denton DA, Di Nisio M, Chong LY, Abraham RP, Al-Assaf AS, et al. Vitamin and mineral supplementation for maintaining cognitive function in cognitively healthy people in mid and late life. The Cochrane database of systematic reviews. 2018;12:CD011906.

64. Rasmussen KL. Plasma levels of apolipoprotein E, APOE genotype and risk of dementia and ischemic heart disease: A review. Atherosclerosis. 2016;255:145-55.

65. Fernández-Calle R, Konings SC, Frontiñán-Rubio J, García-Revilla J, Camprubí-Ferrer L, Svensson M, et al. APOE in the bullseye of neurodegenerative diseases: impact of the APOE genotype in Alzheimer's disease pathology and brain diseases. Mol Neurodegener. 2022;17(1):62.

66. Willett W. Nutritional epidemiology. Oxford: Oxford University Press; 2013.

67. Scarmeas N, Anastasiou CA, Yannakoulia M. Nutrition and prevention of cognitive impairment. The Lancet Neurology. 2018;17(11):1006-15.

68. Solfrizzi V, Custodero C, Lozupone M, Imbimbo BP, Valiani V, Agosti P, et al. Relationships of Dietary Patterns, Foods, and Micro- and Macronutrients with Alzheimer's Disease and Late-Life Cognitive Disorders: A Systematic Review. Journal of Alzheimer's disease : JAD. 2017;59(3):815-49.

69. Taylor MK, Swerdlow RH, Burns JM, Sullivan DK. An Experimental Ketogenic Diet for Alzheimer Disease Was Nutritionally Dense and Rich in Vegetables and Avocado. Curr Dev Nutr. 2019;3(4):nzz003.

70. Zinn C, Rush A, Johnson R. Assessing the nutrient intake of a low-carbohydrate, high-fat (LCHF) diet: a hypothetical case study design. BMJ open. 2018;8(2):e018846.

71. O'Hearn A. Can a carnivore diet provide all essential nutrients? Current opinion in endocrinology, diabetes, and obesity. 2020;27(5):312-6.

72. WHO Guidelines Approved by the Guidelines Review Committee. Risk Reduction of Cognitive Decline and Dementia: WHO Guidelines. Geneva: World Health Organization

© World Health Organization 2019.; 2019.

73. Alles B, Samieri C, Feart C, Jutand MA, Laurin D, Barberger-Gateau P. Dietary patterns: a novel approach to examine the link between nutrition and cognitive function in older individuals. Nutr Res Rev. 2012;25(2):207-22.

74. Cena H, Calder PC. Defining a Healthy Diet: Evidence for The Role of Contemporary Dietary Patterns in Health and Disease. Nutrients. 2020;12(2).

75. Monteiro CA, Cannon G, Moubarac JC, Levy RB, Louzada MLC, Jaime PC. The UN Decade of Nutrition, the NOVA food classification and the trouble with ultra-processing. Public health nutrition. 2018;21(1):5-17.

76. Astrup A, Monteiro CA. Does the concept of "ultra-processed foods" help inform dietary guidelines, beyond conventional classification systems? Debate consensus. The American journal of clinical nutrition. 2022;116(6):1489-91.

77. Aguilera JM. The food matrix: implications in processing, nutrition and health. Crit Rev Food Sci Nutr. 2019;59(22):3612-29.

78. Frayn KN, Evans R. Human Metabolism: A Regulatory Perspective: Wiley; 2019.

79. Stubbs BJ, Cox PJ, Evans RD, Santer P, Miller JJ, Faull OK, et al. On the Metabolism of Exogenous Ketones in Humans. Frontiers in physiology. 2017;8:848.

80. Hervik AK, Svihus B. The Role of Fiber in Energy Balance. Journal of nutrition and metabolism. 2019;2019:4983657.

81. Willett WC, Howe GR, Kushi LH. Adjustment for total energy intake in epidemiologic studies. The American journal of clinical nutrition. 1997;65(4 Suppl):1220S-8S; discussion 9S-31S.

82. Aitchison J. THE STATISTICAL-ANALYSIS OF COMPOSITIONAL DATA. Journal of the Royal Statistical Society Series B-Methodological. 1982;44(2):139-77.

83. Correa Leite ML. Compositional data analysis as an alternative paradigm for nutritional studies. Clin Nutr ESPEN. 2019;33:207-12.

84. Kelly OJ, Gilman JC, Ilich JZ. Utilizing Dietary Micronutrient Ratios in Nutritional Research May be More Informative than Focusing on Single Nutrients. Nutrients. 2018;10(1).

85. Brown AW, Aslibekyan S, Bier D, Ferreira da Silva R, Hoover A, Klurfeld DM, et al. Toward more rigorous and informative nutritional epidemiology: The rational space between dismissal and defense of the status quo. Crit Rev Food Sci Nutr. 2021:1-18.

86. Hall KD. Challenges of human nutrition research. Science (New York, NY). 2020;367(6484):1298-300.

87. Ioannidis JPA. The Challenge of Reforming Nutritional Epidemiologic Research. Jama. 2018;320(10):969-70.

88. Ludwig DS, Ebbeling CB, Heymsfield SB. Improving the Quality of Dietary Research. Jama. 2019.

89. de Souza RJ, Swain JF, Appel LJ, Sacks FM. Alternatives for macronutrient intake and chronic disease: a comparison of the OmniHeart diets with popular diets and with dietary recommendations. The American journal of clinical nutrition. 2008;88(1):1-11.

90. Mente A, Dehghan M, Rangarajan S, McQueen M, Dagenais G, Wielgosz A, et al. Association of dietary nutrients with blood lipids and blood pressure in 18 countries: a cross-sectional analysis from the PURE study. The lancet Diabetes & endocrinology. 2017;5(10):774-87.

91. Zwarenstein M, Treweek S, Gagnier JJ, Altman DG, Tunis S, Haynes B, et al. Improving the reporting of pragmatic trials: an extension of the CONSORT statement. BMJ (Clinical research ed). 2008;337:a2390.

92. Loudon K, Treweek S, Sullivan F, Donnan P, Thorpe KE, Zwarenstein M. The PRECIS-2 tool: designing trials that are fit for purpose. BMJ (Clinical research ed). 2015;350:h2147.

93. Kobayashi M, Hanaoka T, Tsugane S. Validity of a self-administered food frequency questionnaire in the assessment of heterocyclic amine intake using 2-amino-1-methyl-6-phenylimidazo[4,5-b]pyridine (PhIP) levels in hair. Mutat Res. 2007;630(1-2):14-9.

