The effect of phospholipid supplementation on cognitive performance across the lifespan

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The candidate confirms that the work submitted is his/her own and that appropriate credit has been given where reference has been made to the work of others.

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Abstract

Phospholipids (PLs) are found abundantly in mammalian cell membranes and support cellular health and function. Their composition and shape promotes asymmetry within membrane bilayers and affects membrane physiological properties. PLs have the potential to facilitate cognitive function. A systematic review identified ten PL supplementation studies, including two acute and eight chronic (≥ 2 week) interventions. Cognitive benefits, mainly memory enhancement, were reported for a single PL, phosphatidylserine, and a bovine milk-derived PL composite, which was used in the supplementation studies reported in this thesis. The quality of the empirical studies reviewed was compromised by poor study designs and/or analytical approach. Moreover, the review highlighted a lack of empirical studies considering PL supplementation in children and adolescents. The focus of this thesis was to investigate the potential for bovine milk-derived PLs to promote cognitive function. Study 1 (n=70) was the first randomised placebo-controlled trial of the effects of PLs on cognitive performance in school-aged children (6-8 years). This was a six week intervention trial during which the children were tested every 3 weeks on measures of memory, motor skills, executive function and processing speed. Subjective evaluations of appetite, mood, motivation and mental alertness were also measured. The impact of the supplement on cognitive performance was limited. There was also no discernible effect on subjective state. Study 2 (n=50) extended limited existing evidence to examine effects of PL supplementation in middle-aged/older adults with a subjective memory complaint. This randomised placebo controlled trial investigated the acute and chronic effects of PL supplementation over 12 weeks on cognitive measures of memory and executive function and self-reports on the Cognitive Failures Questionnaire. Cognitive performance and the frequency of cognitive failures was measured at week 0 (acute), week 6 and week 12 (chronic). Few effects on cognitive performance following both acute and chronic supplement consumption were observed. Cognitive failures were reduced in participants who received the active supplement and reported greater cognitive failures at baseline. Across both studies, participants' demographic characteristics and baseline performance had a greater impact on cognitive performance than the active supplement. Overall, the findings from the PL intervention studies presented in this thesis add to the existing heterogeneous evidence of the potential for PLs to moderate cognitive performance. Despite strong mechanistic data suggesting PLs could confer beneficial and/or protective effects on cognition, this thesis did not find clear evidence of a benefit of PLs for cognition. Further examination of the potential benefits of PLs in other formulations for cognitive function in young and old samples is warranted.

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Abbreviations

4-HNE	4-Hydroxy-2-Nonenal
AA	Arachidonic acid
AAMI	Age-associated memory impairment
Acetyl-CoA	Acetyl-coenzyme A
ACh	Acetylcholine
AchE	Acetyltransferase
AD	Alzheimer's disease
AICC	Akaike information criterion
Akt	Protein kinase B
ALA	Alpha-linolenic acid
aMCI	Amnesic mild cognitive impairment
Aβ	Amyloid beta
AMPA	Alpha-Amino-3-Hydroxy-5-Methyl-4-Isoxazolepropionic acid
AP-1	Activating protein-1
aPKC	Atypical protein kinase C
APOE	Apolipoprotein E
APOE ε4	Apolipoprotein E ε4 genotype
BAS II	British Ability Scales II
BBB	Blood-brain barrier
BC-PS	Phosphatidylserine sourced from bovine cortex
BDNF	Brain-derived neurotrophic factor
CANTAB	Cambridge Neuropsychological Test Automated Battery
CFQ	Cognitive Failures Questionnaire
CGI-C	Clinical Global Impression Of Change
ChAT	Choline acetyltransferase
CIND	Cognitively impaired-not demented
COXs	Cyclooxygenases
CRP	C-reactive protein
CRT	Choice Reaction Time
CSF	Cerebrospinal fluid
CVLT	Californian Verbal Learning Test
CYP	Cytochromes P450
DAG	Diacylglycerol
DAMPs	Danger-associated molecular patterns
DHA	Docosahexaenoic acid
DM	Dementia
DMN	Default mode network
DPA	Docosapentaenoic acid
DRS	Dementia Rating Scale
DTI	Diffusion tensor imaging
EFSA	European Food Safety Authority
eoxPLs	Enzymatically oxidised phospholipids

EPA	Eicosapentaenoic acid
ETC	Electron transport chain
FA	Fatty acid
FC	Functional connectivity
fMRI	Functional magnetic resonance imaging
GPI	Glycosylphosphatidylinositol
GPL	Glycerophospholipid
HARU	Human Appetite Research Unit
HOMA-IR	Homeostatic model assessment insulin resistance
HVLT	Hopkins Verbal Learning Test
IGF	Insulin-like growth factor
IL-1	Interleukin-1 family of cytokines
IL-6	Interleukin-6
IL-18	Interleukin-18
IL-1β	Interleukin-1 beta
IQ	Intelligence quotient
ITT	Intention to treat
LA	Linoleic acid
LC-PUFA	Long-chain polyunsaturated fatty acid
LOXs	Lipoxygenases
LPS	Lipopolysaccharide
LS-means	Least squares means
LTs	Leukotrienes
LTB4	Leukotriene B4
LTC4	Leukotrienes C4
LTD	Long-term depression
LTP	Long-term potentiation
lysoPC	Lysophosphatidylcholine
lysoPS	Lysophosphatidylserine
MARCKS	Myristoylated alanine-rich C kinase substrate
MCI	Mild cognitive impairment
MCP-1	Monocyte chemoattractant protein-1
MDA	Malondialdehyde
MetS	Metabolic syndrome
Mfsd2a	Major facilitator superfamily domain-containing protein 2A
MGST1	Microsomal glutathione S-transferase 1
MLR	Membrane lipid replacement
MMSE	Mini-Mental State Examination
MoCA	Montreal Cognitive Assessment
MOT	Motor Screening Task
MS	Multiple sclerosis
mtDNA	Mitochondrial DNA
naMCI	Non-amnestic mild cognitive impairment
NF-κB	Nuclear factor kappa-light-chain-enhancer of activated B cells
NMDA	N-Methyl-D-aspartate
NODDI	Neurite orientation dispersion and density imaging

NOS2	Nitric oxide synthase 2
OxPLs	Oxidized phospholipids
PA	Phosphatidic acid
PC	Phosphatidylcholine
PC-DHA	Phosphatidylcholine conjugated to docosahexaenoic acid
PE	Phosphatidylethanolamine
PEMT	Phosphatidylethanolamine N-methyltransferase
PGs	Prostaglandins
PGD2	Prostaglandin D2
PGE2	Prostaglandin E2
PI3Ks	Phosphatidylinositol 3-kinases
PI	Phosphatidylinositol
PIs	Phosphoinositides
PKC	Protein kinase C
PKC-ε	Protein kinase C epsilon
PKC-θ	Protein kinase C theta
PL	Phospholipid
PLD	Phospholipase D
POM-SF	Profile of Mood States – Short Form
PP	Per protocol
PRISMA	Preferred Reporting Items For Systematic Reviews And Meta-Analyses
PROC MIXED	The SAS® Mixed procedure
PRRs	Pattern recognition receptors
PS	Phosphatidylserine
PS-DHA	Phosphatidylserine conjugated to docosahexaenoic acid
PTEN	Phosphatase and tensin homolog deleted on chromosome 10
PUFAs	Polyunsaturated fatty acids
RAVLT	Rey Auditory Verbal Learning Test
RBMT-C	Rivermead Behavioural Memory Test For Children
RCFT	Rey Complex Figure Test
REML	Restricted maximum likelihood
ROS	Reactive oxygen species
RT	Reaction time
RVIP	Rapid Visual Information Processing Task
SACL	Stress Arousal Checklist
SD	Standard deviation
SE	Standard error
SM	Sphingomyelin
SMC	Subjective memory complaint
SPMs	Specialised pro-resolving mediators
SRM	Spatial Recognition Memory
SRT	Simple Reaction Time
SSP	Spatial Span
STAI	State-Trait Anxiety Inventory
STING	Stimulator of interferon genes
T2DM	Type 2 diabetes mellitus

TBI	Traumatic brain injury
TMD	Total mood disturbance
TNF-α	Tumor necrosis factor alpha
TLR4	Toll-like receptor 4
TLR9	Toll-like receptor 9
TXs	Thromboxanes
VVLT	Visual Verbal Learning Test
WASI	Wechsler Abbreviated Scale Of Intelligence
WMHs	White matter hyperintensities

Chapter 1 General Introduction and thesis aims

1.1 Cognition and cognitive function across the lifespan

Cognition is characterised by a complex set of higher mental functions (Nyaradi, Li, Hickling, Foster, & Oddy, 2013) that include memory, perception, attention, executive function and information processing, each of which represent cognitive domains (de Jager et al., 2014; Nouchi & Kawashima, 2014; Wesnes, 2010). Notably, executive function encompasses multiple higher-order cognitive processes, such as response inhibition, working memory, planning and cognitive flexibility (Lezak, Howieson, Bigler, & Tranel, 2012). Cognitive function develops from childhood through to young adulthood (Nouchi & Kawashima, 2014). Importantly, the development and maturation of the brain underlies this development. For example, the development of the prefrontal cortex during adolescence corresponds to sophistication of executive function, including impulse control, behavioural inhibition and planning (Arain et al., 2013). Cognitive development and decline follow different trajectories across the lifespan. For instance, executive function components demonstrate separate developmental trajectories, such that attentional regulation develops from infancy (Berthelsen, Hayes, White, & Williams, 2017), whilst more complex executive skills enabling performance monitoring and relational reasoning begin to evolve during adolescence (Crone & Dahl, 2012). Moreover, there is heterogeneity in when cognitive abilities peak and subsequently begin to deteriorate (Hartshorne & Germine, 2015), with the earliest age-related cognitive decline evident in some domains from twenty years of age in healthy educated adults (Salthouse, 2009). Frequently observed changes in cognitive function with age occur in processing speed, executive function (Murman, 2015) and memory for new information (Lezak et al., 2012). Currently, there is much interest in identifying factors that promote cognitive function during childhood/adolescence and/or prevent cognitive decline during ageing. One such factor which is receiving much attention is the role of diet and the effects of specific nutrients on cognitive function.

1.2 The effect of diet on cognitive function

Diet has the potential to affect cognition and dietary factors are recognised to influence molecular systems and mechanisms that support cognitive functions (Gómez-Pinilla, 2008). Nutrition influences cognition such that improved nutrition is related to optimal brain function

(Nyaradi et al., 2013), with clear evidence of the deleterious effects of nutritional deficiencies and benefits of supplementation to correct these e.g. iron (Falkingham et al., 2010). A number of reviews of observational and interventional studies have reported certain dietary components as favourable for promoting cognitive function in both children (Lam & Lawlis, 2017; Nyaradi et al., 2013; Spencer, Korosi, Layé, Shukitt-Hale, & Barrientos, 2017) and adult samples (Gómez-Pinilla, 2008; Spencer et al., 2017; Vauzour et al., 2017). Hence, there is evidence that dietary manipulations offer an opportunity to enhance or maintain cognition in different age-groups and at different times across the lifespan.

1.2.1 The potential for phospholipids to moderate cognitive performance

There is increasing interest in the potential for milk dairy products and components, such as milk-derived phospholipids (PL), to impact upon cognitive function. PLs are amphiphilic lipids (having both hydrophilic and hydrophobic parts) present in all cell membranes of plants and animals and form lipid bilayers (Küllenberg, Taylor, Schneider, & Massing, 2012). The most common type in eukaryotic cells are glycerophospholipids (GPLs) (Nicolson & Ash, 2017). GLPLs as well as another lipid class, sphingolipids, present in milk are one of the main components of the milk fat globule membrane (MFGM) (Contarini & Povolo, 2013; Zhiqian Liu, Rochfort, & Cocks, 2018). GPLs and sphingolipids are also available from other dietary sources, and soybeans and eggs are particularly good sources of both (Burling & Graverholt, 2008). The composition of PLs is crucial to their potential to promote cognitive function and resolve inflammation associated with cognitive impairment and pathology. Chapter 2, therefore, provides an important comprehensive introduction to cell membrane lipids and their potential facilitative and protective role in cognitive function.

1.2.2 Phospholipid supplementation and cognitive performance

Chapter 3 presents a systematic review of the extant literature exploring GPL supplementation across the lifespan. Across the acute and chronic intervention studies reviewed, there is a preponderance of studies which supplement adults, particularly young healthy adults, with a complete absence of intervention studies in children and adolescents. Overall, this review concluded that there was a lack of consistency with regard to intervention duration, dose of GPL administered and study eligibility criteria. A single GPL, phosphatidylserine (PS), and a composite PL supplement were observed to confer cognitive performance gains in cognitively healthy adults. Methodological issues are discussed and inform recommendations for future GPL supplementation studies.

To address one of the main recommendations that came out of the systematic research review, Chapter 4 reports on a six week intervention study involving the supplementation of 6-8 year old children (n=70) with a bovine milk-derived PL-enriched protein concentrate drink (Study 1). The study conformed to a randomised, double-blind, placebo-controlled, parallel group study design. Children consumed the experimental drinks (active/placebo) in school on weekdays. Cognitive performance and subjective evaluations of appetite, mood, motivation and mental alertness were measured every 3 weeks.

Chapter 5 presents a second intervention study (Study 2) that builds on the limited empirical research concerning GPL supplementation in adults with a subjective memory complaint (SMC). Again, Study 2 conformed to a randomised, double-blind, placebo-controlled, parallel group study design. This was a 12 week intervention in which participants (n=50) aged ≥50 years of age consumed either the bovine milk-derived PL-enriched protein concentrate drink or placebo at home. The study included an acute assessment i.e. cognitive performance was assessed at baseline and again 90 minutes post-first dose, as well as chronic assessment every 6 weeks for 12 weeks. Participants also completed a self-report questionnaire relating to the frequency with which they had experienced cognitive lapses and slips whilst completing everyday tasks (cognitive failures) over the intervening test visit period.

Given the lack of extant empirical research considering the effect of GPL supplementation on cognitive performance in children, the introduction to Chapter 4 features a review of omega-3 fatty acid (FA) interventions in school-aged children. Crucially, FAs are components of GPLs and contribute to the physiological properties of cell membranes. The introduction to Chapter 4 also discusses developmental changes in the brain that take place across childhood and the relationship of these with cognitive development. Similarly, Chapter 5 starts with an introduction to structural and functional brain changes with age and the association of these with cognitive decline. Much of the research discussed in both sections is based upon brain imaging techniques. Such techniques provide insights into brain activity and network connectivity as well as more detailed information, such as fibre-density and myelination. In the context of cognitive development and decline, these techniques provide knowledge of brain alterations, which can be related back to cognitive function. However, these techniques are outside of the scope of this thesis and did not form part of the methodology for either intervention study.

The final Chapter (Chapter 6) provides a summary of the findings from both intervention studies and makes recommendations in terms of potential GPL species and FA composition for future

supplementation studies. Important limitations of the intervention studies and design recommendations are also discussed.

1.3 Summary of thesis aims and hypotheses

Taken together, this thesis aims to explore the impact of PLs (specifically GPLs and sphingomyelin (SM) in combination) on cognitive function to investigate whether supplementation translates to improved cognitive performance both acutely and chronically in children and middle-aged/older adults with a SMC. The specific aims of this thesis are:

1. To systematically review the effects of GPL acute and chronic supplementation on cognitive performance across the lifespan (Systematic research review, Chapter 3).
2. To examine the effects of chronic dietary GPL (also containing SM) intervention on cognitive performance and subjective feelings (mood, alertness, motivation) in children aged 6 – 8 years. It is hypothesised that supplementation with Lacprodan® PL-20 will promote cognitive function, in turn supporting better performance on a cognitive battery that measures memory, motor skills, executive function and processing speed relative to that shown by the control group (Study 1, Chapter 4).
3. To examine the effects of acute dietary GPL (also containing SM) intervention on cognitive performance in adults aged 50 years and over with a SMC. It is hypothesised that supplementation with Lacprodan® PL-20 will facilitate cognitive function and thereby promote better cognitive performance relative to the control group on cognitive measures assessing memory and executive function (Study 2, Chapter 5).
4. To examine the effects of chronic dietary GPL (also containing SM) intervention on cognitive performance and self-reported cognitive failures in adults aged 50 years and over with a SMC. It is hypothesised that supplementation with Lacprodan® PL-20 will enhance cognitive function and therefore augment cognitive performance on cognitive measures assessing memory and executive function and reduce self-reported cognitive failures compared to the control group (Study 2, Chapter 5).

Chapter 2 Introduction to cell membrane lipids and their potential facilitative role in cognitive function

2.1 Glycerophospholipids

Glycerophospholipids (GPLs) are glycerol-based phospholipids (PLs) and are the most prevalent class of lipids in mammalian cells (Blom, Somerharju, & Ikonen, 2011). In addition to their glycerol backbone containing three hydroxyl groups (available in three positions: *sn*-1, *sn*-2 and *sn*-3), GPLs have two chains of fatty acids (FAs) and a phosphate head group (Cui & Decker, 2015). GPLs differ in the FAs attached at the *sn*-1 and *sn*-2 position of the glycerol backbone. Typically, a saturated FA is esterified at the *sn*-1 location whilst a saturated, polyunsaturated, or monounsaturated FA occupies the *sn*-2 position (Fruhwirth, Loidl, & Hermetter, 2007; Manni et al., 2018; Yamashita et al., 2014). The biochemical structure of a GPL containing a saturated FA at the *sn*-1 position and a polyunsaturated FA at the *sn*-2 position is shown in Figure 2.1.