94. Lacalle-Bergeron L, Izquierdo-Sandoval D, Sancho JV, Portolés T. Discovery of Food Intake Biomarkers Using Metabolomics. Methods Mol Biol. 2023;2571:33-43.

95. Nagpal R, Neth BJ, Wang S, Craft S, Yadav H. Modified Mediterranean-ketogenic diet modulates gut microbiome and short-chain fatty acids in association with Alzheimer's disease markers in subjects with mild cognitive impairment. EBioMedicine. 2019;47:529-42.

96. Anstey KJ, Zheng L, Peters R, Kootar S, Barbera M, Stephen R, et al. Dementia Risk Scores and Their Role in the Implementation of Risk Reduction Guidelines. Front Neurol. 2021;12:765454.

97. Dacks PA, Andrieu S, Blacker D, Carman AJ, Green AM, Grodstein F, et al. Dementia Prevention: optimizing the use of observational data for personal, clinical, and public health decision-making. J Prev Alzheimers Dis. 2014;1(2):117-23.

98. Hernán MA. The C-Word: Scientific Euphemisms Do Not Improve Causal Inference From Observational Data. Am J Public Health. 2018;108(5):616-9.

99. Hernán MA, Hsu J, Healy B. A Second Chance to Get Causal Inference Right: A Classification of Data Science Tasks. Chance. 2019;32(1):42-9.

100. Shmueli G. To Explain or to Predict? Statistical Science. 2010;25(3):289-310.

101. Schwingshackl L, Balduzzi S, Beyerbach J, Bröckelmann N, Werner SS, Zähringer J, et al. Evaluating agreement between bodies of evidence from randomised controlled trials and cohort studies in nutrition research: meta-epidemiological study. BMJ (Clinical research ed). 2021;374:n1864.

102. Berding K, Carbia C, Cryan JF. Going with the grain: Fiber, cognition, and the microbiota-gut-brain-axis. Exp Biol Med (Maywood). 2021;246(7):796-811.

103. Hernán MA, Robins JM. Causal Inference: What If: Boca Raton: Chapman & Hall/CRC.; 2020.

104. Lervag A. Editorial: Correlation and causation: to study causality in psychopathology. J Child Psychol Psychiatry. 2019;60(6):603-5.

105. Xu Y, Ritchie SC, Liang Y, Timmers P, Pietzner M, Lannelongue L, et al. An atlas of genetic scores to predict multi-omic traits. Nature. 2023;616(7955):123-31.

106. Ienca M, Vayena E, Blasimme A. Big Data and Dementia: Charting the Route Ahead for Research, Ethics, and Policy. Front Med (Lausanne). 2018;5:13.

107. Pearl J. Theoretical Impediments to Machine Learning With Seven Sparks from the Causal Revolution. 2018.

108. Hill AB. The Environmenta and Disease: Association or Causation? Proc R Soc Med. 1965;58(5):295-300.

109. Phillips CV, Goodman KJ. The missed lessons of Sir Austin Bradford Hill. Epidemiol Perspect Innov. 2004;1(1):3.

110. Tobias DK, Lajous M. What would the trial be? Emulating randomized dietary intervention trials to estimate causal effects with observational data. The American journal of clinical nutrition. 2021;114(2):416-7.

111. Matthews AA, Danaei G, Islam N, Kurth T. Target trial emulation: applying principles of randomised trials to observational studies. BMJ (Clinical research ed). 2022;378:e071108.

112. Hernan MA, Wang W, Leaf DE. Target Trial Emulation: A Framework for Causal Inference From Observational Data. Jama. 2022;328(24):2446-7.

113. Hernán MA. Methods of Public Health Research - Strengthening Causal Inference from Observational Data. N Engl J Med. 2021;385(15):1345-8.

114. Norgren J, Sindi S, Sandebring-Matton A, Kareholt I, Daniilidou M, Akenine U, et al. Ketosis After Intake of Coconut Oil and Caprylic Acid-With and Without Glucose: A Cross-Over Study in Healthy Older Adults. Front Nutr. 2020;7:40.

115. Norgren J, Sindi S, Sandebring-Matton A, Kareholt I, Akenine U, Nordin K, et al. Capillary blood tests may overestimate ketosis: triangulation between three different measures of beta-hydroxybutyrate. American journal of physiology Endocrinology and metabolism. 2020;318(2):E184-E8.

116. Norgren J, Sindi S, Sandebring-Matton A, Ngandu T, Kivipelto M, Kåreholt I. The Dietary Carbohydrate/Fat-ratio and Cognitive Performance: Panel Analyses in Older Adults at Risk for Dementia. Current Developments in Nutrition. 2023.

117. Kivipelto M, Solomon A, Ahtiluoto S, Ngandu T, Lehtisalo J, Antikainen R, et al. The Finnish Geriatric Intervention Study to Prevent Cognitive Impairment and Disability (FINGER): study design and progress. Alzheimer's & dementia : the journal of the Alzheimer's Association. 2013;9(6):657-65.

118. Kivipelto M, Mangialasche F, Ngandu T. Lifestyle interventions to prevent cognitive impairment, dementia and Alzheimer disease. Nat Rev Neurol. 2018;14(11):653-66.

119. Kivimäki M, Singh-Manoux A. Prevention of dementia by targeting risk factors. Lancet (London, England). 2018;391(10130):1574-5.

120. Griffiths TD, Lad M, Kumar S, Holmes E, McMurray B, Maguire EA, et al. How Can Hearing Loss Cause Dementia? Neuron. 2020;108(3):401-12.

121. Muth AK, Park SQ. The impact of dietary macronutrient intake on cognitive function and the brain. Clin Nutr. 2021;40(6):3999-4010.

122. Owen OE, Morgan AP, Kemp HG, Sullivan JM, Herrera MG, Cahill GF, Jr. Brain metabolism during fasting. The Journal of clinical investigation. 1967;46(10):1589-95.

123. Schonfeld P, Reiser G. Why does brain metabolism not favor burning of fatty acids to provide energy? Reflections on disadvantages of the use of free fatty acids as fuel for brain. Journal of cerebral blood flow and metabolism : official journal of the International Society of Cerebral Blood Flow and Metabolism. 2013;33(10):1493-9.

124. Blomqvist G, Thorell JO, Ingvar M, Grill V, Widén L, Stone-Elander S. Use of R-beta-[1-11C]hydroxybutyrate in PET studies of regional cerebral uptake of ketone bodies in humans. Am J Physiol. 1995;269(5 Pt 1):E948-59.

125. Cunnane S, Nugent S, Roy M, Courchesne-Loyer A, Croteau E, Tremblay S, et al. Brain fuel metabolism, aging, and Alzheimer's disease. Nutrition (Burbank, Los Angeles County, Calif). 2011;27(1):3-20.