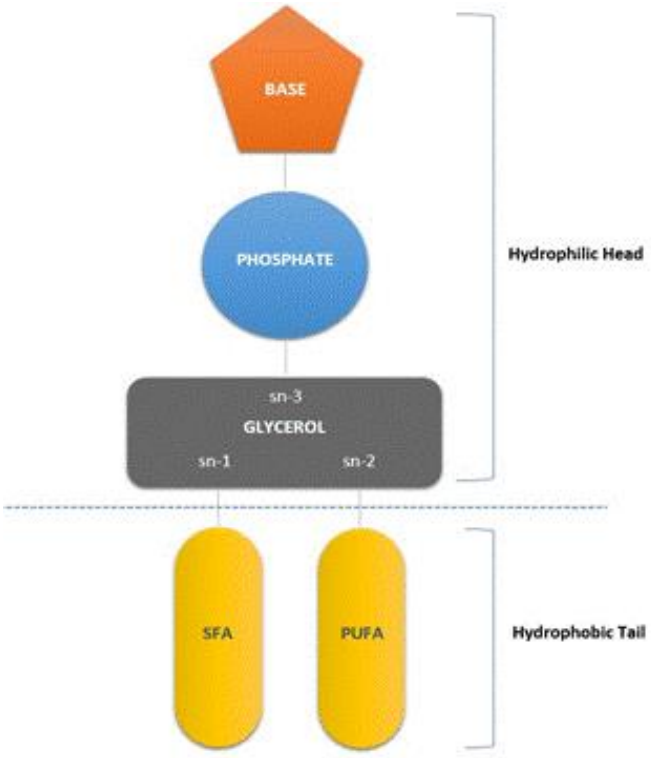


Figure 2.1 Biochemical structure of a glycerophospholipid with a saturated fatty acid and a polyunsaturated fatty acid esterified at the sn-1 and sn-2 position respectively of the glycerol backbone. Adapted from "Glycerophospholipid Supplementation as a Potential Intervention for Supporting Cerebral Structure in Older Adults," by J. M. Reddan, D. J. White, H. Macpherson, A. Scholey and A. Pipingas, 2018, *Frontiers in Aging Neuroscience, 10*, p.49. Copyright 2018 by CC BY. Adapted with permission.

Stearic acid (saturated FA, 18:0[1]) or palmitic acid (saturated FA, 16:0) tends to found at the *sn*-1 position, whilst at the *sn*-2 position, GPLs predominantly contain oleic (omega-9, 18:1), linoleic (LA; omega-6, 18:2) or alpha-linolenic acid (ALA; omega-3, 18:3), arachidonic acid (AA; omega-6, 20:4), or eicosapentaenoic acid (EPA; omega-3, 20:5) and docosahexaenoic acid (DHA; omega-3, 22:6) (Küllenberg et al., 2012; Layé, Nadjar, Joffre, & Bazinet, 2018). The addition of different chemical moieties onto the *sn*-3 position modifies the head group and determines the type of phosphatidyl lipid (Coskun & Simons, 2011). The chemical moieties include choline (phosphatidylcholine; PC), ethanolamine (phosphatidylethanolamine, PE), serine (phosphatidylserine, PS) and inositol (phosphatidylinositol, PI) (Hishikawa, Hashidate, Shimizu, & Shindou, 2014). Importantly, GPLs can differ in their head group and acyl chains (promoting different chain length and saturation properties) (Fruhwirth et al., 2007). GPLs are amphiphilic, the head group being water-loving (hydrophilic) whilst the FA tails are hydrophobic (Küllenberg et al., 2012). This characteristic promotes self-assembly of different structures (e.g. lipid bilayer) when added into aqueous milieu, the assembly of which is governed by the GPL specific properties and conditions (Li et al., 2015). Figure 2.2 is a schematic representation of the lipid bilayer of a eukaryotic cell membrane. This illustrates other features of a cell membrane including proteins, glycolipids and cholesterol. The bend or kink in the FAs attached to the PLs (boldface black lines) indicate double bonds.

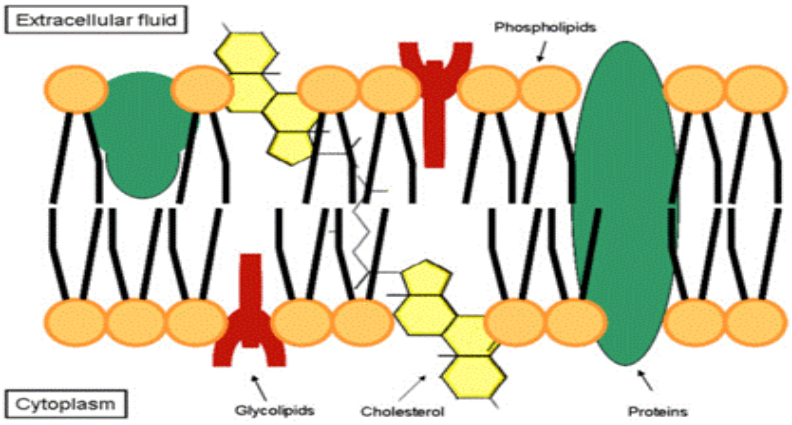


Figure 2.2 Schematic representation of the lipid bilayer of a eukaryotic cell membrane. Reprinted from "Health effects of dietary phospholipids," by D. Küllenberg, L. A. Taylor, M. Schneider and U. Massing, 2012, *Lipids in Health and Disease, 11*(3), p.2. Copyright 2012 by CC BY (Licensee BioMed Central Ltd.). Reprinted with permission.

[1] 18 represents the chain length (18 carbon atoms) and 0 represents the degree of saturation (0 double-bonds), where 0 is saturated, 1 is monounsaturated and ≥ 2 is polyunsaturated (see Appendix 1 for nomenclature, chain length and degree of saturation of fatty acids referred to throughout the thesis).

Membrane lipids facilitate cellular health by providing a matrix allowing chemical and enzymatic reactions to take place in separate membrane compartments, opportunities for energy storage (caloric reserves), sites for localisation of peripheral functional molecules that interact with constituents of the membrane i.e. proteins (Janmey & Kinnunen, 2006; Nicolson & Ash, 2014) as well as bioactive molecules, such as phosphoinositides (PIs), enabling the recruitment of proteins present in the cytosol (intracellular fluid) that are involved in vesicle trafficking (Di Paolo & De Camilli, 2006). Cellular membranes are both complex and varied, and their composition is context-dependent (Sezgin, Levental, Mayor, & Eggeling, 2017). Membrane lipid composition differs among cells, the compartments that exist within a cell and also between the two leaflets (bilayers) of a membrane (Fagone & Jackowski, 2009; Vance, 2015). Transmembrane lipid transporter proteins, flippases and floppases, support membrane asymmetry (difference in the lipid composition of each monolayer of the membrane) by translocating certain lipids in both an ATP-dependent and independent manner, whilst scramblases (a further transmembrane protein) move lipids in a non-selective, energy-independent fashion, leading to disruption in asymmetry (Contreras, Sánchez-Magraner, Alonso, & Goñi, 2010; Kodigepalli, Bowers, Sharp, & Nanjundan, 2015; Williamson & Schlegel, 2002). Differences in the lipid composition both across the leaflets and laterally within a monolayer are closely related to membrane curvature and shape (Yesylevskyy, Rivel, & Ramseyer, 2017) and promote distinct areas of surface charge across the membrane (Whited & Johs, 2015).

2.2 Influence of glycerophospholipids on cell membrane characteristics

2.2.1 Membrane curvature

Remodelling of the lipid composition is one mechanism by which a membrane can alter its shape. Membranes can form highly complex architectures and alterations in healthy cells are well-regulated (Jarsch, Daste, & Gallop, 2016). Importantly, adjustments to a membranes architecture are required for the exchange of molecules and signals from the cytoplasm and extracellular areas (e.g. vesicle formation and trafficking; Bohdanowicz & Grinstein, 2013; Jarsch et al., 2016; Settles, Loftus, McKeown, & Parthasarathy, 2010) and key cellular processes, such as division and differentiation (Yesylevskyy et al., 2017), as well as peripheral protein recruitment (Vanni, Hirose, Barelli, Antonny, & Gautier, 2014). With cells being in a state of flux (Frolov, Shnyrova, & Zimmerberg, 2011), changes in membrane architecture occur on a variety of temporal and spatial scales (Yesylevskyy et al., 2017). GPLs can be grouped by shape due to their headgroup and fatty acyl chains. Specifically, the length and saturation of GPL fatty acyl

chains and the size of the headgroup contribute to GPL shape (McMahon & Boucrot, 2015). Both PC and PS are cylindrical in shape, forming flat monolayers, whereas PE and phosphatidic acid (PA: a PL and a precursor of GPLs) are roughly conical in shape, creating negative curvature (inverse) (McMahon & Boucrot, 2015). Alternatively, PI creates positive curvature due to its inverted conical shape (Zimmerberg & Kozlov, 2006). Crucially, when GPLs of a similar shape gather, the monolayer can spontaneously curve (McMahon & Boucrot, 2015). This may result in the whole membrane altering its shape (i.e. bilayer coupling; McMahon & Gallop, 2005).

2.2.2 Membrane fluidity

The extent to which molecules can diffuse within the membrane (Vígh & Maresca, 2002) i.e. membrane fluidity, is essential for the proper functioning of cell membranes (Fan & Evans, 2015; García et al., 2014). The presence of unsaturated FAs in membrane lipids promotes membrane fluidity (Los & Murata, 2004). Indeed, an increase in the availability of unsaturated FAs leads to an increase in membrane fluidity (Schmitz & Ecker, 2008; Weijers, 2015), whereas damage to polyunsaturated fatty acids (PUFAs) can decrease membrane fluidity (García et al., 2014). Crucially, the acyl chain composition determines membrane fluidity, where saturated FAs, with their linear acyl chains, are able to pack together tightly (De Marothy & Elofsson, 2015). Alternatively, unsaturated FAs have double bonds, introducing a bend in their tails, resulting in less denser packing in the monolayer (McMahon & Boucrot, 2015). The composition of the lipid membrane is continuously altered to modify membrane fluidity following changes in the physiochemical environment (Maulucci et al., 2016). Optimal membrane functionality requires the maintenance of a narrow range of membrane physical properties (Levental, Malmberg, & Levental, 2018). Importantly, incorporation of FAs into the membrane depends upon their availability and abundance, meaning membrane homeostasis (and therefore the preservation of membrane physical properties) relies upon a balanced pool of FAs (Maulucci et al., 2016). This highlights the relationship between diet and the FA profiles of GPLs, where dietary intake can influence GPL profiles (Saadatian-Elahi et al., 2009; Zheng et al., 2019).

2.2.3 Membrane surface charge

PE, PC are all zwitterionic (contain both positive and negative charged groups; net charge is neutral), whilst PS, PI and PA are negatively charged lipids (Ingólfsson et al., 2014). This results in ion concentration gradients being present at the membrane surface that are known to support electrostatic interactions between peripheral proteins and lipid headgroups (Whited & Johs, 2015). With the abundance of PS present in the cytoplasmic side (inner-leaflet) of the

membrane, this confers a higher anionic charge relative to the exofacial side (outer-leaflet; Marquardt, Geier, & Pabst, 2015). The presence of negative charges influence membrane characteristics (Tanguy, Kassas, & Vitale, 2018) by promoting separate membrane territories, supporting the localisation of peripheral proteins with cationic clusters (Bigay & Antonny, 2012). Importantly, the interaction between proteins and lipids depend on their respective charge, and attenuation of the charge due to membrane PL metabolism or redistribution can lead to peripheral proteins relocalising, resulting in different reactions (Yeung et al., 2008).

Transmembrane helices enable transmembrane proteins to attach themselves to the plasma membrane (Baker, Wong, Eisenhaber, Warwicker, & Eisenhaber, 2017). The positive inside rule denotes the enrichment of positively charged residues in cytoplasmic loops and at the cytoplasmic edge of transmembrane helices (Baker et al., 2017; De Marothy & Elofsson, 2015; von Heijne, 1989), which help proteins to orientate towards the inner-leaflet (De Marothy & Elofsson, 2015; Elazar, Weinstein, Prilusky, & Fleishman, 2016; Marquardt et al., 2015; van Geest & Lolkema, 2000). Membrane potential also influences the structure and function of transmembrane proteins (Bohdanowicz & Grinstein, 2013). For example, the function of voltage-gated potassium channels (transmembrane channels) involved in physiological processes such as cell proliferation, neuronal excitability and neurotransmitter release exemplifies how the charge from lipid headgroups modulates their stability and operation (Escribá et al., 2008).

2.2.4 Membrane rafts

Lateral segregation of membrane constituents represented by membrane sub-compartmentalisation illustrates the dynamic ability of membranes to form membrane rafts (Simons & Sampaio, 2011). Membrane rafts are represented by a liquid-ordered phase, stabilised by the presence of cholesterol (Sonnino & Prinetti, 2013) and show tighter packing relative to non-raft phases of the membrane (Rajendran & Simons, 2005). Membrane rafts serve many functions, such as cellular trafficking and signal transduction (Ariga, McDonald, & Yu, 2008; Hanzal-Bayer & Hancock, 2007). Consistent with this, membrane rafts have been implicated as platforms for assembly and initiating signalling cascades (Staubach & Hanisch, 2011). Membrane rafts also act as sorting platforms for targeted protein traffic and are implicated in protein endocytosis (Staubach & Hanisch, 2011). Additionally, they have also been found to support growth cone (neuronal growth) expansion as well as axon formation (Davare et al., 2009; Grider, Park, Spencer, & Shine, 2009; Head, Patel, & Insel, 2014; Kamiguchi, 2006) and guidance (Kamiguchi, 2006).

The original composition of the membrane raft model reflects the close affinity between sphingolipids (see section 2.4) and cholesterol (Simons & Ikonen, 1997). This association between sphingolipids and cholesterol is due, in part, to their strong hydrogen bonding (Slotte, 2013; Sodt, Pastor, & Lyman, 2015). The saturated hydrocarbon chains of sphingolipids contribute to their easy integration into areas of high acyl chain order (consistent with the presence of cholesterol in the membrane; Róg, Pasenkiewicz-Gierula, Vattulainen, & Karttunen, 2009), unlike GPLs, which tend to have unsaturated FAs and therefore bends in their tails (Hanzal-Bayer & Hancock, 2007). Both gangliosides (Simons & Toomre, 2000; Sandro Sonnino, Mauri, Chigorno, & Prinetti, 2007), which have mostly saturated FAs attached (Kolter, 2012) and relatively saturated PLs have also been associated with raft-like environments (Sezgin et al., 2017).

2.3 Types of glycerophospholipids

2.3.1 Phosphatidylserine (PS)

PS represents approximately 10% of the total lipids present in lipid membranes (Vance, 2003) and is unequally distributed across the cellular membrane (van Meer, Voelker, & Feigenson, 2008). The chemical strucure of PS is diplayed in Figure 2.3.

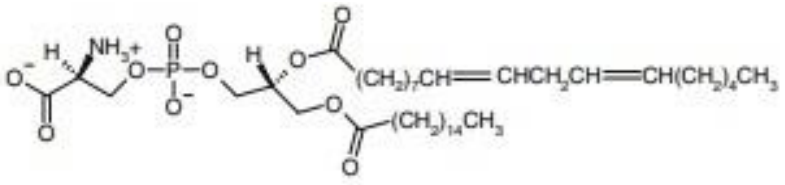


Figure 2.3 Chemical structure of phosphatidylserine. Reprinted by permission from Springer Nature Customer Service Centre GmbH: Springer. Chromatographia.“Feasibility of Phospholipids Separation by Packed Column SFC with Mass Spectrometric and Light Scattering Detection,” by H. S. H. Yip, M. Ashraf-Khorassani and L. T. Taylor. Copyright 2007. Reprinted with permisson.

PS is found mainly on the cytoplasmic side of the membrane in healthy eukaryotic cells, enabling various regulatory and structural membrane-bound enzymes to interact with it, where it acts as a co-factor (Zwaal, Comfurius, & Bevers, 2005). However, during apoptosis, PL scrambling takes place leading to random distribution across the membrane (Williamson, 2015). This disruption in asymmetry leads to the presence of PS on the outer leaflet, which is needed for effective ingestion (phagocytosis) of apoptotic cells by macrophages (Nagata, Suzuki, Segawa, & Fujii, 2016; Segawa & Nagata, 2015). Importantly, this programmed cell death is different to that

which is brought on by pathology, in that there is no risk of harmful intracellular enzymes and antigens being released (Leventis & Grinstein, 2010).

As discussed in section 2.2.3, PS is a anionic GPL (Vance & Tasseva, 2013), which creates membrane electrostatics (Platre & Jaillais, 2017), such that an increase in the concentration of PS results in an increase in the negative electrostatic potential of the cellular membrane (Kooijman & Burger, 2009), making the membrane attractive to cationic proteins (Goldenberg & Steinberg, 2010). PS is known to be a primary binding site (Lemmon, 2008) and there are a large number of proteins that bind PS, such as annexins and synaptotagmin. This implicates PS in a number of cellular processes including signal transduction, membrane trafficking and neurotransmitter release (Stace & Ktistakis, 2006). PS also binds protein kinase C (PKC), a family of protein kinase enzymes, as discussed in section 2.7.3.