126. Mattson MP, Moehl K, Ghena N, Schmaedick M, Cheng A. Intermittent metabolic switching, neuroplasticity and brain health. Nature reviews Neuroscience. 2018;19(2):63-80.

127. Croteau E, Castellano CA, Richard MA, Fortier M, Nugent S, Lepage M, et al. Ketogenic Medium Chain Triglycerides Increase Brain Energy Metabolism in Alzheimer's Disease. Journal of Alzheimer's disease : JAD. 2018;64(2):551-61.

128. Neth BJ, Craft S. Insulin Resistance and Alzheimer's Disease: Bioenergetic Linkages. Front Aging Neurosci. 2017;9:345.

129. Cunnane S, Courchesne-Loyer A, St-Pierre V, Vandenberghe C, Croteau E, Castellano C-A. Glucose and Ketone Metabolism in the Aging Brain: Implications for Therapeutic Strategies to Delay the Progression of Alzheimer's Disease. . In: Masino SA, editor. Ketogenic Diet and Metabolic Therapies Oxford University Press; 2017. p. 113-32.

130. Kullmann S, Heni M, Hallschmid M, Fritsche A, Preissl H, Haring HU. Brain Insulin Resistance at the Crossroads of Metabolic and Cognitive Disorders in Humans. Physiological reviews. 2016;96(4):1169-209.

131. Xin LJ, Ipek O, Beaumont M, Shevlyakova M, Christinat N, Masoodi M, et al. Nutritional Ketosis Increases NAD(+)/NADH Ratio in Healthy Human Brain: An in Vivo Study by P-31-MRS. Frontiers in Nutrition. 2018;5.

132. Klosinski LP, Yao J, Yin F, Fonteh AN, Harrington MG, Christensen TA, et al. White Matter Lipids as a Ketogenic Fuel Supply in Aging Female Brain: Implications for Alzheimer's Disease. EBioMedicine. 2015;2(12):1888-904.

133. Roy M, Fortier M, Rheault F, Edde M, Croteau E, Castellano CA, et al. A ketogenic supplement improves white matter energy supply and processing speed in mild cognitive impairment. Alzheimer's & dementia (New York, N Y). 2021;7(1):e12217.

134. Youm YH, Nguyen KY, Grant RW, Goldberg EL, Bodogai M, Kim D, et al. The ketone metabolite beta-hydroxybutyrate blocks NLRP3 inflammasome-mediated inflammatory disease. Nat Med. 2015;21(3):263-9.

135. Shimazu T, Hirschey MD, Newman J, He W, Shirakawa K, Le Moan N, et al. Suppression of oxidative stress by beta-hydroxybutyrate, an endogenous histone deacetylase inhibitor. Science (New York, NY). 2013;339(6116):211-4.

136. Edwards C, Copes N, Bradshaw PC. D-ss-hydroxybutyrate: an anti-aging ketone body. Oncotarget. 2015;6(6):3477-8.

137. Newman J. Ketone Body Signaling in Health and Aging. Metabolic Health Summit; Los Angeles, USA2020.

138. Cunnane SC. Ketones, omega-3 fatty acids and the Yin-Yang balance in the brain: insights from infant development and Alzheimer's disease, and implications for human brain evolution. Ocl-Oilseeds and Fats Crops and Lipids. 2018;25(4).

139. Balasse EO, Fery F. Ketone body production and disposal: effects of fasting, diabetes, and exercise. Diabetes/metabolism reviews. 1989;5(3):247-70.

140. Bach AC, Babayan VK. Medium-chain triglycerides: an update. The American journal of clinical nutrition. 1982;36(5):950-62.

141. Vandenberghe C, St-Pierre V, Pierotti T, Fortier M, Castellano CA, Cunnane SC. Tricaprylin Alone Increases Plasma Ketone Response More Than Coconut Oil or Other Medium-Chain Triglycerides: An Acute Crossover Study in Healthy Adults. Current Developments in Nutrition. 2017;1(4).

142. Owen OE, Felig P, Morgan AP, Wahren J, Cahill GF. Liver and Kidney Metabolism During Prolonged Starvation. Journal of Clinical Investigation. 1969;48(3):574-&.

143. Colagiuri S, Sandbaek A, Carstensen B, Christensen J, Glumer C, Lauritzen T, et al. Comparability of venous and capillary glucose measurements in blood. Diabetic Medicine. 2003;20(11):953-6.

144. Brooke J, Stiell M, Ojo O. Evaluation of the Accuracy of Capillary Hydroxybutyrate Measurement Compared with Other Measurements in the Diagnosis of Diabetic Ketoacidosis: A Systematic Review. International journal of environmental research and public health. 2016;13(9).

145. Alkedeh O, Priefer R. The Ketogenic Diet: Breath Acetone Sensing Technology. Biosensors (Basel). 2021;11(1).

146. Taylor MK, Sullivan DK, Mahnken JD, Burns JM, Swerdlow RH. Feasibility and efficacy data from a ketogenic diet intervention in Alzheimer's disease. Alzheimer's & dementia (New York, N Y). 2018;4:28-36.

147. Brandt J, Buchholz A, Henry-Barron B, Vizthum D, Avramopoulos D, Cervenka MC. Preliminary Report on the Feasibility and Efficacy of the Modified Atkins Diet for Treatment of Mild Cognitive Impairment and Early Alzheimer's Disease. Journal of Alzheimer's disease : JAD. 2019;68(3):969-81.

148. Krikorian R, Shidler MD, Dangelo K, Couch SC, Benoit SC, Clegg DJ. Dietary ketosis enhances memory in mild cognitive impairment. Neurobiology of aging. 2012;33(2):425 e19-27.

149. Henderson ST, Vogel JL, Barr LJ, Garvin F, Jones JJ, Costantini LC. Study of the ketogenic agent AC-1202 in mild to moderate Alzheimer's disease: a randomized, double-blind, placebo-controlled, multicenter trial. Nutr Metab (Lond). 2009;6:31.

150. Henderson ST, Morimoto BH, Cummings JL, Farlow MR, Walker J. A Placebo-Controlled, Parallel-Group, Randomized Clinical Trial of AC-1204 in Mild-to-Moderate Alzheimer's Disease. Journal of Alzheimer's disease : JAD. 2020;75(2):547-57.

151. Avgerinos KI, Egan JM, Mattson MP, Kapogiannis D. Medium Chain Triglycerides induce mild ketosis and may improve cognition in Alzheimer's disease. A systematic review and meta-analysis of human studies. Ageing research reviews. 2020;58:101001.