2.3.2 Phosphatidylcholine (PC)

PC is the most abundant PL present in plasma membranes, contributing approximately 40-50% of total cellular membrane lipids (Vance, 2015). PC contributes to the synthesis of sphingomyelin (SM), in which the choline-phosphate head group is transferred to a ceramide lipid anchor (Gault, Obeid, & Hannun, 2010), the importance of which is discussed in section 2.4.1. The chemical strucure of PC is diplayed in Figure 2.4.

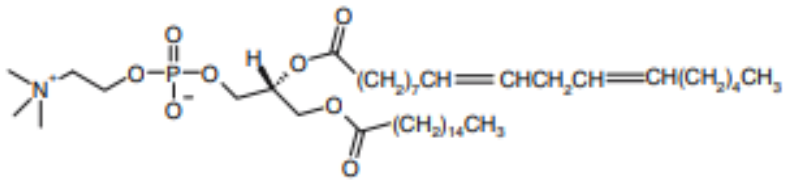


Figure 2.4 Chemical structure of phosphatidylcholine. Reprinted by permission from Springer Nature Customer Service Centre GmbH: Springer. Chromatographia.“Feasibility of Phospholipids Separation by Packed Column SFC with Mass Spectrometric and Light Scattering Detection,” by H. S. H. Yip, M. Ashraf-Khorassani and L. T. Taylor. Copyright 2007. Reprinted with permisson.

The breakdown of PC, by, for example, the enzyme phospholipase D (PLD), leads to the production of PA and free choline (Park et al., 2014). Crucially, PA is a precursor of all membrane GPLs and is essential in the transmission, amplification and regulation of many intracellular signalling and cellular functions such as vesicle trafficking (Ammar, Kassas, Bader, & Vitale, 2014). Moreover, like PS, PA is negatively charged, thus supporting the binding of PA-binding protein domains with PA (Tanguy et al., 2018). The importance of choline is discussed in section 2.7.4.

2.3.3 Phosphatidylethanolamine (PE)

PE along with PC form more than 50% of all PLs in eukaryotic cell membranes (Gibellini & Smith, 2010). PE makes up ~45% of brain total PLs (Vance & Tasseva, 2013) and is enriched in the internal layer of the cell membrane bilayer (Castro-Gómez, Garcia-Serrano, Visioli, & Fontecha, 2015). The chemical structure of PE is displayed in Figure 2.5.

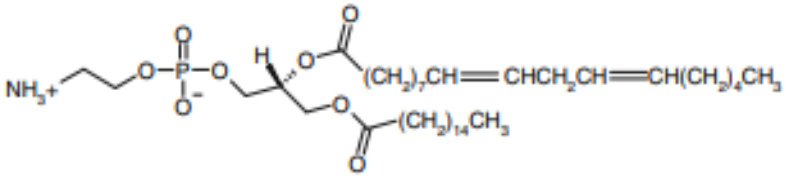


Figure 2.5 Chemical structure of phosphatidylethanolamine. Reprinted by permission from Springer Nature Customer Service Centre GmbH: Springer. Chromatographia.“Feasibility of Phospholipids Separation by Packed Column SFC with Mass Spectrometric and Light Scattering Detection,” by H. S. H. Yip, M. Ashraf-Khorassani and L. T. Taylor. Copyright 2007. Reprinted with permisson.

The recruitment of PE to the plasma membrane enables cells to regulate their fluidity such that the increased presence of PE fosters rigidity of the bilayer (Dawaliby et al., 2016). Further, PE is known to affect protein behaviour. Due to its conical shape, PE causes membrane lateral pressure (stress), which has been coupled to transmembrane protein structure and function, such that alterations in pressure affect protein activation (Almeida, Preto, Koukos, Bonvin, & Moreira, 2017; van den Brink-van der Laan, Antoinette Killian, & de Kruijff, 2004). Secondly, the conical shape induces packing defects randomly distributed across the outer-leaflet of various sizes, which act as binding sites for exposed hydrophobic residues of peripheral proteins (Vamparys et al., 2013; van den Brink-van der Laan et al., 2004; Vanni et al., 2013). Moreover, PE is a precursor of the glycosylphosphatidylinositol (GPI) anchor (anchor for various transmembrane proteins; Kinoshita, 2016), enabling the anchorage of these to the outer-leaflet of the membrane (Paulick & Bertozzi, 2008). PE also acts as a chaperone by facilitating the folding of proteins to enable their proper function (Bogdanov, Mileykovskaya, & Dowhan, 2008; Patel & Witt, 2017). Also, evidence indicates that PE supports autophagy (cellular recycling) and therefore promotes the optimisation of cellular health and longevity (Madeo, Tavernarakis, & Kroemer, 2010).

2.3.4 Phosphatidylinositol (PI)

PI is located mainly in the inner-leaflet of the plasma membrane and represents less than 15% of all PLs and the smallest amount of any of the other main GPLs in the plasma membrane (Di Paolo & De Camilli, 2006; Fadeel & Xue, 2009; Yamaji-Hasegawa & Tsujimoto, 2006). The chemical structure of PI is shown in Figure 2.6.

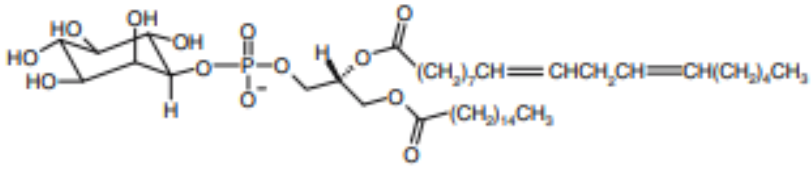


Figure 2.6 Chemical structure of phosphatidylinositol. Reprinted by permission from Springer Nature Customer Service Centre GmbH: Springer. Chromatographia.“Feasibility of Phospholipids Separation by Packed Column SFC with Mass Spectrometric and Light Scattering Detection,” by H. S. H. Yip, M. Ashraf-Khorassani and L. T. Taylor. Copyright 2007. Reprinted with permisson.

Despite its low prevalence in the membrane, the phosphorylated derivatives of PI, phosphoinositides (PIs), influence most elements of a cells life, including being universal signalling entities, modulating vesicle trafficking and transmembrane proteins, such as ion channels, pumps and transporters, and importantly, regulating lipid distribution and metabolism via lipid transfer proteins (Balla, 2013; Clarke et al., 2015). Crucially, each compartment within the membrane has a characteristic suite of PIs, which act as a specific lipid signature, attracting a specific complement of functionally significant, loosely bound peripheral proteins existing within the cytoplasm (Falkenburger, Jensen, Dickson, Suh, & Hille, 2010). Membrane lipid remodelling is associated with a turnover of peripheral proteins, where a new unique set of peripheral proteins with their own enzymatic and signalling functions will associate with remodelled compartments (Di Paolo & De Camilli, 2006; Falkenburger et al., 2010).

PI kinases can phosphorylate the 3, 4, and 5-hydroxyl groups of the inositol head group to produce 7 structurally related but distinct PIs, which include phosphatidylinositol-monophosphate (PI3P, PI4P, and PI5P), phosphatidylinositol-bisphosphate ($PI(3,4)P_2$, $PI(3,5)P_2$, and $PI(4,5)P_2$) and phosphatidylinositol-trisphosphate ($PI(3,4,5)P_3$) (Antonietta De Matteis, Di Campli, & Godi, 2005). PIs play an extensive number of roles, a number of which are known to support cognitive function. These are discussed in section 2.7.

2.4 Sphingolipids

Sphingolipids are highly dynamic lipids that have a sphingoid base backbone (Merrill, 2011), this being sphingosine in mammals (Pralhada Rao et al., 2013). FA acyl chains in sphingolipids vary in length between C16 and ≥ C28, and in most mammalian tissues, the most frequent types are

palmitic acid and lignoceric acid (saturated FA, 24:0) and nervonic acid (monounsaturated FA, 24:1) (Sassa, Suto, Okayasu, & Kihara, 2012). The addition of different headgroups forms complex sphingolipids, for example, the addition of phosphorylcholine creates SM (Young, Mina, Denny, & Smith, 2012). The nervous system is particularly enriched in SM (Cutler & Mattson, 2001) and the amount present within the brain changes throughout life (Posse de Chaves & Sipione, 2010).

2.4.1 Sphingomyelin (SM)

SM is a complex sphingolipid and the most abundant of the complex sphingolipids within mammalian cells (Castro-Gómez et al., 2015; Gault et al., 2010). The chemical structure of SM is shown in Figure 2.7.

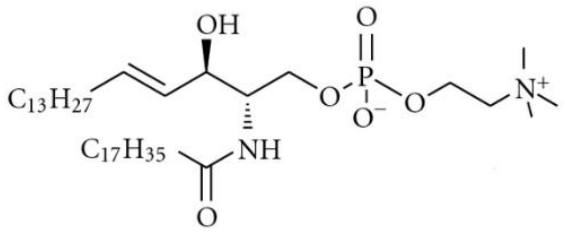


Figure 2.7 Chemical structure of sphingomyelin. Adapted from "Sphingolipid and Ceramide Homeostasis: Potential Therapeutic Targets," by S. A. Young, J. G. Mina, P. W. Denny and T. K. Smith, 2012, *Biochemistry Research International, 2012,* p.2. Copyright 2012 by CC BY. Adapted with permisson.

Oligodendrocytes (myelinating cells of the central nervous system) and myelin (sheath surrounding axons) are particularly enriched with SM (Capodivento et al., 2017; Don et al., 2014). SM is most frequently located within the outer layer of the bilayer membrane and in myelin sheaths, where it supports stability and preserves chemical resistance (Castro-Gómez et al., 2015). The sphingolipid profile in humans features SM from axonal / dendritic branching and synaptogenesis through to adulthood (Olsen & Færgeman, 2017). The presence of long-chain SM in the plasma membrane contributes to membrane thickness by conferring increased thickness. This may support the integration of transmembrane proteins into the membrane owing to the length of their transmembrane domains (Slotte, 2013). Various channels including voltage-gated potassium channels are modulated by SM (Combs, Shin, Xu, Ramu, & Lu, 2013; Milescu et al., 2009).

2.5 Dietary sources of glycerophospholipids and sphingolipids, and their bioavailability

In addition to de novo synthesis, GPLs and sphingolipids are available from diet and can be taken as supplements (Küllenberg et al., 2012). The typical dietary daily intake of PLs is between 2 and

8 grams, with the body absorbing a greater amount of PC (>90%) (Cohn, Wat, Kamili, & Tandy, 2008). In respect of spingolipids, it has been suggested that a western diet provides 0.2-0.4 grams per day, with most coming from animal sources (Vesper et al., 1999). GPLs and sphingolipids are available in different concentrations in red and white meat, dairy products, egg yolk, fish, shellfish, roe, krill, vegetables, cereals and oils (Fong et al., 2016; Küllenberg et al., 2012; Restuccia et al., 2012; Weihrauch & Son, 1983). Particularly rich sources include soybeans, eggs and milk (Burling & Graverholt, 2008). In respect of the relative concentration of GPLs present in these food items, soybeans are equally high in PC, PE and PI, whilst egg yolk is particularly high in PC, whereas milk is enriched in PC, PE and SM (Küllenberg et al., 2012). Bile from the liver provides GPLs for intestinal absorption also; notably 10 – 15 grams per day, which is mostly PC (Wang, Liu, Portincasa, & Wang, 2013). Table 2.1 presents the total PL (g kg^{-1} total food) and respective PL (g kg^{-1} total phospholipids) content in common foods.

Table 2.1 Total phospholipid and respective phospholipid content of common foods[a]

Food	PL	PC	PE	PS	PI	SM
Chicken whole egg	34.9	770	166			24
Bovine whole milk	0.2	327	285	141[b]		230
Beef	7.0	493[c]	180	139[d]	46	64
Pork	6.0	429	267	49	68	75
Chicken breast	4.0	610	194	40	67	55
Salmon (head)	5.4	547	140	104	25	83
Tuna	6.0	379	210	54	85	40
Soybean	20	450	263	50[e]	141	
Peanut	6.0	435	81	40[e]	242	
Soybean lecithin		386	164	06	192	
Egg lecithin		754	183			19

Notes. Adapted from "Phospholipids in foods: prooxidants or antioxidants?" by L. Cui and E. A. Decker, 2015, *Journal of the Science of Food and Agriculture*, *96*, p.22. Copyright 2015 by Society of Chemical Industry, Wiley Publishing. Adapted with permission. PL: total phospholipids, PC: phosphatidylcholine, PE, phosphatidylethanolamine, PS: phosphatidylserine, PI: phosphatidylinositol, SM: sphingomyelin.

[a]The values of total phospholipids are in g kg^{-1} total food. The values of individual phospholipids are in g kg^{-1} total phospholipids. [b]The value includes PI. [c]The value includes lysophosphatidylcholine. [d]The value includes phosphatidic acid and cardiolipin. [e]The values include phosphatidic acid.

GPLs have good bioavailability due to their amphiphilic nature (Li et al., 2015). In the small intestine, GPLs along with cholesterol and other lipids form a fat-water emulsion, leading to the formation of micelles in the presence of bile salts, which are optimal for lipid intestinal absorption (Boyer, 2013; Fricker et al., 2010). Digestion of SM has been observed as being more than 80% following supplementation with a 250 mg dose of milk SM (Ohlsson et al., 2010).

2.6 Polyunsaturated fatty acid components of glycerophospholipids

In the brain, which has a comparatively unique FA composition relative to other organs (Chouinard-Watkins, Lacombe, Metherel, Masoodi, & Bazinet, 2019), PS and PE are particularly enriched with DHA and docosapentaenoic acid (DPA; omega-3, 22:5), whereas EPA is esterified to PI (Chen, Liu, Ouellet, Calon, & Bazinet, 2009). GPLs in cell membranes within the brain have specific PUFA profiles (Layé et al., 2018). DHA and AA are prevalent in the brain, with DHA being reported to be the predominant omega-3 PUFA, contributing over 40% of total brain PUFA (Lacombe, Chouinard-Watkins, & Bazinet, 2018). As already discussed, assimilation of FAs into PL membranes has consequences for cellular membrane composition, and the types of FAs present in cellular membranes influence membrane fluidity, membrane raft formation, membrane-generated signalling cascades (Raphael & Sordillo, 2013), vesicle budding and fusion, and protein function (Schmitz & Ecker, 2008). Lipid remodelling (i.e. Lands' cycle) depends, amongst other things, upon the availability of FAs, and so there needs to be a balanced pool of available FAs to serve the homeostatic requirements of membranes, especially to aid recovery following stress (Maulucci et al., 2016; Nicolson & Ash, 2017). The notion of dietary fatty acids altering phospholipid composition is well supported and discussed in section 2.7.6.2.

AA, EPA, DPA and DHA can be obtained directly from diet or synthesised from shorter-chain FAs. Specifically, omega-6 LA is required for the synthesis of AA, whereas omega-3 ALA is needed for the synthesis of EPA, DPA and DHA (Kaur, Chugh, & Gupta, 2014). LA and ALA are classed as essential FA's in that they cannot be produced in vivo by mammals and are therefore obtained exclusively from diet (Bazinet & Layé, 2014; Di Pasquale, 2009). As the same metabolic pathways are used for the synthesis of long-chain PUFA from ALA and LA, this introduces competition (i.e. for desaturases and elongases) (Calder, 2016; Kaur et al., 2014). Notably, the end products are, to a limited extent, proportional to their precursors (Layé et al., 2018). As humans lack specific desaturase enzymes, they cannot synthesise omega-3 PUFA from omega-6 (Simopoulos, 2006). The omega-6:omega-3 ratio in tissue PLs directly reflects diet composition (Guillou, Zadravec, Martin, & Jacobsson, 2010). Endogenous synthesis of EPA, DPA and DHA within the brain is poor relative to uptake from the plasma free (unesterified) FA pool (DeMar et al., 2006; DeMar, Ma,

Chang, Bell, & Rapoport, 2005), suggesting that the brain maintains levels of these FAs through uptake from dietary and/or liver sources available in plasma (Dyall, 2015). The poor synthesis of EPA, DPA and DHA from ALA may partly be explained by the high proportion of ALA undergoing oxidisation (β-oxidation) in the brain (Anderson & Ma, 2009; DeMar et al., 2005; Kaur et al., 2014). The Western diet typically includes a low intake of EPA and DHA (Bradbury, 2011; van Elst et al., 2014). The ideal ratio of omega 6:omega 3 intake is 1-4:1 (Patterson, Wall, Fitzgerald, Ross, & Stanton, 2012), however, researchers suggest that the Western diet provides a ratio between 10-20:1 (Molendi-Coste, Legry, & Leclercq, 2011; Zárate, el Jaber-Vazdekis, Tejera, Pérez, & Rodríguez, 2017). Importantly, a low intake of ALA with a high intake of LA results in omega-6 accumulation, including AA (Layé et al., 2018). Whilst it is true that some omega-6 FAs are required for health, the Western diet provides an excessive quantity, which can lead to the displacement of DHA from membrane PLs (Bradbury, 2011).