152. Bohnen JLB, Albin RL, Bohnen NI. Ketogenic interventions in mild cognitive impairment, Alzheimer's disease, and Parkinson's disease: A systematic review and critical appraisal. Frontiers in Neurology. 2023;14.

153. Giannos P, Prokopidis K, Lidoriki I, Triantafyllidis KK, Kechagias KS, Celoch K, et al. Medium-chain triglycerides may improve memory in non-demented older adults: a systematic review of randomized controlled trials. BMC Geriatr. 2022;22(1):817.

154. Dangour AD, Allen E. Do omega-3 fats boost brain function in adults? Are we any closer to an answer? The American journal of clinical nutrition. 2013;97(5):909-10.

155. Fogelholm M. New Nordic Nutrition Recommendations are here. Food & nutrition research. 2013;57.

156. Phillips JA. Dietary Guidelines for Americans, 2020-2025. Workplace Health Saf. 2021;69(8):395.

157. Abbatecola AM, Russo M, Barbieri M. Dietary patterns and cognition in older persons. Current opinion in clinical nutrition and metabolic care. 2018;21(1):10-3.

158. Townsend RF, Logan D, O'Neill RF, Prinelli F, Woodside JV, McEvoy CT. Whole Dietary Patterns, Cognitive Decline and Cognitive Disorders: A Systematic Review of Prospective and Intervention Studies. Nutrients. 2023;15(2).

159. Campbell AP. DASH Eating Plan: An Eating Pattern for Diabetes Management. Diabetes Spectr. 2017;30(2):76-81.

160. Chiu S, Bergeron N, Williams PT, Bray GA, Sutherland B, Krauss RM. Comparison of the DASH (Dietary Approaches to Stop Hypertension) diet and a higher-fat DASH diet on blood pressure and lipids and lipoproteins: a randomized controlled trial. The American journal of clinical nutrition. 2016;103(2):341-7.

161. Nishi SK, Babio N, Gomez-Martinez C, Martinez-Gonzalez MA, Ros E, Corella D, et al. Mediterranean, DASH, and MIND Dietary Patterns and Cognitive Function: The 2-Year Longitudinal Changes in an Older Spanish Cohort. Front Aging Neurosci. 2021;13:782067.

162. Martinez-Gonzalez MA, Hershey MS, Zazpe I, Trichopoulou A. Transferability of the Mediterranean Diet to Non-Mediterranean Countries. What Is and What Is Not the Mediterranean Diet. Nutrients. 2017;9(11).

163. Glans I, Sonestedt E, Nägga K, Gustavsson AM, González-Padilla E, Borne Y, et al. Association Between Dietary Habits in Midlife With Dementia Incidence Over a 20-Year Period. Neurology. 2023;100(1):e28-e37.

164. Akbaraly TN, Singh-Manoux A, Dugravot A, Brunner EJ, Kivimäki M, Sabia S. Association of Midlife Diet With Subsequent Risk for Dementia. Jama. 2019;321(10):957-68.

165. Dearborn-Tomazos JL, Wu A, Steffen LM, Anderson CAM, Hu EA, Knopman D, et al. Association of Dietary Patterns in Midlife and Cognitive Function in Later Life in US Adults Without Dementia. JAMA Netw Open. 2019;2(12):e1916641.

166. Kjeldsen EW, Thomassen JQ, Rasmussen KL, Nordestgaard BG, Tybjærg-Hansen A, Frikke-Schmidt R. Adherence to dietary guidelines and risk of dementia: a prospective cohort study of 94 184 individuals. Epidemiol Psychiatr Sci. 2022;31:e71.

167. Devranis P, Vassilopoulou E, Tsironis V, Sotiriadis PM, Chourdakis M, Aivaliotis M, et al. Mediterranean Diet, Ketogenic Diet or MIND Diet for Aging Populations with Cognitive Decline: A Systematic Review. Life (Basel). 2023;13(1).

168. Loughrey DG, Lavecchia S, Brennan S, Lawlor BA, Kelly ME. The Impact of the Mediterranean Diet on the Cognitive Functioning of Healthy Older Adults: A Systematic Review and Meta-Analysis. Advances in nutrition (Bethesda, Md). 2017;8(4):571-86.

169. Petersson SD, Philippou E. Mediterranean Diet, Cognitive Function, and Dementia: A Systematic Review of the Evidence. Advances in nutrition (Bethesda, Md). 2016;7(5):889-904.

170. Valls-Pedret C, Sala-Vila A, Serra-Mir M, Corella D, de la Torre R, Martinez-Gonzalez MA, et al. Mediterranean Diet and Age-Related Cognitive Decline: A Randomized Clinical Trial. JAMA Intern Med. 2015;175(7):1094-103.

171. Guasch-Ferre M, Babio N, Martinez-Gonzalez MA, Corella D, Ros E, Martin-Pelaez S, et al. Dietary fat intake and risk of cardiovascular disease and all-cause mortality in a population at high risk of cardiovascular disease. The American journal of clinical nutrition. 2015;102(6):1563-73.

172. Estruch R, Ros E, Salas-Salvadó J, Covas MI, Corella D, Arós F, et al. Primary prevention of cardiovascular disease with a Mediterranean diet. N Engl J Med. 2013;368(14):1279-90.

173. Agarwal A, Ioannidis JPA. PREDIMED trial of Mediterranean diet: retracted, republished, still trusted? BMJ (Clinical research ed). 2019;364:l341.

174. Estruch R, Ros E, Salas-Salvadó J, Covas MI, Corella D, Arós F, et al. Retraction and Republication: Primary Prevention of Cardiovascular Disease with a Mediterranean Diet. N Engl J Med 2013;368:1279-90. N Engl J Med. 2018;378(25):2441-2.

175. Staubo SC, Aakre JA, Vemuri P, Syrjanen JA, Mielke MM, Geda YE, et al. Mediterranean diet, micronutrients and macronutrients, and MRI measures of cortical thickness. Alzheimers & Dementia. 2017;13(2):168-77.

176. Corrêa Leite ML. Log-ratio transformations for dietary compositions: numerical and conceptual questions. J Nutr Sci. 2021;10:e97.

177. Zhang J, McKeown RE, Muldoon MF, Tang SH. Cognitive performance is associated with macronutrient intake in healthy young and middle-aged adults. Nutritional Neuroscience. 2006;9(3-4):179-87.

178. Phillips MCL, Deprez LM, Mortimer GMN, Murtagh DKJ, McCoy S, Mylchreest R, et al. Randomized crossover trial of a modified ketogenic diet in Alzheimer's disease. Alzheimer's Research & Therapy. 2021;13(1).