2.7 Potential role of glycerophospholipids in the protection and facilitation of cognitive function

2.7.1 Relationship between glycerophospholipids and insulin resistance

Insulin resistance (failure of cells to respond to insulin/to suppress hepatic gluconeogenesis and glucose release into the blood) and impaired glucose tolerance (IGT - where following a glucose load, cells are not able to clear glucose from the blood effectively) lead to disrupted glucose homeostasis (Ruud, Steculorum, & Brüning, 2017). Insulin resistance is a characteristic of metabolic syndrome (MetS) (Geragotou et al., 2016). Both insulin resistance and MetS are predictive of type 2 diabetes mellitus (T2DM) (Guo et al., 2018; Taylor, 2012) and all three increase the likelihood of experiencing cognitive decline and cognitive impairment, particularly dementia (DM) (Chang & Chieh Yen, 2018; Grover, 2018; Laws et al., 2017; Manschot et al., 2006; Mayeda, Whitmer, & Yaffe, 2015; Neergaard et al., 2017; Umegaki, 2014; Yates, Sweat, Yau, Turchiano, & Convit, 2012; Zilliox, Chadrasekaran, Kwan, & Russell, 2016). Previous research has observed a relationship between FA composition of skeletal muscle phospholipids and insulin sensitivity, such that reduced insulin sensitivity was found to be related to decreased PUFA concentrations (Borkman et al., 1993) and the proportion of palmitic acid (Vessby, Tengblad, & Lithell, 1994) present in skeletal muscle phospholipids. More recently, a number of plasma PC species, namely 32:2, 34:1, 34:2, 34:3, 40:5, 42:4 and 42:5 as well as SM 16:0 and 24:1 were significantly associated with reduced odds of homeostatic model assessment insulin resistance (HOMA-IR) in older adults without diabetes (Semba et al., 2018). In a prospective study, serum PC 32:1, 36:1, 38:3, and 40:5 were all significantly positively associated with risk of

T2DM, whilst SM 16:1, lysophosphatidylcholine (lysoPC) - a product of PC hydrolysis by phospholipase A_2 (PLA_2) - 18:2 and PC 34:3, 40:6, 42:5, 44:4 and 44:5 were significantly inversely associated with risk of T2DM (Floegel et al., 2013). Similarly, lower concentrations of lysoPC 18:2 was found to be predictive of both impaired glucose tolerance and T2DM in older adults (Wang-Sattler et al., 2012). Although research shows an association between PLs and insulin sensitivity, there is no evidence at this time to determine whether changes in in PLs lead to or are a result of insulin resistance (Chang, Hatch, Wang, Yu, & Wang, 2019).

Impairment in the activation of atypical protein kinase C (aPKC) contributing to muscle insulin resistance in those with IGT and T2DM is related to reduced responsiveness of aPKC to $PI(3,4,5)P_3$ (Beeson et al., 2003). Indeed, $PI(3,4,5)P_3$-mediated activation of protein kinase B (Akt) and aPKC in muscle promotes the translocation of the glucose transporter, GLUT4 (insulin-regulated glucose transporter in adipose tissue and muscle cells essential for glucose uptake and are central to whole-body glucose homeostatis; Gao, Chen, Gao, Wang, & Xiong, 2017) from the cytoplasm to the plasma membrane, affording increased glucose uptake (Thong, Dugani, & Klip, 2005). In its basal state, GLUT4 is present in cytoplasmic vehicles, however, in response to insulin hormone, GLUT4 translocates to the plasma membrane to facilitate cellular glucose entry (van Dam, Govers, & James, 2005). Alternatively, impaired $PI(3,4,5)P_3$ signalling in muscle has been found to lead to lower levels of GLUT4 translocation and poorer glucose uptake resulting in diabetic pathophysiology (Tremblay, Lavigne, Jacques, & Marette, 2001). Moreover, in high glucose treated adipocytes, exogenous supplementation of $PI(3,4,5)P_3$ was found to upregulate GLUT4 and increase glucose uptake and utilisation. Furthermore, supplementation with $PI(3,4,5)P_3$ and insulin was found to enhance glucose uptake and utilisation in adipocytes relative to $PI(4,5)P_2$ and insulin or insulin alone; therefore, reduced cellular $PI(3,4,5)P_3$ concentration may augment impaired insulin sensitivity in diabetes (Manna & Jain, 2013).

2.7.2 Role of glycerophospholipids in synaptic plasticity and neuronal communication

$PI(4,5)P_2$ is the most abundant phosphorylated PI (Dickson & Hille, 2019) and the most dominant PI located in the plasma membrane (Sohn et al., 2018), specifically in the inner leaflet of the plasma membrane (Ye et al., 2018). $PI(4,5)P_2$ is known to control approximately 100 ion channels and transporters (Dickson, 2019) including voltage-gated potassium and calcium channels (Hille, Dickson, Kruse, Vivas, & Suh, 2015; Logothetis, Petrou, Adney, & Mahajan, 2010), therefore, changes in the availability and/or distribution of $PI(4,5)P_2$ has consequences for neuron electrical activity (Dickson & Hille, 2019; Kruse, Vivas, Traynor-Kaplan, & Hille, 2016; Suh & Hille, 2005). $PI(4,5)P_2$ interacts with hundreds of effector proteins and is implicated in a variety of cellular

functions, such as membrane fusion and exocytosis (Martin, 2015; Tan, Thapa, Choi, & Anderson, 2015). Membrane fusion involves the coordinated merging of two bilayers (Martens & McMahon, 2008), whereas exocytosis concerns the regulated release of neurotransmitters or hormones that exist within secretory vesicles that promotes neuronal or hormonal communication (Wen et al., 2011). $PI(4,5)P_2$ has been observed to be involved in priming, a stage that follows vesicle docking and is prior to Ca^{2+}-triggered fusion (Martin, 2012). Specifically, $PI(4,5)P_2$ recruits and activates multifunctional $PI(4,5)P_2$ binding proteins, such as synaptotagmin-1 and Munc13-1/2, to control SNARE protein function that mediates vesicle fusion (Ammar, Kassas, Chasserot-Golaz, Bader, & Vitale, 2013; Martin, 2012, 2015; Walter et al., 2017). Further, vesicle exocytosis has been observed at areas of high-concentration of $PI(4,5)P_2$ (nanodomains) on the plasma membrane of neuroendocrine cells (Martin, 2015), and exocytosis and synaptic vehicle recycling at presynaptic sites are sensitive to $PI(4,5)P_2$ availability (Cremona & De Camilli, 2001; Di Paolo & De Camilli, 2006).

N-methyl-D-aspartate (NMDA) and alpha-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) receptors are types of glutamate receptors that facilitate synaptic plasticity through their expression in pre- and post-synaptic membranes of the hippocampus (Voglis & Tavernarakis, 2006). These receptors are crucial in evoking long-term potentiation (LTP) and long-term depression (LTD), both of which are thought to constitute the molecular mechanisms that form the basis of learning and memory (Lüscher & Malenka, 2012). Moreover, both are complementary in promoting the fine-tuning of neural circuitry (Bruel-Jungerman, Davis, & Laroche, 2007). During NMDA receptor-dependent LTP and LTD, the synthesis of $PI(3,4,5)P_3$ by phosphatidylinositol 3-kinases (PI3Ks) in dendritic spines from CA1 hippocampal cultured neurons was found to be upregulated. However, phosphatase and tensin homolog deleted on chromosome 10 (PTEN) activity appears to prevent $PI(3,4,5)P_3$ accumulation during LTD (Arendt et al., 2014). This finding is consistent with the notion that synaptic potentiation tends to be associated with upregulation of the $PI(3,4,5)P_3$ pathway whilst synaptic depression is related to downregulation of the pathway (Knafo & Esteban, 2012; Peineau et al., 2007). Moreover, regular turnover of $PI(3,4,5)P_3$ at postsynaptic terminals preserves AMPA receptor clustering and controls synaptic function in rat hippocampal neurons under basal conditions (Arendt et al., 2010). Similarly, $PI(4,5)P_2$ availability and activity has been implicated in LTP in hippocampal membranes in old mice. Improved LTP was found to be dependent upon the interaction between $PI(4,5)P_2$ and myristoylated alanine-rich C kinase substrate (MARCKS), a $PI(4,5)P_2$-clustering molecule, such that increasing the levels of MARCKS resulted in improved LTP and memory retention (Trovò et al., 2013). $PI(4,5)P_2$ has also been found to be necessary for LTD

induction in the mouse hippocampal CA3-CA1 pathway (Kim et al., 2017). Notably, the CA1 subregion of the hippocampus has been implicated in pattern separation (i.e. differentiation of similar patterns of neural activity; Hanert, Pedersen, & Bartsch, 2019), whilst the CA3 subregion is crucial for the rapid encoding of new memories (Leutgeb & Leutgeb, 2007) and object recognition (Dillon et al., 2017).

2.7.3 Role of glycerophospholipids in facilitating cognitive function via protein kinase C (PKC) activation

PKC plays a key role in a variety of cellular signalling pathways that regulate cell differentiation, growth, transformation, apoptosis and tumorigenicity (Cosentino-Gomes, Rocco-Machado, & Meyer-Fernandes, 2012). There are different types of PKC which have different roles which merit some exploration in order to understand their potential to influence cognition. $PI(4,5)P_2$ and PS have both been recognised as PKC activators. Phospholipase C hydrolyzes $PI(4,5)P_2$ to generate inositol triphosphate (IP_3) and diacylglycerol (DAG); subsequent to this, Ca^{2+} is released following IP_3 binding with intracellular receptors (Sun & Alkon, 2012). Together, Ca^{2+} and DAG stimulate the conventional (also known as classical) PKC (cPKC) and PS mediates its activation (Dries & Newton, 2008). In brief, cPKC has four isoenzymes (PKC-α, βI, βII, and γ), each containing four homologous domains (C1-C4), of which the tandem C1 domain mediates diacylglycerol (DAG) and PS binding in a Ca^{2+}-dependent manner (Antal & Newton, 2014; Johnson, Giorgione, & Newton, 2000). It has been reported that PS is a requirement for the activation of all PKC isozymes (Black & Black, 2013; Kanno et al., 2006; Loegering & Lennartz, 2011) and PKC is known to have the greatest binding affinity with membranes containing PS when in the presence of activators (Alzamora, Brown, & Harvey, 2007; Newton, 1995). PI(4,5)P2 is also known to directly bind to PCK-Epsilon (PKC-ε) promoting neuron induction activity (Shirai, Murakami, Kuramasu, Iijima, & Saito, 2007). Further, PKC-ε supports synaptogenesis and synaptic maturation by activating brain-derived neurotrophic factor (BDNF), nerve growth factor, and insulin-like growth factor (IGF) (Sen, Hongpaisan, Wang, Nelson, & Alkon, 2016). PKC isoenzymes are particularly expressed in the hippocampus and have therefore been implicated in memory and learning (Sun & Alkon, 2009, 2014; Talman, Pascale, Jäntti, Amadio, & Tuominen, 2016). In preclinical studies, PKC activators have improved the availability and activity of PKC isoenzymes leading to the restoration of PKC signalling and downstream activity, such as synaptic remodelling and the stimulation of neurotrophic activity in areas including the hippocampus (Sun & Alkon, 2012). Pharmacological activation of PKC has been shown to inhibit amyloid beta (Aβ)-induced toxicology of hippocampal neurons in vitro (Garrido, Godoy, Alvarez, Bronfman, & Inestrosa, 2002; Han, Zheng, Bastianetto, Chabot, & Quirion, 2004). Furthermore,

PKC signalling activity has also decreased the accumulation of neurotoxic Aβ aggregates and tau protein hyperphosphorylation (Etcheberrigaray et al., 2004; Isagawa et al., 2000; Sun & Alkon, 2006). Indeed, activation of PKC-ε and PKC-α has been observed to prevent amyloid plaques and synaptic loss, and to maintain cognitive function in Alzheimer's disease (AD) transgenic mice (Hongpaisan, Sun, & Alkon, 2011).

2.7.4 Contribution of glycerophospholipids to Choline availability

Although choline, an essential nutrient (Blusztajn, Slack, & Mellott, 2017; Wallace et al., 2018), is obtained primarily though diet, PC contributes to choline availability via the methylation of PE to PC catalysed by PE *N*-methyltransferase (PEMT), followed by the action of phospholipases on PC (see also section 2.3.2) (Li & Vance, 2008; Zeisel & da Costa, 2009). Choline is required for the synthesis of acetylcholine (ACh), the transportation of lipids and in cell-membrane signalling (da Costa, Gaffney, Fischer, & Zeisel, 2005; Penry & Manore, 2008). The enzyme choline acetyltransferase (ChAT) is responsible for the synthesis of ACh from choline and acetyl-coenzyme A (acetyl-CoA), the latter being supplied by mitochondria (Ferreira-Vieira, Guimaraes, Silva, & Ribeiro, 2016). Importantly, in the brain, ACh mediates neuronal excitability as well as facilitates synaptic transmission and induces synaptic plasticity (Picciotto, Higley, & Mineur, 2012). ACh is used by cholinergic neurons, which innervate almost all regions of the brain (Woolf & Butcher, 2011). Cholinergic signalling promotes anti-inflammatory properties, upregulation of BDNF, neurogenesis and LTP in the hippocampus, thereby having beneficial consequences for memory (Maurer & Williams, 2017). Notably, the modulation of memory by ACh may be specific to hippocampus-dependent memory, such as episodic and semantic memory rather than procedural memory (Haam & Yakel, 2017). Moreover, ACh has also been proposed to regulate top-down control of attention in the prefrontal and parietal cortex (Klinkenberg, Sambeth, & Blokland, 2011). At present, cholinesterase inhibitors (e.g. Donepezil and Rivastigmine; Gawel et al., 2016), which prevent acetylcholinesterase from breaking down ACh thereby increasing the levels of ACh at synapses, are used as standard care for the symptomatic treatment of AD (Hampel et al., 2019). The rationale for this is that cholinergic neurons of the basal forebrain (Gratwicke et al., 2013; Hampel et al., 2018; Liu, Chang, Pearce, & Gentleman, 2015) and cholinergic medial septum neurons (Hampel et al., 2019) are particularly vulnerable to neuropathology in AD.

Choline is also irreversibly metabolised to betaine (Obeid, 2013; Sivanesan, Taylor, Zhang, & Bakovic, 2018) and betaine donates methyl groups to homocysteine, resulting in homocysteine being metabolised to methionine (McRae, 2013). Reducing levels of homocysteine is favourable for cognitive function with elevated levels of plasma total homocysteine concentrations

associated with reduced cognitive performance, independent of any structural brain alterations in non-demented older adults (Prins et al., 2002). Indeed, a review of 111 studies reported a positive association between increased plasma homocysteine levels and cognitive decline both in healthy and cognitively impaired patients, which was confirmed in meta-analyses (Setién-Suero, Suárez-Pinilla, Suárez-Pinilla, Crespo-Facorro, & Ayesa-Arriola, 2016).

2.7.5 Restoration of mitochondrial function by glycerophospholipid supplementation following oxidative stress

PLs can be oxidised enzymatically (eOxPL) or non-enzymatically (OxPL). Lipoxygenases (LOXs) or cyclooxygenases (COXs) enzymes oxidise PLs in response to injury/infection in activated immune cells and platelets. eOxPLs are formed acutely through the coupling of eicosanoid and prostaglandin pathways with enzymes involved in the Lands' cycle (where a preformed oxidised FA is inserted into a lysophospholipid) or by direct oxidation (O'Donnell, Aldrovandi, Murphy, & Krönke, 2019). eOxPLs include a small number of specific molecular species and are formed via controlled processes in immune cells involving enzymes that are conserved among all mammalian species (O'Donnell & Murphy, 2012). Enzymic-oxidation of omega 3 FAs exert anti-inflammatory and inflammation resolving properties in addition to immunomodulatory effects as seen in model systems and most likely in humans also (Sottero et al., 2019). Alternatively, OxPLs are created during chronic inflammation and atherosclerosis via uncontrolled processes leading to the generation of diverse species that tend to have damaging bioactivities (Aldrovandi & O'Donnell, 2013). The non-enzymic oxidation is hard to control, as it is sustained by free-radical chain reactions (Sottero et al., 2019). Importantly, OxPLs tend to be related to later-stage disease (O'Donnell & Murphy, 2012). The generation of eOxPLs and OxPLs involves the removal of hydrogen from PL-bound PUFAs followed by the addition of oxygen to the acyl chain (O'Donnell et al., 2019; Smith & Murphy, 2008), which reflects the initiation phase of lipid peroxidation (Reis & Spickett, 2012). In the case of eOxPLs, oxygen insertion is determined by the enzyme pathway (O'Donnell & Murphy, 2012).