179. Cao GY, Li M, Han L, Tayie F, Yao SS, Huang Z, et al. Dietary Fat Intake and Cognitive Function among Older Populations: A Systematic Review and Meta-Analysis. J Prev Alzheimers Dis. 2019;6(3):204-11.

180. Ruan Y, Tang J, Guo X, Li K, Li D. Dietary Fat Intake and Risk of Alzheimer's Disease and Dementia: A Meta-Analysis of Cohort Studies. Curr Alzheimer Res. 2018;15(9):869-76.

181. Zhu RZ, Chen MQ, Zhang ZW, Wu TY, Zhao WH. Dietary fatty acids and risk for Alzheimer's disease, dementia, and mild cognitive impairment: A prospective cohort meta-analysis. Nutrition (Burbank, Los Angeles County, Calif). 2021;90:111355.

182. Tomova GD, Arnold KF, Gilthorpe MS, Tennant PWG. Adjustment for energy intake in nutritional research: a causal inference perspective. The American journal of clinical nutrition. 2021.

183. Chlebowski RT, Rapp S, Aragaki AK, Pan K, Neuhouser ML, Snetselaar LG, et al. Low-fat dietary pattern and global cognitive function: Exploratory analyses of the Women's Health Initiative (WHI) randomized Dietary Modification trial. EClinicalMedicine. 2020;18:100240.

184. Roberts RO, Roberts LA, Geda YE, Cha RH, Pankratz VS, O'Connor HM, et al. Relative Intake of Macronutrients Impacts Risk of Mild Cognitive Impairment or Dementia. Journal of Alzheimers Disease. 2012;32(2):329-39.

185. Ding B, Xiao R, Ma W, Zhao L, Bi Y, Zhang Y. The association between macronutrient intake and cognition in individuals aged under 65 in China: a cross-sectional study. BMJ open. 2018;8(1):e018573.

186. McKenzie BL, Harris K, Peters SAE, Webster J, Woodward M. The association of energy and macronutrient intake with all-cause mortality, cardiovascular disease and dementia: findings from 120 963 women and men in the UK Biobank. The British journal of nutrition. 2021:1-10.

187. Ludwig DS, Willett WC, Volek JS, Neuhouser ML. Dietary fat: From foe to friend? Science (New York, NY). 2018;362(6416):764-70.

188. Keys A, Anderson JT, Grande F. Serum cholesterol response to changes in the diet: IV. Particular saturated fatty acids in the diet. Metabolism: clinical and experimental. 1965;14(7):776-87.

189. Hu FB, Manson JE, Willett WC. Types of dietary fat and risk of coronary heart disease: a critical review. J Am Coll Nutr. 2001;20(1):5-19.

190. Mozaffarian D, Ludwig DS. The 2015 US Dietary Guidelines Lifting the Ban on Total Dietary Fat. Jama-Journal of the American Medical Association. 2015;313(24):2421-2.

191. Astrup A, Magkos F, Bier DM, Brenna JT, de Oliveira Otto MC, Hill JO, et al. Saturated Fats and Health: A Reassessment and Proposal for Food-Based Recommendations: JACC State-of-the-Art Review. J Am Coll Cardiol. 2020;76(7):844-57.

192. Koyama T, Yoshita K, Okuda N, Saitoh S, Sakata K, Okayama A, et al. Overall nutrient and total fat intake among Japanese people: The INTERLIPID Study Japan. Asia Pacific Journal of Clinical Nutrition. 2017;26(5):837-48.

193. Nwaru BI, Dierkes J, Ramel A, Arnesen EK, Thorisdottir B, Lamberg-Allardt C, et al. Quality of dietary fat and risk of Alzheimer's disease and dementia in adults aged >= 50 years: a systematic review. Food & nutrition research. 2022;66.

194. Kelly OJ, Gilman JC, Ilich JZ. Utilizing Dietary Nutrient Ratios in Nutritional Research: Expanding the Concept of Nutrient Ratios to Macronutrients. Nutrients. 2019;11(2).

195. Taylor MK, Sullivan DK, Swerdlow RH, Vidoni ED, Morris JK, Mahnken JD, et al. A high-glycemic diet is associated with cerebral amyloid burden in cognitively normal older adults. The American journal of clinical nutrition. 2017;106(6):1463-70.

196. Philippou E, Constantinou M. The influence of glycemic index on cognitive functioning: a systematic review of the evidence. Advances in nutrition (Bethesda, Md). 2014;5(2):119-30.

197. Philippou E, Pot GK, Heraclides A, Richards M, Bendayan R. Dietary glycaemic index and cognitive function: prospective associations in adults of the 1946 British birth cohort. Public health nutrition. 2018;22(8):1-10.

198. Bayer-Carter JL, Green PS, Montine TJ, VanFossen B, Baker LD, Watson GS, et al. Diet intervention and cerebrospinal fluid biomarkers in amnestic mild cognitive impairment. Archives of neurology. 2011;68(6):743-52.

199. Hanson AJ, Bayer JL, Baker LD, Cholerton B, VanFossen B, Trittschuh E, et al. Differential Effects of Meal Challenges on Cognition, Metabolism, and Biomarkers for Apolipoprotein E ε4 Carriers and Adults with Mild Cognitive Impairment. Journal of Alzheimer's disease : JAD. 2015;48(1):205-18.

200. Shan ZL, Rehm CD, Rogers G, Ruan MY, Wang DD, Hu FB, et al. Trends in Dietary Carbohydrate, Protein, and Fat Intake and Diet Quality Among US Adults, 1999-2016. Jama-Journal of the American Medical Association. 2019;322(12):1178-87.

201. Valsta LM, Tapanainen H, Kortetmaki T, Sares-Jaske L, Paalanen L, Kaartinen NE, et al. Disparities in Nutritional Adequacy of Diets between Different Socioeconomic Groups of Finnish Adults. Nutrients. 2022;14(7).

202. Willett W, Rockström J, Loken B, Springmann M, Lang T, Vermeulen S, et al. Food in the Anthropocene: the EAT-Lancet Commission on healthy diets from sustainable food systems. Lancet (London, England). 2019;393(10170):447-92.

203. Dalile B, Kim C, Challinor A, Geurts L, Gibney ER, Galdos MV, et al. The EAT–Lancet reference diet and cognitive function across the life course. The Lancet Planetary Health. 2022;6(9):e749-e59.

204. Kristensen NB, Madsen ML, Hansen TH, Allin KH, Hoppe C, Fagt S, et al. Intake of macro- and micronutrients in Danish vegans. Nutr J. 2015;14:115.

205. Paivarinta E, Itkonen ST, Pellinen T, Lehtovirta M, Erkkola M, Pajari AM. Replacing Animal-Based Proteins with Plant-Based Proteins Changes the Composition of a Whole Nordic Diet-A Randomised Clinical Trial in Healthy Finnish Adults. Nutrients. 2020;12(4).

206. Katonova A, Sheardova K, Amlerova J, Angelucci F, Hort J. Effect of a Vegan Diet on Alzheimer's Disease. International journal of molecular sciences. 2022;23(23).

207. Tsai JH, Huang CF, Lin MN, Chang CE, Chang CC, Lin CL. Taiwanese Vegetarians Are Associated with Lower Dementia Risk: A Prospective Cohort Study. Nutrients. 2022;14(3).

208. Giem P, Beeson WL, Fraser GE. The incidence of dementia and intake of animal products: preliminary findings from the Adventist Health Study. Neuroepidemiology. 1993;12(1):28-36.

209. Bush CL, Blumberg JB, El-Sohemy A, Minich DM, Ordovás JM, Reed DG, et al. Toward the Definition of Personalized Nutrition: A Proposal by The American Nutrition Association. J Am Coll Nutr. 2020;39(1):5-15.

210. Huebbe P, Rimbach G. Evolution of human apolipoprotein E (APOE) isoforms: Gene structure, protein function and interaction with dietary factors. Ageing research reviews. 2017;37:146-61.

211. Zannis VI, Nicolosi RJ, Jensen E, Breslow JL, Hayes KC. Plasma and hepatic apoE isoproteins of nonhuman primates. Differences in apoE among humans, apes, and New and Old World monkeys. Journal of lipid research. 1985;26(12):1421-30.

212. National Research Council 2003. *Nutrient Requirements of Nonhuman Primates: Second Revised Edition*. Washington, DC: The National Academies Press. https://doi.org/10.17226/9826.

213. Gerstner KF, Pruetz JD. Wild Chimpanzee Welfare: A Focus on Nutrition, Foraging and Health to Inform Great Ape Welfare in the Wild and in Captivity. Animals (Basel). 2022;12(23).

214. Finch CE, Stanford CB. Meat-adaptive genes and the evolution of slower aging in humans. Quarterly Review of Biology. 2004;79(1):3-50.

215. Henderson ST. High carbohydrate diets and Alzheimer's disease. Med Hypotheses. 2004;62(5):689-700.

216. Graeser AC, Boesch-Saadatmandi C, Lippmann J, Wagner AE, Huebbe P, Storm N, et al. Nrf2-dependent gene expression is affected by the proatherogenic apoE4 genotype-studies in targeted gene replacement mice. J Mol Med (Berl). 2011;89(10):1027-35.

217. Ben-Dor M, Sirtoli R, Barkai R. The evolution of the human trophic level during the Pleistocene. Am J Phys Anthropol. 2021.

218. Abondio P, Sazzini M, Garagnani P, Boattini A, Monti D, Franceschi C, et al. The Genetic Variability of APOE in Different Human Populations and Its Implications for Longevity. Genes (Basel). 2019;10(3).

219. Singh PP, Singh M, Mastana SS. APOE distribution in world populations with new data from India and the UK. Ann Hum Biol. 2006;33(3):279-308.

220. Zhang H, Greenwood DC, Risch HA, Bunce D, Hardie LJ, Cade JE. Meat consumption and risk of incident dementia: cohort study of 493,888 UK Biobank participants. The American journal of clinical nutrition. 2021;114(1):175-84.

221. Wasserstein RL, Schirm AL, Lazar NA. Moving to a World Beyond "p < 0.05". The American Statistician. 2019;73(sup1):1-19.

222. Munafo MR, Tilling K, Taylor AE, Evans DM, Davey Smith G. Collider scope: when selection bias can substantially influence observed associations. International journal of epidemiology. 2018;47(1):226-35.

223. Farmer BC, Johnson LA, Hanson AJ. Effects of apolipoprotein E on nutritional metabolism in dementia. Curr Opin Lipidol. 2019;30(1):10-5.

224. Egert S, Rimbach G, Huebbe P. ApoE genotype: from geographic distribution to function and responsiveness to dietary factors. Proceedings of the Nutrition Society. 2012;71(3):410-24.

225. Lane-Donovan C, Herz J. High-Fat Diet Changes Hippocampal Apolipoprotein E (ApoE) in a Genotype- and Carbohydrate-Dependent Manner in Mice. PloS one. 2016;11(2).

226. Mehlig K, Lapidus L, Thelle DS, Waern M, Zetterberg H, Björkelund C, et al. Low fasting serum insulin and dementia in nondiabetic women followed for 34 years. Neurology. 2018;91(5):e427-e35.

227. Peila R, Rodriguez BL, White LR, Launer LJ. Fasting insulin and incident dementia in an elderly population of Japanese-American men. Neurology. 2004;63(2):228-33.

228. Morris JK, Uy RAZ, Vidoni ED, Wilkins HM, Archer AE, Thyfault JP, et al. Effect of APOE epsilon4 Genotype on Metabolic Biomarkers in Aging and Alzheimer's Disease. Journal of Alzheimer's disease : JAD. 2017;58(4):1129-35.

229. Hershey MS, Martínez-González M, Álvarez-Álvarez I, Martínez Hernández JA, Ruiz-Canela M. The Mediterranean diet and physical activity: better together than apart for the prevention of premature mortality. The British journal of nutrition. 2022;128(7):1413-24.

230. Ngandu T, Lehtisalo J, Solomon A, Levalahti E, Ahtiluoto S, Antikainen R, et al. A 2 year multidomain intervention of diet, exercise, cognitive training, and vascular risk monitoring versus control to prevent cognitive decline in at-risk elderly people (FINGER): a randomised controlled trial. Lancet (London, England). 2015;385(9984):2255-63.

231. Hafdi M, Hoevenaar-Blom MP, Richard E. Multi-domain interventions for the prevention of dementia and cognitive decline. The Cochrane database of systematic reviews. 2021;11(11):Cd013572.

232. Kivipelto M, Mangialasche F, Snyder HM, Allegri R, Andrieu S, Arai H, et al. World-Wide FINGERS Network: A global approach to risk reduction and prevention of dementia. Alzheimer's & dementia : the journal of the Alzheimer's Association. 2020;16(7):1078-94.

233. Sibbald B, Roberts C. Understanding controlled trials. Crossover trials. BMJ (Clinical research ed). 1998;316(7146):1719.