2.7.5.1 Reactive oxygen species, oxidative stress and lipid peroxidation

Reactive oxygen species (ROS) can generate OxPLs (Smith & Murphy, 2008). Cellular metabolism in living organisms generates ROS from molecular oxygen (Birben, Sahiner, Sackesen, Erzurum, & Kalayci, 2012). Specifically, aerobic metabolism produces ROS as a by-product (Schieber & Chandel, 2014). Complexes I and III of the mitochondrial electron transport chain (ETC) containing several redox centres are well known for generating ROS in living cells such as superoxide anion radical, a precursor of most ROS (Ademowo, Dias, Burton, & Griffiths, 2017; Pamplona, 2008). In brief, oxidative phosphorylation in the synthesis of adenosine triphosphate

(ATP – energy for cells) involves electron flow between enzymes (Chaban, Boekema, & Dudkina, 2014). During oxidative phosphorylation, electrons removed from biological fuels including fatty acids and glucose go through the mitochondrial ETC to generate energy (Cheng et al., 2017). When electrons exit the chain before being reduced to water (electron leak), superoxide and hydrogen peroxide are produced (Treberg, Braun, Zacharias, & Kroeker, 2018), both of which are highly reactive species that cause nonspecific oxidative damage (Lobo, Patil, Phatak, & Chandra, 2010; Murphy, 2013).

Importantly, as well as energy production for cells (e.g. ATP), mitochondria have a central role in intracellular Ca^{2+} signalling and cell growth (Kann & Kovács, 2007; Zhao et al., 2019). As discussed, ROS are a by-product of normal mitochondrial metabolism and homeostasis (Zorov, Juhaszova, & Sollott, 2014). ROS are involved in multiple cellular pathways (Dan Dunn, Alvarez, Zhang, & Soldati, 2015) and low levels of ROS are beneficial for the maintenance of physiological functions, such as signal transduction, gene expression, immunity and proliferation (Schieber & Chandel, 2014). To an extent, ROS metabolism is subtly regulated by enzymes (Zuo, Zhou, Pannell, Ziegler, & Best, 2015). Cellular maintenance under conditions of low lipid peroxidation rates is performed via constitutive antioxidants defence systems that scavenge radicals. However, when lipid peroxidation rates are higher, the repair capacity is overwhelmed by the degree of oxidative damage. In such cases, cells induce apoptosis or necrosis programmed cell death, which causes molecular damage and potentially increases the risk of pathology and accelerated ageing (Ayala, Muñoz, & Argüelles, 2014). Dysregulation of the antioxidant systems or overproduction of oxidising molecules triggers oxidative stress (Angelova & Abramov, 2018; Gaschler & Stockwell, 2017; Scialò, Fernández-Ayala, & Sanz, 2017). Oxidative stress (i.e. excessive amounts of ROS) represents an imbalance between production and metabolism of ROS and manifests an oxidative environment (Poljsak, Šuput, & Milisav, 2013). This imbalance is a universal condition in neurodegeneration (Niedzielska et al., 2016). Excessive ROS disrupts redox homeostasis and results in damage to DNA, proteins and lipids (He et al., 2017), and injuries seen in diabetes (Figueroa-Romero, Sadidi, & Feldman, 2008; Kaneto, Katakami, Matsuhisa, & Matsuoka, 2010; Lee, Yu, Yang, Jiang, & Ha, 2003; Volpe, Villar-Delfino, Dos Anjos, & Nogueira-Machado, 2018), ageing (Davalli, Mitic, Caporali, Lauriola, & D'Arca, 2016; Santos, Sinha, & Lindner, 2018) and neurodegeneration (Angelova & Abramov, 2018; Liu, Zhou, Ziegler, Dimitrion, & Zuo, 2017; Wu, Du, Xue, Wu, & Zhou, 2012). ROS are able to penetrate mitochondrial membranes and propagate outside of cells to cause widespread tissue damage (Offen, Gilgun-Sherki, & Melamed, 2004). Therefore, the extent to which the presence of ROS is beneficial or otherwise depends upon ROS levels (Cheng et al., 2018).

Lipids are extremely vulnerable to ROS (Niki, Yoshida, Saito, & Noguchi, 2005). Oxygen-derived free radicals can oxidise membrane lipid components such as PLs causing lipid peroxidation, and this is particularly the case for PLs that contain PUFAs (Catalá & Díaz, 2016), such as LA, AA, and DHA (Gaschler & Stockwell, 2017). Indeed, unsaturated FAs are major targets of free radicals leading to oxidative moderation, unlike SFAs, which are more resilient (Bochkov et al., 2017; Reis & Spickett, 2012). Specifically, lipids containing carbon-carbon double-bonds are particularly vulnerable (Ayala et al., 2014). Lipid peroxidation impacts membrane physicochemical properties, fluidity and permeability, membrane-initiated signalling pathways, lipid-lipid and lipid-protein interaction dynamics, ion and nutrient transport and metabolic processes resulting in cell dysfunction and death (Adibhatla & Hatcher, 2010; Catalá, 2009; Volinsky & Kinnunen, 2013). Notably, the oxidant species, the type of linkage of the FA to the glycerol backbone and the fatty acyl chain present influence the OxPL profile (Reis & Spickett, 2012). Exposure of biological membranes to ROS leads to a mixture of distinct OxPLs (Bretscher et al., 2015). Moreover, ROS are able to generate secondary oxidative products including aldehydes, which further promote and spread ROS-initiated damage (Hill & Bhatnagar, 2009). 4-hydroxy-2-nonenal (4-HNE) and malondialdehyde (MDA) are the most studied species of aldehydes (Ademowo et al., 2017; Gaschler & Stockwell, 2017; Hauck & Bernlohr, 2016), with 4-HNE receiving attention due to the part it plays in the aetiology of neurodegeneration and cardiovascular disease (Barrera et al., 2015; Mali & Palaniyandi, 2014; Nègre-Salvayre et al., 2017; Perluigi, Coccia, & Butterfield, 2012). Oxidative stress and lipid peroxidative end-products are recognised as biomarkers of diabetes, inflammation and neurodegeneration (Adibhatla & Hatcher, 2010; Bigagli & Lodovici, 2019; Niedzielska et al., 2016).

2.7.5.2 Mitochondrial dysfunction in the central nervous system

Brain tissues consume approximately ten times more glucose and oxygen than other tissues (Angelova & Abramov, 2018). The high levels of aerobic metabolism along with the large lipid content make the brain especially susceptible to oxidative damage (Adibhatla & Hatcher, 2010). Neurons require large amounts of ATP to maintain ionic gradients across cellular membranes and for communication (e.g. vesicle pool cycling; Kann & Kovács, 2007). Neurons also transport mitochondria to distal synapses, therefore placing further demands on energy resources (Zhao et al., 2019). As such, neuronal function and health are sensitive to mitochondrial dysfunction (Zhao et al., 2019). High energy consumption levels within the brain places demand on mitochondria for energy production, with many mitochondrial mutations and toxins leading to tissue damage and pathology (Gandhi & Abramov, 2012). Both mitochondrial dysfunction and mitochondrial ROS formation in the CNS are causative factors of acute brain insults and chronic

neurodegenerative states (Chinopoulos & Adam-Vizi, 2006; Johri & Beal, 2012; Kalogeris, Bao, & Korthuis, 2014). Mitochondrial dysfunction with accompanying elevation in lipid peroxidation products has been observed in several age-related diseases (Ademowo et al., 2017). All aggregated misfolded proteins including Aβ, tau, α-synuclein and huntingtin that are characteristic of neurodegenerative disorders, inhibit mitochondrial function and cause further oxidative stress (Abramov, Berezhnov, Fedotova, Zinchenko, & Dolgacheva, 2017; Angelova & Abramov, 2017).

Mitochondrial bioenergetics are largely conditional upon the physiology of the inner mitochondrial membrane, which is the location of redox complexes and phosphorylation apparatus involved in ATP production (Monteiro, Oliveira, & Jurado, 2013). The integrity of the inner membrane is pivotal to mitochondrial function and depends on the provision of proteins and PLs (Schenkel & Bakovic, 2014). The inner mitochondrial membrane is particularly rich in proteins (Aufschnaiter et al., 2017). A significant proportion of these make up the oxidative phosphorylation system and their activity and stability is sensitive to their interactions with PLs (Schenkel & Bakovic, 2014). As well as cardiolipin, a GPL found exclusively in mitochondrial inner membranes, mitochondrial membranes are characteristically enriched in PC and PE; however, PS, PI and PA are also present (Horvath & Daum, 2013). Several lipid species including GPLs regulate mitochondrial shape and function through the recruitment of proteins, management of protein interactions and alterations of membrane structure and curvature (Frohman, 2015). As discussed, excess ROS can affect PL membrane biophysical properties by affecting their structure and interfering with intracellular functions (Ademowo et al., 2017). This will likely disrupt mitochondrial function, which has been shown to be restored following consumption of membrane lipid replacement (MLR) supplements (Nicolson & Ash, 2017). MLR supplements seek to replace damaged PLs (Nicolson et al., 2016). Ageing and illness (acute and chronic) tend to be accompanied by oxidative damage to cellular membranes (Liguori et al., 2018; Uttara, Singh, Zamboni, & Mahajan, 2009) and lipid provision from diet often fails to provide the quantities required for preserving cellular membranes in an undamaged state in such cases (Nicolson, 2016). In addition to GPLs, MLR supplements may provide antioxidants to protect the unsaturated FAs and GPLs from oxidation both during storage and ingestion, and fructooligosaccharides to protect the GPLs from the effects of temperature in the environment and enzymes and bile present in the gastrointestinal system (Nicolson & Ash, 2014). Notably, there is some empirical evidence of MLR supplementation benefiting cognitive function, and reducing homocysteine levels and risk factors for MetS (reviewed in Nicolson & Ash, 2017).

2.7.6 Glycerophospholipids in the amelioration of inflammation

Inflammation is part of the response against pathogenic organisms and toxic compounds, and is an essential component of the immune response following tissue injury (Raphael & Sordillo, 2013). Importantly, inflammation is typically self-limiting, with negative feedback systems such as secretion of pro-resolving lipid mediators and inhibition of pro-inflammatory signalling cascades resulting in its resolution (Calder, 2015). Disruption or loss of these regulatory processes can lead to inappropriate, excessive or continued (chronic) inflammation that can cause irreversible tissue damage in the host, including in the brain (Leyrolle, Layé, & Nadjar, 2016). Chronic inflammation is typically low-grade and persistent (Franceschi & Campisi, 2014). Systemic inflammation results in elevated circulating pro-inflammatory cytokines including tumor necrosis factor alpha (TNF-α), C-reactive protein (CRP) and interleukin 6 (IL-6), which interact with the CNS (Lin et al., 2018). Elevated levels of these pro-inflammatory cytokines are related to impairments in older adults in global cognition (Schram et al., 2007), executive function (Heringa et al., 2014; Schram et al., 2007), memory (Schram et al., 2007; Teunissen et al., 2003) and processing speed (Bettcher et al., 2014; Heringa et al., 2014). Chronic inflammation is a pervasive feature of ageing (referred to as inflammageing) (Franceschi & Campisi, 2014). Indeed, increases in plasma/serum levels of several inflammatory mediators can be 2- to 4-fold in the elderly (Krabbe, Pedersen, & Bruunsgaard, 2004). However, the association between elevated levels of pro-inflammatory cytokines and cognitive impairment has also been observed in young (Brydon, Harrison, Walker, Steptoe, & Critchley, 2008) and middle-aged adults (Marsland et al., 2015, 2006).

In the CNS, the initial inflammatory response is coordinated by activated microglia, with chronic activation typically seen in neurodegenerative disease, which has the potential to cause neuronal injury due to continued production of cytokines and ROS (van Horssen, van Schaik, & Witte, 2017). In aged mice, microglia show an exacerbated inflammatory response and greater expression of cytokines. Moreover, during ageing, increased DNA oxidative damage and greater intracellular ROS production have been observed; the latter activating nuclear factor kappa light chain enhancer of activated B cells (NF-κB), which serves to promote further neuroinflammation and cognitive impairment (von Bernhardi, Eugenín-von Bernhardi, & Eugenín, 2015). A sustained inflammatory response in the brain has been found to accelerate core AD pathologies (Kinney et al., 2018). Notably, chronic inflammation has been associated with Aβ accumulation, tau pathology, and impairment of synaptic plasticity and neuronal loss (Newcombe et al., 2018). Recently, systemic inflammation in middle-age has been found to be related to cognitive decline over a 20-year period. Participants (n=12,336) who had a midlife inflammation composite score

in the top quartile demonstrated steeper decline in their cognitive function than those in the lowest quartile. This was also the case for those that had elevated inflammatory markers in midlife (which was found to be consistently related with memory decline) compared to those with lower levels, and both findings did not differ by Apolipoprotein E ε4 genotype (APOE ε4) i.e. risk of incident AD (higher for APOE ε4 carriers) did not influence the reported relationship (Walker et al., 2019).

2.7.6.1 Potential of glycerophospholipid supplementation to reduce inflammation by restoring mitochondrial function

Inflammasomes are multi-protein signalling complexes that activate inflammatory caspases, of which, nucleotide-binding oligomerization domain-like receptor protein 3 (NLRP3) is well characterised (Jo, Kim, Shin, & Sasakawa, 2016). NLPR3 inflammasome is known to regulate the maturation of pro-inflammatory interleukin (IL)-1 family cytokines interleukin-1 beta (IL-1β) and interleukin-18 (IL-18) via caspase-1 activation (Davis, Wen, & Ting, 2011; De Nardo & Latz, 2011). Both IL-1β and IL-18 drive inflammatory responses, which can result in neuronal damage (Song, Pei, Yao, Wu, & Shang, 2017). The NLPR3 inflammasome signalling pathway largely contributes to neuroinflammation in the CNS and mitochondrial dysfunction has been observed as increasing the NLRP3 inflammasome-driven pro-inflammatory cascade in microglia (Sarkar et al., 2017). This may be due to the associated increase in ROS generation, which is known to activate the NLPR3 inflammasome (Heid et al., 2013; Zhou, Yazdi, Menu, & Tschopp, 2011). Importantly, microglial NLRP3 inflammasome activation plays a critical role in dopaminergic neuronal loss (Lee et al., 2019) and contributes to pathogenesis following cerebral ischemia, traumatic brain injury (TBI) and haemorrhagic stroke (Song et al., 2017). In respect of AD, Aβ can promote the maturation of IL-1β by inducing augmented caspase-1 and NLRP3 activity via mitochondria ROS, causing an elevation in microglial neurotoxicity (Parajuli et al., 2013).

Another route by which mitochondrial dysfunction induces an inflammatory response is via danger-associated molecular patterns (DAMPs). DAMPS are released following cellular (including neuronal) stress or death (Bajwa, Pointer, & Klegeris, 2019) and interact with pattern recognition receptors (PRRs) to activate the host's immune system (Roh & Sohn, 2018). Oxidised mitochondrial DNA (mtDNA), a consequence of excessive ROS generation (Bhatti, Bhatti, & Reddy, 2017), is acknowledged as being subtype of the DAMP family (Mathew et al., 2012). Oxidised mtDNA is released from mitochondria into the cytoplasm following the opening of the mitochondrial transition pore (ROS induced) and under conditions of cellular stress or necrosis, are further released into extracellular fluid (Boyapati, Tamborska, Dorward, & Ho, 2017; Nakayama & Otsu, 2018). Once in the cytoplasm or extracellular space, mtDNA is able to evoke

a pro-inflammatory response (West & Shadel, 2017). Specifically, mtDNA activates several innate immune pathways involving NLRP3, Toll-like receptor 9 (TLR9) and stimulator of interferon genes (STING) signalling (Fang, Wei, & Wei, 2016). This in turn leads to the enhancement of the transcriptional activity of inflammatory cytokines and interferons (Nakayama & Otsu, 2018).

Restoration of mitochondrial function though MLR supplementation discussed in section 2.7.5 may be an important therapeutic strategy against the role of mitochondrial dysfunction in inflammation. MLR supplementation of GPLs can restore the structure and function of various cellular membranes (Nicolson & Ash, 2014). In doing so, MLR has the potential to improve mitochondrial function and reduce levels of ROS. This in turn would result in better management of inflammation and therefore may contribute to the management of inflammation-driven diseases (Cruz & Kang, 2018).

2.7.6.2 Fatty acid composition of phospholipids and the consequences for inflammation

Overall, it is thought that the effect of FAs on inflammatory cell responses involves their incorporation into PLs within the cell membrane (Calder, 2008b). Crucially, dietary intake impacts the FA profiles of PLs (Saadatian-Elahi et al., 2009; Zheng et al., 2019) and increased intake of marine omega-3 FAs has been observed to augment EPA and DHA concentrations in membrane PLs of blood cells that engage in inflammatory processes (Browning et al., 2012; Faber et al., 2011; Kew et al., 2004; Rees et al., 2006). Further, incorporation of marine omega-3 FAs into membrane PLs of cells that participate in inflammation is both time- and dose-dependent (Browning et al., 2012; Rees et al., 2006). Moreover, increased incorporation of DHA and EPA from diet or following supplementation results in the displacement of omega-6 PUFAs, particularly AA, from cell membrane PLs (Calder, 2013; Walker et al., 2015; Wood et al., 2010), which is favourable for attenuating the pro-inflammatory response in the context of inflammation (Innes & Calder, 2018). Specific examples of how the FA content of cell membrane PLs influence inflammation concern their impact on lipid raft formation and by being precursors of various bioactive lipids following being released from the lipid membrane by, for example, the action of phospholipases (Calder, 2012, 2013; Raphael & Sordillo, 2013).