234. Koo TK, Li MY. A Guideline of Selecting and Reporting Intraclass Correlation Coefficients for Reliability Research. J Chiropr Med. 2016;15(2):155-63.

235. van de Pol MV, Wright J. A simple method for distinguishing within- versus between-subject effects using mixed models. Animal Behaviour. 2009;77(3):753-8.

236. Gunasekara FI, Richardson K, Carter K, Blakely T. Fixed effects analysis of repeated measures data. International journal of epidemiology. 2014;43(1):264-9.

237. Puska P. From Framingham to North Karelia From Descriptive Epidemiology to Public Health Action. Progress in Cardiovascular Diseases. 2010;53(1):15-20.

238. Mannisto S, Laatikainen T, Helakorpi S, Valsta LM. Monitoring diet and diet-related chronic disease risk factors in Finland. Public health nutrition. 2010;13(6a):907-14.

239. World Health Rankings; Data Source: WHO 2020 [Available from: https://www.worldlifeexpectancy.com/cause-of-death/alzheimers-dementia/by-country/, assessed April 15, 2023]

240. Eiser AR. Why does Finland have the highest dementia mortality rate? Environmental factors may be generalizable. Brain research. 2017;1671:14-7.

241. Kievit RA, Frankenhuis WE, Waldorp LJ, Borsboom D. Simpson's paradox in psychological science: a practical guide. Front Psychol. 2013;4:513.

242. Hall KD, Guo J, Courville AB, Boring J, Brychta R, Chen KY, et al. Effect of a plant-based, low-fat diet versus an animal-based, ketogenic diet on ad libitum energy intake. Nat Med. 2021;27(2):344-53.

243. Ludwig DS, Dickinson SL, Henschel B, Ebbeling CB, Allison DB. Do Lower-Carbohydrate Diets Increase Total Energy Expenditure? An Updated and Reanalyzed Meta-Analysis of 29 Controlled-Feeding Studies. The Journal of nutrition. 2021;151(3):482-90.

244. Heidt C, Fobker M, Newport M, Feldmann R, Fischer T, Marquardt T. Beta-Hydroxybutyrate (BHB), Glucose, Insulin, Octanoate (C8), and Decanoate (C10) Responses to a Medium-Chain Triglyceride (MCT) Oil with and without Glucose: A Single-Center Study in Healthy Adults. Nutrients. 2023;15(5).

245. Fortier M, Castellano CA, St-Pierre V, Myette-Cote E, Langlois F, Roy M, et al. A ketogenic drink improves cognition in mild cognitive impairment: Results of a 6-month RCT. Alzheimer's & dementia : the journal of the Alzheimer's Association. 2020.

246. Baumeister A, Gardemann J, Fobker M, Spiegler V, Fischer T. Short-Term Influence of Caffeine and Medium-Chain Triglycerides on Ketogenesis: A Controlled Double-Blind Intervention Study. Journal of nutrition and metabolism. 2021;2021:1861567.

247. Vandenberghe C, St-Pierre V, Courchesne-Loyer A, Hennebelle M, Castellano CA, Cunnane SC. Caffeine intake increases plasma ketones: an acute metabolic study in humans. Canadian journal of physiology and pharmacology. 2016:1-4.

248. Fernando WM, Martins IJ, Goozee KG, Brennan CS, Jayasena V, Martins RN. The role of dietary coconut for the prevention and treatment of Alzheimer's disease: potential mechanisms of action. The British journal of nutrition. 2015;114(1):1-14.

249. Watanabe S, Tsujino S. Applications of Medium-Chain Triglycerides in Foods. Front Nutr. 2022;9:802805.

250. Mett J, Lauer AA, Janitschke D, Griebsch LV, Theiss EL, Grimm HS, et al. Medium-Chain Length Fatty Acids Enhance Abeta Degradation by Affecting Insulin-Degrading Enzyme. Cells. 2021;10(11).

251. Eyres L, Eyres MF, Chisholm A, Brown RC. Coconut oil consumption and cardiovascular risk factors in humans. Nutrition reviews. 2016;74(4):267-80.

252. Hu E, Du H, Zhu X, Wang L, Shang S, Wu X, et al. Beta-hydroxybutyrate Promotes the Expression of BDNF in Hippocampal Neurons under Adequate Glucose Supply. Neuroscience. 2018;386:315-25.

253. Pang PT, Teng HK, Zaitsev E, Woo NT, Sakata K, Zhen S, et al. Cleavage of proBDNF by tPA/plasmin is essential for long-term hippocampal plasticity. Science (New York, NY). 2004;306(5695):487-91.

254. Chriett S, Dąbek A, Wojtala M, Vidal H, Balcerczyk A, Pirola L. Prominent action of butyrate over β-hydroxybutyrate as histone deacetylase inhibitor, transcriptional modulator and anti-inflammatory molecule. Scientific reports. 2019;9(1):742.

255. Reger MA, Henderson ST, Hale C, Cholerton B, Baker LD, Watson GS, et al. Effects of beta-hydroxybutyrate on cognition in memory-impaired adults. Neurobiology of aging. 2004;25(3):311-4.

256. Avgerinos KI, Mullins RJ, Egan JM, Kapogiannis D. Ketone Ester Effects on Biomarkers of Brain Metabolism and Cognitive Performance in Cognitively Intact Adults ≥ 55 Years Old. A Study Protocol for a Double-Blinded Randomized Controlled Clinical Trial. J Prev Alzheimers Dis. 2022;9(1):54-66.

257. Lieberman HR, Fulgoni VL, Agarwal S, Pasiakos SM, Berryman CE. Protein intake is more stable than carbohydrate or fat intake across various US demographic groups and international populations. The American journal of clinical nutrition. 2020;112(1):180-6.

258. Leite ML. Applying compositional data methodology to nutritional epidemiology. Stat Methods Med Res. 2016;25(6):3057-65.

259. Speakman JR, de Jong JMA, Sinha S, Westerterp KR, Yamada Y, Sagayama H, et al. Total daily energy expenditure has declined over the past three decades due to declining basal expenditure, not reduced activity expenditure. Nat Metab. 2023;5(4):579-88.

260. Kinjo M, Chia-Cheng Lai E, Korhonen MJ, McGill RL, Setoguchi S. Potential contribution of lifestyle and socioeconomic factors to healthy user bias in antihypertensives and lipid-lowering drugs. Open heart. 2017;4(1):e000417.

261. Berry D, Willoughby MT. On the Practical Interpretability of Cross-Lagged Panel Models: Rethinking a Developmental Workhorse. Child Dev. 2017;88(4):1186-206.