2.7.6.2.1 Lipid raft formation

Lipid rafts in the plasma membrane that concentrate membrane proteins and lipids such as cholesterol, SM and saturated FAs, serving as signalling platforms, can be affected by the saturation and length of FAs in cell membrane PLs (Endo & Arita, 2016). As discussed, lipid rafts are found in a highly structured liquid-ordered states (Stillwell, 2006). However, omega-3 PUFA

acyl chains are highly disordered (Shaikh, Kinnun, Leng, Williams, & Wassall, 2015) and indicate conformational flexibility, thereby are able to affect lipid raft organisation by disturbing molecular order and acyl chain packing (Shaikh, 2012). Indeed, omega-3 PUFA have a low affinity for cholesterol (Kučerka et al., 2010; Shaikh, 2012). Alternatively, studies using quantitative imaging with polarity sensitive probes suggest omega-3 PUFA enhance the molecular order of rafts. For example, using mouse models, omega-3 PUFA promoted the formation of ordered lipid microdomains and enhanced the molecular order of the immunological synapse on the T cell side, a cholesterol-dependent interface. Importantly, these alterations in the molecular order influence the recruitment of select proteins into the synapse, resulting in suppression of downstream activation and proliferation of T cells (Kim, Barhoumi, McMurray, & Chapkin, 2014; Kim et al., 2008).

DHA and/or EPA have been observed to incorporate directly into membrane fractions crudely corresponding to rafts in a variety of cell types (Briolay, Jaafar, Nemoz, & Bessueille, 2013; Li et al., 2005; Schley, Brindley, & Field, 2007; Shaikh, Rockett, Salameh, & Carraway, 2009). Consistent with this, earlier in vivo studies that supplemented mice with an omega-3 PUFA enriched fish-corn oil mixture (Fan, McMurray, Ly, & Chapkin, 2003) or enriched fish-corn oil mixture or DHA ethyl ester (Fan, Ly, Barhoumi, McMurray, & Chapkin, 2004) (compared to corn oil as placebo) reported incorporation of omega-3 PUFA in lipid raft and soluble membrane PLs in murine splenic T-cells, which resulted in reduced raft SM content. Moreover, omega-3 PUFA were observed to suppress the partitioning of PKC theta (PKC-θ) into lipid rafts, which was related to reduced activation of transcription factors Activating protein-1 (AP-1) and NF-κB and suppression of IL-2 production, a T cell growth factor (Fan et al., 2004). Activation of AP-1 and NF-κB promotes pro-inflammatory cytokine production and elevates pro-inflammatory signalling (Luo & Zheng, 2016; Pugazhenthi, Zhang, Bouchard, & Mahaffey, 2013; Turner, Nedjai, Hurst, & Pennington, 2014).

Further evidence of how omega-3 PUFA incorporation can impact the function of membrane proteins includes the observation that DHA is able to inhibit the dimerization and recruitment of Toll-Like receptor 4 (TLR4) into membrane lipid raft fractions (Ciesielska & Kwiatkowska, 2015; Hwang, Kim, & Lee, 2016; Rogero & Calder, 2018; Wong et al., 2009). TLR4 plays a pivotal role in the escalation of the inflammatory response (Molteni, Gemma, & Rossetti, 2016) and mediates both infection-induced inflammation as well as sterile inflammation caused by endogenous molecules (Wong et al., 2009). Importantly, TLR4 is known to trigger pro-

inflammatory transcription via adaptor proteins, in turn inducing transcription factors AP-1 and NF-κB (Loegering & Lennartz, 2011; Troutman, Bazan, & Pasare, 2012).

2.7.6.2.2 Pro-inflammatory, pro-resolving and anti-inflammatory lipid mediators

PUFA within cell membrane PLs are selectively released from their *sn*-2 position leading to unesterified PUFA (and a lysophospholipid), which are precursors of metabolically active lipid mediators (Lacombe et al., 2018). AA is a major substrate for the biosynthesis of eicosanoids, which are highly bioactive lipid mediators (Raphael & Sordillo, 2013). AA is frequently found in membrane PLs of macrophages, neutrophils and lymphocytes, making AA a typical eicosanoid precursor (Calder, 2013). Eicosanoid mediators including numerous leukotrienes (LTs), prostaglandins (PGs) and thromboxanes (TXs), can be created following the oxidation of AA by LOX, COX and cytochrome P450 (CYP) enzymes or through non-enzymatic free radical mechanisms (Calder, 2015; Dennis & Norris, 2015). Notably, COX-1 is thought to support cellular housekeeping functions (Calder, 2015), whilst COX-2 is recognised as a significant mediator of inflammatory pathways (Gandhi, Khera, Gaur, Paul, & Kaul, 2017). PGs, TXs and LTs tend to be pro-inflammatory (Das, 2018). For example, prostaglandin E_2 (PGE_2) is able to induce IL-6, whilst leukotriene B_4 (LTB_4) produced by macrophages and neutrophils supports inflammatory mediator synthesis including superoxide and inflammatory cytokines leukotrienes C_4 (LTC_4), D4 (LTD4) and E4 (LTE4) (Innes & Calder, 2018). The metabolism of AA takes place following esterified AA being cleaved from membrane PLs, which is carried out by three main phospholipases families, such as PLA2, resulting in increased free AA (Hanna & Hafez, 2018). PLA2 can be activated by inflammatory stimuli (Sun et al., 2010). Although typically associated with inflammation, eicosanoids do have homeostatic functions (Dennis & Norris, 2015). However, excessive or inappropriate production of AA-derived eicosanoids is related to disease (Calder, 2006; Chiurchiù, Leuti, & Maccarrone, 2018).

2.7.6.2.2.1 Pro-resolving lipid mediators

Contrary to pro-inflammatory lipid mediators, specialised pro-resolving mediators (SPMs) act as immunoresolvents and include AA-derived lipoxins (LXs: LXA_4 and LXB_4), resolvins (E-series synthesised from EPA: RvE1–3 and D-series synthesised from DHA: $RvD_{1–6}$), protectins/neuroprotectins (protectins generated in neural tissue) (PD_1/NPD_1 and PDX), maresins from DHA (MaR_1 and MaR_2) and DPA-derived 13-series resolvins ($RvT_{1–4}$) (Dalli, Chiang, & Serhan, 2015; Serhan, 2014; Serhan & Chiang, 2013; Serhan, Chiang, & Dalli, 2018). Following acute inflammation, SPMs support various pro-resolving processes including the termination of further leukocyte infiltration, inhibition of pro-inflammatory cytokines and promotion of anti-

inflammatory mediator production, activation of endogenous resolution programs and aiding tissue regeneration (Buckley, Gilroy, & Serhan, 2014; Serhan, 2014). In view of chronic inflammation, SPMs have been observed to directly regulate adaptive immune cells, such as B and T lymphocytes, both of which are heavily involved in chronic inflammation (Ariel, Chiang, Arita, Petasis, & Serhan, 2003; Ariel et al., 2005; Chiurchiù et al., 2016; Kim et al., 2016; Ramon, Gao, Serhan, & Phipps, 2012).

Interestingly, several neurodegenerative diseases, such as AD and multiple sclerosis (MS) that are typically characterised by chronic inflammation, also appear to be related to failure of activating pro-resolving mechanisms (Chiurchiù et al., 2018). Evidence for dysfunction of inflammation resolution comes from post-mortem hippocampal tissue and cerebrospinal fluid (CSF) of AD patients and healthy controls. Dysfunction was evidenced by reduced LXA_4 in the CSF and the hippocampus and elevations of two SPM receptors in AD brains. Moreover, levels of LXA_4 and RvD_1 in the CSF associated with scores on the Mini-Mental State Examination (MMSE) (Wang et al., 2015). In another study that involved AD patients and age-matched controls, MaR_1, PD_1 and RvD_5 were found to be lower in the entorhinal cortex of AD patients (one of the first regions to show neurodegeneration in AD; Howett et al., 2019), however, levels of prostaglandin D_2 (PGD_2), a pro-inflammatory lipid mediator, were higher in AD. Furthermore, it was also reported that certain lipids were associated with specific characteristics; LXA_4, MaR_1, RvD_1 and PDX induced neuroprotective activity, MaR_1 and RvD_1 down-regulated Aβ42-generated inflammation in microglia and MaR_1 stimulated Aβ42 uptake by microglia (Zhu et al., 2016). Additionally, treatment of primary neuronal and glial cultures with another DHA derived SPM, namely NPD_1, has been identified to both decrease amyloidogenic and upregulate non-amyloidogenic processing of amyloid precursor protein (Zhao et al., 2011). Further evidence of the pro-resolving characteristics of SPMs, particularly SPMs supporting neuronal survival and Aβ uptake by microglia has been reported by in vitro studies (Fiala, Halder, et al., 2015; Fiala, Terrando, & Dalli, 2015; Mizwicki et al., 2013).

2.7.6.2.2.2 Anti-inflammatory characteristics of omega-3 polyunsaturated fatty acids

Omega-3 PUFAs DHA and EPA also demonstrate anti-inflammatory properties. This is exemplified by DHA and EPA competing with AA for metabolism by PLA_2 (Wada et al., 2007) and COX-2, and as consequence, limiting the production of AA-derived pro-inflammatory lipid mediators (Endo & Arita, 2016; Ye & Ghosh, 2018). Crucially, the extent of the competition is dependent upon the concentration of the competing lipids (Wada et al., 2007). Notably, EPA is also a precursor for eicosanoids, however, those derived from EPA are structurally distinct from AA-derived eicosanoids rendering those produced from EPA less potent pro-inflammatory

mediators (Das, 2018). Therefore, EPA availability promotes decreased generation of potent AA-derived eicosanoids whilst increasing the production of weak eicosanoids (Calder, 2015). Additionally, given that increased content of DHA and EPA in cell membranes is associated with reduced content of AA, the increased incorporation means there will be less AA available to act as a substrate of pro-inflammatory eicosanoids (Calder, 2013; Rees et al., 2006).

Crucially, the incorporation of DHA and EPA into inflammatory cell membranes also enables the modulation of inflammatory gene expression via interaction with numerous nuclear receptors and transcription factors (Calder, 2008a). Previous evidence in support of this reported DHA and EPA reduced the production of pro-inflammatory cytokines (Baumann et al., 1999), whilst more recently, an in vitro study revealed DHA and EPA treated macrophages showed decreased expression of IL-6, TNF-α and monocyte chemoattractant protein-1 (MCP-1) (Wang et al., 2009). Importantly, MCP-1, a chemokine, is integral in regulating migration and infiltration of macrophages (Deshmane, Kremlev, Amini, & Sawaya, 2009). Moreover, basal effects of DHA, EPA and EPA + DHA on the expression of ten genes associated with inflammation in unstimulated cultured THP-1-derived macrophages have been explored. Collectively, DHA or EPA or both in combination (at different doses) reduced the expression of genes concerned with the NF-κB pathway, cytokine production including TNF-α expression and reduced nitric oxide synthase 2 (NOS2) expression, a pro-oxidative gene, and enhanced microsomal glutathione S-transferase 1 (MGST1) expression, an anti-oxidative gene (Allam-Ndoul, Guénard, Barbier, & Vohl, 2016). A similar study reported DHA and EPA affecting the inflammatory nature of activated THP-1-derived macrophages across a range of lipopolysaccharide (LPS) concentrations (responsible for inducing inflammation). Both DHA and EPA down-regulated the generation of pro-inflammatory cytokines related to the NF-κB pathway, however, individually DHA was found to be more potent compared to EPA in attenuating IL-1β and IL-6 secretion, whereas EPA modulated TNF-α to a greater extent than DHA (Mullen, Loscher, & Roche, 2010).

2.8 Summary

GPLs are major constituents of cell membranes and include PS, PC, PE and PI, with the latter being the precursor of 7 structurally related but distinct PIs (see section 2.3.4). SM, a sphingolipid (PL species), is also found in cell membranes. Cells can have different organisations of GPLs in their membranes (and bilayers), for example, mitochondria are particularly enriched with PC, PE and cardiolipin. The composition of GPLs vary in respect of the phosphate head group and FA chains. This promotes asymmetry within and across membrane bilayers. Such

diversity has implications for membrane electrostatics, lipid-protein interactions, membrane shape and curvature, membrane fluidity and lateral segregation of membrane constituents illustrated by lipid rafts. Importantly, cell membrane lipids support cellular health and function. Empirical evidence suggests numerous key properties of GPLs (and SM) that facilitate cellular function. These include (not exhaustive) PS acting as a primary binding site for peripheral proteins and PE affecting transmembrane protein structure and function by causing membrane lateral pressure. Further, PC hydrolysis promotes increased PA availability in turn likely facilitating vesicle trafficking, whereas SM supports lipid raft formation as well as myelin stability and transmembrane channels, such as voltage-gated potassium channels.

There are various potential mechanisms by which GPLs may support cognitive function. Insulin resistance, a feature of MetS, is associated with cognitive impairment. The PI PI(3,4,5)P_3 is reported to support GLUT4 translocation to the plasma membrane by activation of aPKC and Akt in muscle leading to increased glucose uptake. The same result was found when high glucose treated adipocytes were supplemented with PI(3,4,5)P_3. PI(3,4,5)P_3 has also been observed to be upregulated during LTP and LTD. For example, regular turnover of PI(3,4,5)P_3 at postsynaptic terminals preserves AMPA receptor clustering, a glutamate receptor that facilitates synaptic plasticity in the hippocampus. Another PI, PI(4,5)P_2, has also been identified as supporting LTP via its interaction with a protein localised to the plasma membrane. This same PI has been implicated in neuron function through its regulation of voltage-gated potassium and calcium channels and its involvement in the preparation of synaptic vesicles following vesicle docking and prior to vesicle fusion. Moreover, PI(4,5)P_2 and PS are recognised for their role in PKC activation and therefore have the potential to promote synaptic remodelling, stimulate neurotrophic activity in the hippocampus and inhibit Aβ-induced toxicology. Another GPL, PC, indirectly supports neuronal excitability, synaptic transmission and plasticity by contributing to ACh synthesis from choline. Membrane lipid replacement supplementation featuring GPLs aims to restore mitochondrial function, which may offer therapeutic benefits following lipid peroxidation by oxidative stress. Restoration of mitochondrial function may also serve as a strategy for reducing systemic inflammation, which has been associated with cognitive impairment in young, middle-aged and older adults. The FA profile of GPLs has also been studied in the context of inflammation. Incorporation of omega-3 PUFA into lipid rafts has been related to reduced activation of transcription factors, activation of which is known to promote pro-inflammatory cytokine production and pro-inflammatory signalling. Also, DHA and EPA released from the cell membranes act as precursors of SPMs that support various pro-resolving processes. Crucially, lower levels of SPMs have been associated with AD.

Chapter 3 Systematic review of glycerophospholipid supplementation studies across the life span and their cognitive outcomes

3.1 Introduction

This chapter reports on a systematic review of the effects of GPL supplementation on cognitive outcomes across the lifespan, including both acute and chronic supplementation studies. To date, no review has reviewed these studies systematically. The aim of the systematic review was to address the following questions:

a) Is there evidence that specific GPLs or a GPL composite can confer a benefit for cognitive performance?
b) Is acute GPL supplementation sufficient to enhance cognitive performance?
c) Does the literature suggest a dose-response effect and/or an ideal duration of supplementation (chronic)?
d) Does supplementation support a particular cognitive domain or are benefits seen across multiple domains?

3.2 Methods

This systematic review followed the preferred reporting items for systematic reviews and meta-analyses (PRISMA) 2009 checklist and is registered in PROSPERO. The registration number is CRD42019148939.

3.2.1 Search strategy and search terms

The following electronic databases were searched, CINAHL 1 January 1960 – 3 January 2020, Embase Classic + Embase 1947 – 3 January 2020, Ovid MEDLINE(R) 1946 – 3 January 2020, and PubMed on 3 January 2020. Details of the cognitive search terms, limits and tags are located in Appendix 2. For each search conducted, the relationship was: (cognitive terms combined with OR) AND (PL terms combined with OR). The reference lists of identified articles were examined individually to supplement the electronic search. Further, an inventory of existing references obtained from on-going citation alerts was also examined.

3.2.2 Inclusion and exclusion criteria

This review was limited to articles published in peer-reviewed journals in English. Case reports, abstracts and conference proceedings were not included. Articles were included or excluded in this review using the criteria in Table 3.1.

Table 3.1 Inclusion and exclusion criteria used for the assessment of study eligibility

	Inclusion criteria	Exclusion criteria
Population	✓ Cognitively healthy participants or participants expressing subjective complaints concerning their memory or cognition or classed as having age-associated memory impairment/age-associated cognitive impairment, of any age. ✓ Human samples. ✓ Minimum reporting requirements included sample size and composition.	× Studies including participants with a psychiatric or medical disorder that could interfere with cognitive function, including a neurological disorder, such as dementia, Parkinson's disease, stroke, focal brain lesions, multiple sclerosis or epilepsy. × Non-human samples. × No detail of sample.
Intervention	✓ Single or multiple GPL(s) – composite - as the active supplement. Where another ingredient is present in the active supplement, this must also be present in the placebo to the same quantity. ✓ Interventions can be either acute or chronic and can be delivered in any form e.g. liquid, gel capsule, powder etc. In the case of cross-over studies, there is no restriction on the length of wash-out period. ✓ Both researchers and their sample are blinded to the treatment condition(s) (double-blind). ✓ Minimum reporting requirements included GPL dose and duration of intervention.	× Non-GPL ingredient present in the active or placebo supplement not given in equal quantity in the comparator. × Open label or single-blind study. × No detail concerning GPL dose nor the duration of intervention.
Comparison	✓ A placebo condition. Where a placebo contains an active ingredient (for example, a GPL or a vitamin), this same ingredient must be administered to the same quantity within the active supplement. Other experimental conditions are acceptable in addition to the placebo condition.	× No placebo condition. × Use of a non-inert substance as the placebo or the addition of an active ingredient not matched in active supplement.
Outcome	✓ Studies including at least one objective measure of cognitive performance will be considered. Paper or computerised measures are acceptable.	× No objective measure of cognitive performance.