262. Norwitz NG, Saif N, Ariza IE, Isaacson RS. Precision Nutrition for Alzheimer's Prevention in ApoE4 Carriers. Nutrients. 2021;13(4).

263. Mathias RA, Fu W, Akey JM, Ainsworth HC, Torgerson DG, Ruczinski I, et al. Adaptive evolution of the FADS gene cluster within Africa. PloS one. 2012;7(9):e44926.

264. Li J, Chen Y, Wu H, Li L. Apolipoprotein E (Apo E) gene polymorphisms and recurrent pregnancy loss: a meta-analysis. J Assist Reprod Genet. 2014;31(2):139-48.

265. Martinez-Martinez AB, Torres-Perez E, Devanney N, Del Moral R, Johnson LA, Arbones-Mainar JM. Beyond the CNS: The many peripheral roles of APOE. Neurobiology of disease. 2020;138:104809.

266. van Exel E, Koopman JJE, Bodegom DV, Meij JJ, Knijff P, Ziem JB, et al. Effect of APOE ε4 allele on survival and fertility in an adverse environment. PloS one. 2017;12(7):e0179497.

267. Corbo RM, Ulizzi L, Scacchi R, Martínez-Labarga C, De Stefano GF. Apolipoprotein E polymorphism and fertility: a study in pre-industrial populations. Mol Hum Reprod. 2004;10(8):617-20.

268. Ellis S, Franks DW, Nattrass S, Cant MA, Bradley DL, Giles D, et al. Postreproductive lifespans are rare in mammals. Ecol Evol. 2018;8(5):2482-94.

269. O'Connell J F, Hawkes K, Blurton Jones NG. Grandmothering and the evolution of homo erectus. J Hum Evol. 1999;36(5):461-85.

270. Caspari R, Lee SH. Older age becomes common late in human evolution. Proc Natl Acad Sci U S A. 2004;101(30):10895-900.

271. Hawkes K, O'Connell J, Blurton Jones N. Hunter-gatherer studies and human evolution: A very selective review. Am J Phys Anthropol. 2018;165(4):777-800.

272. Wu L, Zhang X, Zhao L. Human ApoE Isoforms Differentially Modulate Brain Glucose and Ketone Body Metabolism: Implications for Alzheimer's Disease Risk Reduction and Early Intervention. The Journal of neuroscience : the official journal of the Society for Neuroscience. 2018;38(30):6665-81.

273. Qiu WQ, Folstein MF. Insulin, insulin-degrading enzyme and amyloid-beta peptide in Alzheimer's disease: review and hypothesis. Neurobiology of aging. 2006;27(2):190-8.

274. Raulin AC, Doss SV, Trottier ZA, Ikezu TC, Bu G, Liu CC. ApoE in Alzheimer's disease: pathophysiology and therapeutic strategies. Mol Neurodegener. 2022;17(1):72.

275. Miyashita A, Kikuchi M, Hara N, Ikeuchi T. Genetics of Alzheimer's disease: an East Asian perspective. J Hum Genet. 2022.

276. Elmadfa I, Kornsteiner M. Dietary fat intake--a global perspective. Ann Nutr Metab. 2009;54 Suppl 1:8-14.

277. Gureje O, Ogunniyi A, Baiyewu O, Price B, Unverzagt FW, Evans RM, et al. APOE epsilon4 is not associated with Alzheimer's disease in elderly Nigerians. Ann Neurol. 2006;59(1):182-5.

278. Naslavsky MS, Suemoto CK, Brito LA, Scliar MO, Ferretti-Rebustini RE, Rodriguez RD, et al. Global and local ancestry modulate APOE association with Alzheimer's neuropathology and cognitive outcomes in an admixed sample. Mol Psychiatry. 2022;27(11):4800-8.

279. Lehtisalo J, Ngandu T, Valve P, Antikainen R, Laatikainen T, Strandberg T, et al. Nutrient intake and dietary changes during a 2-year multi-domain lifestyle intervention among older adults: secondary analysis of the Finnish Geriatric Intervention Study to Prevent Cognitive Impairment and Disability (FINGER) randomised controlled trial. The British journal of nutrition. 2017;118(4):291-302.

280. Beal T, Gardner CD, Herrero M, Iannotti LL, Merbold L, Nordhagen S, et al. Friend or Foe? The Role of Animal-Source Foods in Healthy and Environmentally Sustainable Diets. The Journal of nutrition. 2023.

281. Ylilauri MPT, Hantunen S, Lönnroos E, Salonen JT, Tuomainen TP, Virtanen JK. Associations of dairy, meat, and fish intakes with risk of incident dementia and with cognitive performance: the Kuopio Ischaemic Heart Disease Risk Factor Study (KIHD). Eur J Nutr. 2022;61(5):2531-42.

282. Jin X, Xiong S, Yuan C, Gong E, Zhang X, Yao Y, et al. Apolipoprotein E Genotype, Meat, Fish, and Egg Intake in Relation to Mortality Among Older Adults: A Longitudinal Analysis in China. Front Med (Lausanne). 2021;8:697389.

283. Pekkala T, Hall A, Mangialasche F, Kemppainen N, Mecocci P, Ngandu T, et al. Association of Peripheral Insulin Resistance and Other Markers of Type 2 Diabetes Mellitus with Brain Amyloid Deposition in Healthy Individuals at Risk of Dementia. Journal of Alzheimer's disease : JAD. 2020;76(4):1243-8.

284. Duara R, Loewenstein DA, Lizarraga G, Adjouadi M, Barker WW, Greig-Custo MT, et al. Effect of age, ethnicity, sex, cognitive status and APOE genotype on amyloid load and the threshold for amyloid positivity. Neuroimage Clin. 2019;22:101800.

285. Zhao Y, Li Y, Wang W, Song Z, Zhuang Z, Li D, et al. Low-carbohydrate diets, low-fat diets, and mortality in middle-aged and older people: A prospective cohort study. J Intern Med. 2023.

286. Crosby L, Davis B, Joshi S, Jardine M, Paul J, Neola M, et al. Ketogenic Diets and Chronic Disease: Weighing the Benefits Against the Risks. Front Nutr. 2021;8:702802.

287. Watanabe M, Tuccinardi D, Ernesti I, Basciani S, Mariani S, Genco A, et al. Scientific evidence underlying contraindications to the ketogenic diet: An update. Obesity reviews : an official journal of the International Association for the Study of Obesity. 2020;21(10):e13053.

288. Cho H, Kim YE, Chae W, Kim KW, Kim JW, Kim HJ, et al. Distribution and clinical impact of apolipoprotein E4 in subjective memory impairment and early mild cognitive impairment. Scientific reports. 2020;10(1):13365.