3.2.3 Study selection process

The literature search yielded a total of 7242 citations. Following removal of 439 duplicates, a total of 6803 citations were retrieved for possible inclusion in the review. The titles and abstracts of these citations were screened by one reviewer (CC) to remove obviously irrelevant reports (such as animal studies, review papers, case studies, abstracts/posters/chapters, n=742), resulting in retention of 6061 papers. Another reviewer (DH) independently screened, at random, 5% of the titles and abstracts to establish agreement about the inclusion and exclusion of studies. The inter-rater agreement was 95%. Any disagreements during this process were resolved by discussion and a consensus decision was reached. The full-text versions of 103 articles plus citations identified through other sources (n=4) were retrieved and examined for eligibility based on the inclusion and exclusion criteria, and authors were contacted to clarify any missing information. Inter-rater agreement was 100%. As a result of this second screening process, a further 100 articles were excluded leaving a total of 7 articles included in the review. A flow diagram (Figure 3.1) presents the study selection process.

In addition to the 7 included articles, three further articles based on intervention studies that supplemented Lacprodan® PL-20 (Boyle et al., 2019; Hellhammer, Waladkhani, Hero, & Buss, 2010; Schubert, Contreras, Franz, & Hellhammer, 2011) were also included in the review. These studies did not meet the inclusion and exclusion criteria, as sphingomyelin (a sphingolipid, see section 2.4.1), a constituent of the active supplement, was not incorporated in the placebo. However, as the intervention studies reported in this thesis supplemented Lacprodan® PL-20, it is important to consider these.

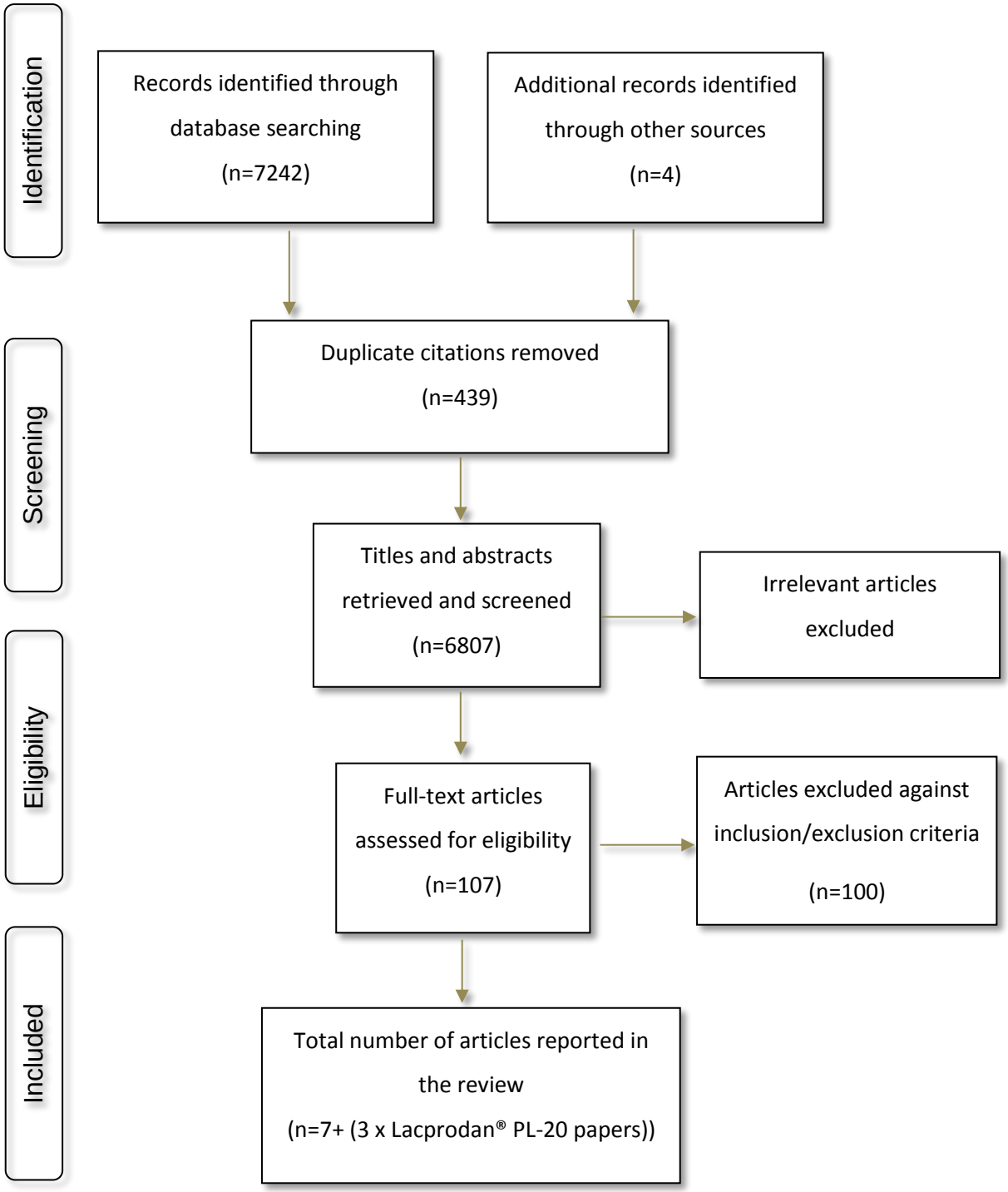


Figure 3.1 Study selection flow diagram

3.2.4 Data extraction

The Cochrane data extraction form was modified for the purposes of this review. Data were extracted by one researcher (CC), and authors were contacted when insufficient information was provided in the published paper. Half (50%) of these articles were then double data

extracted by another researcher (DH). Any disagreements were resolved by discussion, and a consensus decision was reached.

The following information was extracted from the reviewed studies: *Ingredient*: Details the specific GPL(s) provided and the source, where this was available. *Sample characteristics*: The sample size and composition (sex, age). All reviewed studies included cognitively healthy samples. Five of the studies recruited samples based upon specific inclusion criteria, such as meeting the criteria for age-associated memory impairment. *Design and intervention*: Study design and details of the active and placebo supplements as well as any other ingredients provided as part of the intervention. Specifics include supplement composition, form, dose and frequency of administration and intervention length (acute or chronic). The cognitive testing schedule and/or any specific details concerning the design of the study that are noteworthy can also be found here. *Cognitive measures*: A summary of the objective cognitive measure(s) used including a brief description where the nature of the measure is not obvious from the title and/or where there are multiple parts (test battery). Where any Clinical Rating Scales were administered or biological samples taken to explore the effects of supplementation (e.g. changes in GPL levels in plasma), details of these have also been included for completeness. *Reported findings:* A summary of any significant differences by condition as well as marginally significant differences (≥ .05 - .07) and/or trends (> .07 - < .10) in favour of GPL supplementation or otherwise. *Subgroup / further analysis*: A summary of any findings indicating significant differences, marginally significant differences and/or trends following any further analyses or subgroup analyses. *Effect summary*: The reported effects of GPL supplementation were summarised as positive (+), no effect (x), negative effect (-) and (?) where there was any doubt regarding the reported outcome.

3.3 Key characteristics of the studies reviewed

A summary of the ten reviewed studies is provided in Table 3.A (Appendix 3). The experimental design of the studies included in this review conformed to a randomised, double-blind, placebo-controlled design. Of the ten studies reviewed, one study recruited healthy pregnant women and tested their infants at 10 and 12 months of age (Cheatham et al., 2012), seven studies recruited young adults (18-44 years) (Baumeister et al., 2008; Boyle et al., 2019; Harris, Dysken, Fovall, & Davis, 1983; Hellhammer et al., 2010; Parker et al., 2011; Rosadini, Sannita, Nobili, & Cenacchi, 1990; Schubert et al., 2011), two of which followed an acute supplementation strategy (Harris et al., 1983; Rosadini et al., 1990), whilst one study recruited middle-aged adults (45-64

years) (Crook, Tinklenberg, Yesavage, Petrie, Nunzi, Massari, et al., 1991) and one included older-adult samples (65 years +) (Vakhapova, Cohen, Richter, Herzog, & Korczyn, 2010). Where sample age ranges were across two periods, such as 50 – 90 years of age, as in the case of Vakhapova et al. (2010), average age (within each condition where sample average age was not given) was used to determine which age category the study was assigned to.

PS was supplemented most frequently, being administered in five of the ten studies (Baumeister et al., 2008; Crook et al., 1991; Parker et al., 2011; Rosadini et al., 1990; Vakhapova et al., 2010). Supplements were supplied as an oral dose in five studies (Cheatham et al., 2012; Crook et al., 1991; Harris et al., 1983; Parker et al., 2011; Vakhapova et al., 2010), intravenously in one study (Rosadini et al., 1990), in food form in another study (nutritional bar; Baumeister et al., 2008) or as a drink in three studies (milk; Boyle et al., 2019; Hellhammer et al., 2010; Schubert et al., 2011).

Of the studies that assessed the benefit(s) of chronic administration, the longest intervention duration was from 18 weeks gestation to 90 days postpartum (approx. 8 months; Cheatham et al., 2012) whereas the shortest was 2 weeks (Parker et al., 2011). Finally, four of the ten reviewed studies used neuropsychological tests[2] (or a component of these) to identify an effect of treatment (Cheatham et al., 2012; Crook et al., 1991; Harris et al., 1983; Vakhapova et al., 2010).

Within Table 3.A (Appendix 3), the cognitive outcomes are reported as per the original publication, however, these have been re-categorised according to their domain in line with the work of Galioto and Spitznagel (2016) presented in Table 3.2 'Overview of study findings by cognitive domain' and in the remainder of this Chapter. The framework used by Galioto and Spitznagel (2016) is adapted from Lezak, Howieson, Bigler and Tranel (2012); widely accepted as the standard reference that provides comprehensive coverage on the measurement of cognitive domains. Importantly, Galioto and Spitznagel (2016) set out descriptions of cognitive domains and subdomains, as well as examples of tasks associated with each domain that are relevant for studies reported in this review. One exception concerns verbal fluency, which Galioto and Spitznagel (2016) categorise as a function of language. However, this review considers verbal

[2] Digit symbol substitution of the Wechsler Adult Intelligence Scale was used by Harris et al. (1983); Mullen Scales of Early Learning was used by Cheatham et al. (2012); NexAde™ cognitive battery was used by Vakhapova et al. 2010; Benton Visual Retention Test, Wechsler Memory Scale Logical Memory Subtest (Form A) and Wechsler Memory Scale Associative Learning Subtest (Form A) was used by Crook et al. (1991).

fluency to represent executive function, as per Lezak et al. (2012). The following section provides an overview of the cognitive outcomes and the effects of GLPs reported in the reviewed studies.

3.3.1 Risk of bias assessment

Each study was assessed for risk of bias using the Cochrane Collaboration's tool for assessing risk of bias in randomised trials (Higgins et al., 2011). This assessment tool covers 6 domains of bias, where a judgement is taken of high, low or unclear risk of bias (Jørgensen et al., 2016): Selection bias (random sequence generation and allocation concealment), performance bias (blinding of participants and personnel), detection bias (blinding of outcome assessment), attrition bias (incomplete outcome data), reporting bias (selective reporting) and other sources of bias. Two reviewers (CC and DH) independently assessed each study against each of the 6 domains based on the predetermined criteria. Any disagreements during this process were resolved by discussion, and a consensus decision was reached. Risk of bias assessments for each of the ten studies are provided in Figure 3.2.

Details on the randomisation technique was not elaborated in six of the ten studies and therefore a judgement of unclear risk of bias was awarded. Allocation concealment was described in four of the studies. Specifics as to any measures undertaken to blind study participants and personnel from knowledge of which treatment a participant had received were also lacking in 5 of the studies. Further, blinding of outcome assessment was unclear in most studies. Insufficient information was provided regarding risk for incomplete outcome data to permit a judgement of low or high risk in five of the studies. Although two of the ten studies (Boyle et al., 2019; Cheatham et al., 2012) have been registered on the clinical trials database, there was insufficient detail to make an informed decision regarding selective reporting. Due to this and the absence of a published study protocol for each of the respective studies meant a judgement of unclear risk of bias was taken for the domain of reporting bias. A small sample size (Baumeister et al., 2008; Harris et al., 1983; Rosadini et al., 1990) and the absence of a measure of compliance to monitor supplement intake from experimental procedures (Baumeister et al., 2008; Crook et al., 1991; Hellhammer et al., 2010; Parker et al., 2011; Schubert et al., 2011) were typically recorded under the domain of other sources of bias. Fundamentally, a judgment of low risk of bias was taken against the latter only when no other source(s) of bias could be listed otherwise a judgement of high risk of bias was taken.

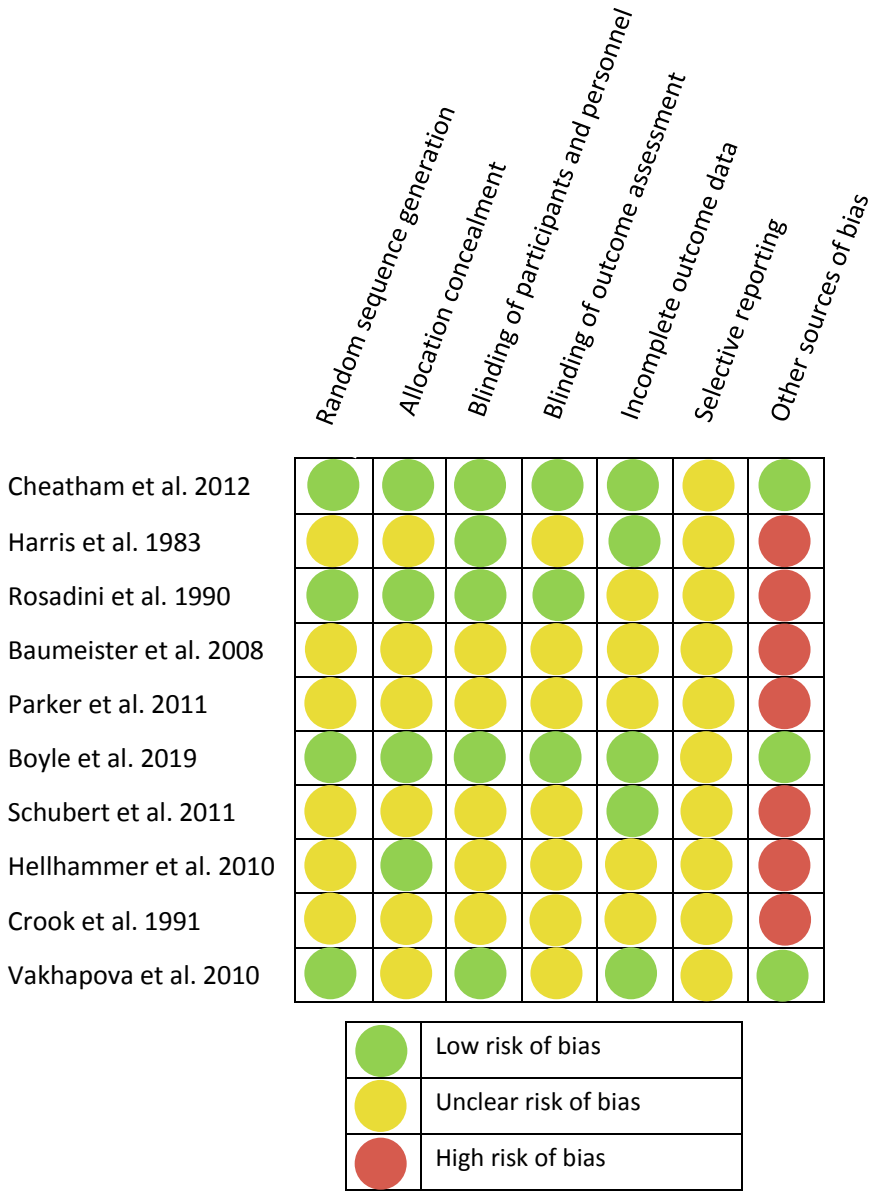


Figure 3.2 Risk of bias assessments for each of the ten studies

3.4 Cognitive outcomes of acute and chronic supplementation: Overview of reported results

3.4.1 Gestation and infancy (n=1)

One study considered the effects of supplementing pregnant women with six Nutrasal PhosChol gel capsules per day, each containing 833 mg PC, from 18 week gestation until 90 days postpartum on neurodevelopment (Cheatham et al., 2012). Only women who intended to breastfeed until 90 days postpartum were recruited to the study (only mothers were supplemented postpartum). Infants were tested at 10 and 12 months of age, with all infants

demonstrating developmental age effects (better performance with age). No treatment effects were reported other than marginal significance for better performance on a measure of delayed recall (deferred action imitation) by those in the placebo condition at 12 months (p = .056). However, this finding did not persist when dietary betaine intake (measured by food diary) at 30-week gestation was added to the analysis as a covariate, found to be significantly higher in the PhosChol condition (p < .05). In a separate stepwise regression analysis using backward elimination, betaine intake at 30-week gestation was a negative predictor of immediate visuospatial recall at 10 months when the task was novel (p = .061), whereas betaine intake at 45 days postpartum was a significant positive predictor of immediate visuospatial recall when the task was novel (p = .058), language development (p = .017) and global development (composite measure) (p = .026) at 12 months. Analysis of choline intake at 45 days postpartum revealed the same patterns of prediction.

3.4.2 Young adults (n=7)

3.4.2.1 Acute administration (n=2)

Two acute cross-over studies administered a single dose of 20 g of lecithin[3] (PC) or placebo orally to healthy adults five hours before cognitive testing (Harris et al., 1983) or 25 mg, 50 mg, 75 mg of PS or matching placebo intravenously to healthy male adults following EEG recordings at 10, 30, 90, 180 and 360 minutes post PS or placebo administration (Rosadini et al., 1990). Despite plasma choline levels being raised in the lecithin condition to nearly double that of the placebo condition during the test session (+ 5 hours post lecithin consumption), this did not confer any cognitive benefit (Harris et al., 1983). Similarly, there was no significant difference in cognitive performance following the administration of PS at 25 mg, 50 mg or 75 mg, or placebo (Rosadini et al., 1990).

3.4.2.2 Chronic administration (n=5)

Five of the studies reviewed recruited young adults to explore whether supplementation provides stress-buffering effects to attenuate stress-induced cognitive performance impairments (Baumeister et al., 2008; Boyle et al., 2019; Hellhammer et al., 2010; Parker et al., 2011; Schubert et al., 2011). Acute stress is known to have divergent effects on cognitive performance, which can be, amongst other things, dependent upon the cognitive domain (Boyle et al., 2019). For instance, post-acute stress induction, cortisol responders have been found to

[3] The GPL and FA profiles depend upon the raw material sources. Typically consists of PC, PE, PS and PI. Originally assigned to pure PC (van Hoogevest & Wendel, 2014).

demonstrate better performance on a measure of attention (Plieger et al., 2017) and pattern separation relative to non-responders when stress was induced during memory consolidation (Jiang, Tran, Madison, & Bakker, 2019).

Baumeister et al. (2008) gave healthy males one IQ PLUS brain bar containing 200 mg soy-based PS or a placebo bar for 6 weeks. Both bars were equivalent in energy and macronutrient (protein, carbohydrate, fat) and vitamin content. After 6 weeks of supplementation (with participants tested at baseline and endpoint), no benefit of PS was found on a measure of inhibitory control nor on a measure of vigilance / focus following acute stress. However, both conditions demonstrated practice effects.

Parker at al. (2011) recruited healthy men meeting the criterion of being physically active for a cross-over trial and gave participants IQPLUS Foods LLC containing 200 mg soy-based PS (per serving; 400 mg per day) or a matching placebo for 2 weeks. Each cognitive testing session took place after each 2-week intervention period in which a measure of working memory was assessed pre-acute exercise bout, then again +5 and +60 minutes post-acute exercise bout. A beneficial trend for PS supplementation was found for average time per correct calculation relative to placebo at pre-acute exercise bout only (treatment x time interaction, p = .007). When cognitive performance was assessed at pre-acute exercise bout only, a significant benefit of PS was found for average time per correct calculation (p = .001) and marginal significance was found for accuracy (number of correct calculations; p = .07). No significant differences were found for serum cortisol, total testosterone, or cortisol to testosterone ratio by condition or condition and time.

Three included studies that also induced acute stress as part of their experimental procedure supplemented their sample with either Lacprodan® PL-20 milk drink containing PC, PE, PS, PI and the sphingolipid SM or a placebo milk drink. In all cases, the drinks were prepared from bovine milk. Boyle et al. (2019) gave healthy males who rated themselves as high on perfectionism (and are therefore likely to be high stress vulnerable) either the PL milk drink (2.7 g of PLs per day) or placebo for 6 weeks. Testing was carried out at baseline and following the intervention, and a benefit of the PL milk drink was found for reaction time on a measure of vigilance / focus post-acute stress (p = .01). Interestingly, the PL milk drink was not found to attenuate the cortisol response but there was a marginally significant difference between those that had consumed the PL milk drink for 6 weeks who showed reduced anticipatory subjective stress ratings post-intervention vs. the placebo (p = .06). Conversely, those in the PL milk condition also reported significantly increased subjective arousal during peak stress exposure following 6 weeks of intervention (p = .03).

Similarly, the PL milk drink enriched with either 1.0% (300 mg) or 0.5% (150 mg) PLs or placebo was given to chronically stressed healthy men for 6 weeks (Schubert et al., 2011). However, unlike the study by Boyle et al. (2019), testing was conducted post-intervention only, prior to and following acute stress exposure. No effect of treatment was detected. After exploratory data analysis, the sample was split by age using a median split at 41 years. It was found that the older participants (41-51 years) who had received 1.0% PL milk drink demonstrated superior performance on a measure of immediate visual recall relative to older participants who had received either 0.5% PL or placebo post-acute stress exposure (p = .042). Moreover, participants in the 1.0% PL milk drink condition demonstrated higher cortisol levels, whereas the placebo had the lowest levels, before and after acute stress exposure, but this was not significant.

A similar experimental procedure was conducted by Hellhammer et al. (2010) who supplemented healthy male adults matched on age and socio-economic status with either the PL milk drink (13.5 g of PLs per day) or placebo for 3 weeks, and again exposed participants to acute stress exposure, with cognitive testing sessions taking place 20 minutes before and 10 minutes after this (post-intervention). A trend was found towards a difference in the performance of participants in both conditions on a measure of verbal recognition memory, with those in the PL composite milk condition demonstrating faster reaction times compared to the placebo condition (p = .09). After controlling for inter-individual variation in cortisol concentration, this difference became marginally significant (p = .06) in the same direction. Further subgroup analysis determined that the PL milk drink also dampened endocrine and psychological stress response but only in highly stressed individuals.

Table 3.2 Overview of study findings by cognitive domain

Cognitive domains and subcomponents	Task	Number of reported findings in favour of supplement	Supplement	Supplement duration	Age of sample	Condition of sample
Attention and processing speed						
Attentional capacity	Forward digit span	0/1[a]	PS-DHA/EPA	15 weeks	Older adults	Memory complaints
Attentional capacity	Divided attention	0/1[b]	BC-PS	12 weeks	Middle aged	AAMI
		0/2				
Vigilance / focus	D2 concentration test	0/1[c]	PS from soy	6 weeks	Young adults	Healthy
Vigilance / focus	Attention switch task	1/1[d]	PL-20	6 weeks	Young adults	Healthy (high stress)
Vigilance / focus	Symbol spotting of the NexAde™	0/1[a]	PS-DHA/EPA	15 weeks	Older adults	Memory complaints
		1/3				
Processing speed	Digit-symbol substitution	0/2[e,a,f]	1. PS-DHA/EPA[a]; 2. Lecithin (PC)[f]	1. 15 weeks[a]; 2. Acute[f]	1. Older adults[a]; 2. Young adults[f]	1. Memory complaints[a]; 2. Healthy[f]
		0/2				
Executive function						
Reasoning	Pattern identification of the NexAde™	0/1[a]	PS-DHA/EPA	15 weeks	Older adults	Memory complaints
Inhibitory control	Stroop word-colour interference	0/1[c]	PS from soy	6 weeks	Young adults	Healthy
Working memory	N-back (memory load: 2)	0/1[d]	PL-20	6 weeks	Young adults	Healthy (high stress)

Cognitive domains and subcomponents	Task	Number of reported findings in favour of supplement	Supplement	Supplement duration	Age of sample	Condition of sample
Working memory	Serial subtraction task	1/1[g]	PS from soy	2 weeks	Young adults	Healthy (physically active)
Working memory	Backward Digit Span	0/2[h,a,i]	1. PS-DHA/EPA[a]; 2. PS[i]	1. 15 weeks[a]; 2. Acute[i]	1. Older adults[a]; 2. Young adults[i]	1. Memory complaints[a]; 2. Healthy[i]
Verbal fluency	Category noun generation	0/1[f]	Lecithin (PC)	Acute	Young adults	Healthy
		1/7				
Language						
Language development	MacArthur-Bates Short Form Vocabulary Checklist	0/1[j]	Nutrasal PhosChol	18-week gestation - 90 days postpartum	Infants	Healthy
Receptive and expressive	Mullen Scales of Early Learning	0/1[j]	Nutrasal PhosChol	18-week gestation - 90 days postpartum	Infants	Healthy
		0/2				
Psychomotor						
Fine and gross	Mullen Scales of Early Learning	0/1[j]	Nutrasal PhosChol	18-week gestation - 90 days postpartum	Infants	Healthy
		0/1				

Cognitive domains and subcomponents	Task	Number of reported findings in favour of supplement	Supplement	Supplement duration	Age of sample	Condition of sample
Visual ability						
Visual reception	Mullen Scales of Early Learning	0/1[j]	Nutrasal PhosChol	18-week gestation - 90 days postpartum	Infants	Healthy
		0/1				
Visuospatial ability						
Visuospatial ability	Rey Complex Figure Test (copy time)	1/1[k,a]	PS-DHA/EPA	15 weeks	Older adults	Memory complaints
Visuospatial ability	Rey Complex Figure Test (copy quality)	0/1[a]	PS-DHA/EPA	15 weeks	Older adults	Memory complaints
		1/2				
Memory and learning						
Immediate recall: verbal / visual	Short-term visual spatial memory delayed response task	0/1[j]	Nutrasal PhosChol	18-week gestation - 90 days postpartum	Infants	Healthy
Immediate recall: verbal / visual	Name Face Association	1/1[k,b]	BC-PS	12 weeks	Middle aged	AAMI
Immediate recall: verbal / visual	Telephone number recall	1/1[l,b]	BC-PS	12 weeks	Middle aged	AAMI

Cognitive domains and subcomponents	Task	Number of reported findings in favour of supplement	Supplement	Supplement duration	Age of sample	Condition of sample
Immediate recall: verbal / visual	Selective reminding	0/1[b]	BC-PS	12 weeks	Middle aged	AAMI
Immediate recall: verbal / visual	First-Last Names	0/1[b]	BC-PS	12 weeks	Middle aged	AAMI
Immediate recall: verbal / visual	Categorised serial learning task	0/1[f]	Lecithin (PC)	Acute	Young adults	Healthy
Immediate recall: verbal / visual	Paired-associates learning task	0/1[f]	Lecithin (PC)	Acute	Young adults	Healthy
Immediate recall: verbal / visual	Short-Term Retention Test for sequences of letters	0/1[i]	PS	Acute	Young adults	Healthy
Immediate recall: verbal / visual	Immediate Retention Test for sequences of colours	0/1[i]	PS	Acute	Young adults	Healthy
Immediate recall: verbal / visual	VISGED Visual Memory Test	1/1[n]	PL-20	6 weeks	Young adults	Healthy (chronically stressed)
Immediate recall: verbal / visual	Rey Auditory Verbal Learning Test (trial 1)	1/1[m,a]	PS-DHA/EPA	15 weeks	Older adults	Memory complaints
Immediate recall: verbal / visual	Rey Auditory Verbal Learning Test- verbal total learning (sum of scores of trials 1-5)	1/1[l,a]	PS-DHA/EPA	15 weeks	Older adults	Memory complaints
Immediate recall: verbal / visual	Rey Complex Figure Test	0/1[a]	PS-DHA/EPA	15 weeks	Older adults	Memory complaints
Immediate recall: verbal / visual	Recall a pattern of the NexAde™	0/1[a]	PS-DHA/EPA	15 weeks	Older adults	Memory complaints
		5/14				

Cognitive domains and subcomponents	Task	Number of reported findings in favour of supplement	Supplement	Supplement duration	Age of sample	Condition of sample
Delayed recall: verbal / visual	Deferred imitation	0/1[j]	Nutrasal PhosChol	18-week gestation - 90 days postpartum	Infants	Healthy
Delayed recall: verbal / visual	Name Face Association	1/1[k,b]	BC-PS	12 weeks	Middle aged	AAMI
Delayed recall: verbal / visual	Misplaced objects recall	1/1[l,b]	BC-PS	12 weeks	Middle aged	AAMI
Delayed recall: verbal / visual	Selective reminding	0/1[b]	BC-PS	12 weeks	Middle aged	AAMI
Delayed recall: verbal / visual	Categorised serial learning task	0/1[f]	Lecithin (PC)	Acute	Young adults	Healthy
Delayed recall: verbal / visual	Rey Auditory Verbal Learning Test	1/1[l,a]	PS-DHA/EPA	15 weeks	Older adults	Memory complaints
Delayed recall: verbal / visual	Rey Complex Figure Test	0/1[a]	PS-DHA/EPA	15 weeks	Older adults	Memory complaints
Delayed recall: verbal / visual	Delayed pattern recall of the NexAde™	0/1[a]	PS-DHA/EPA	15 weeks	Older adults	Memory complaints
		3/8				
Recognition: verbal / visual	Facial Recognition	1/1[k,b]	BC-PS	12 weeks	Middle aged	AAMI
Recognition: verbal / visual	Uncategorised word recognition task	0/1[f]	Lecithin (PC)	Acute	Young adults	Healthy
Recognition: verbal / visual	Uppercase letter recognition	1/1[o]	PL-20	3 weeks	Young adults	Healthy
Recognition: verbal / visual	Rey Auditory Verbal Learning Test	0/1[a]	PS-DHA/EPA	15 weeks	Older adults	Memory complaints
		2/4				

Cognitive domains and subcomponents	Task	Number of reported findings in favour of supplement	Supplement	Supplement duration	Age of sample	Condition of sample
Neuropsychological tests not otherwise categorised						
Benton Visual Retention Test	-	0/1[b]	BC-PS	12 weeks	Middle aged	AAMI
Wechsler Memory Scale Logical Memory Subtest (Form A)	-	1/1[l,b]	BC-PS	12 weeks	Middle aged	AAMI
Wechsler Memory Scale Associative Learning Subtest (Form A)	-	0/1[b]	BC-PS	12 weeks	Middle aged	AAMI
		1/3				

[a]Vakhapova et al. (2010); [b]Crook et al. (1991); [c]Baumesiter et al. (2008); [d]Boyle et al. (2019); [e]0/1 component of NexAde™ / 0/1 subtask of WAIS; [f]Harris et al. (1983); [g]Parker et al. (2011); [h]0/1 component of NexAde™; [i]Rosadini et al. (1990); [j]Cheatham et al. (2012); [k]Significant treatment effect also found following analysis of subgroup data; [l]Significant treatment effect found following analysis of subgroup data only; [m]Significant treatment effect also found following analysis of subgroup data and a trend following intention to treat analysis (of whole sample); [n]Schubert et al. (2011); [o]Hellhammer et al. (2010). AAMI: Age-associated memory impairment; BC-PS: bovine cortex phosphatidylserine; PC: Phosphatidylcholine; PS: Phosphatidylserine, PS-DHA/EPA: Phosphatidylserine-Docosahexaenoic acid/Eicosapentaenoic acid.

3.4.3 Middle aged adults (n=1)

Adults (n=149) who met the criteria for age-associated memory impairment (AAMI) were given 300 mg of bovine cortex PS per day or a matched placebo for 12 weeks (Crook et al., 1991). Paper based neuropsychological tests were administered at baseline and endpoint; also, a computerised psychometric battery was undertaken at baseline, then again at weeks 3, 6, 9, 12 and 16 (the latter being 4 weeks post-endpoint). Also, a Clinical Global Rating Scale was undertaken by a study psychologist/registered nurse during an interview with participants at weeks 12 and 16 to explore improvements since treatment initiation. Condition related performance differences were found in favour of PS supplementation. Specifically, those in the PS condition demonstrated significantly better performance than those in the placebo condition on immediate visual and verbal associative recall at weeks 3 and 6 ($p < .001$), delayed visual and verbal associative recall at weeks 3 ($p = .04$) and 6 ($p = .03$), and on recognition of visual items at week 12 ($p = .01$), however, at week 3, this was marginally significant ($p = .05$). In addition, cluster analysis of baseline data was performed, which identified two subgroups differentiated by memory function at baseline i.e. good vs. poor. Those identified as having poor memory function at baseline consistently showed treatment effects, so a separate analysis was conducted on the data from these participants alone (n=57). Again, those in the PS condition performed significantly better than those in the placebo condition on immediate visual and verbal associative recall at weeks 3 ($p = .01$), 6 ($p = .04$) and 12 ($p = .01$), delayed visual and verbal associative recall at week 12 ($p = .04$), with marginal significance at week 3 ($p = .05$), on recognition of visual items at weeks 3 ($p = .03$), 6 ($p = .02$), 12 ($p = .02$) and 16 ($p = .01$), on immediate verbal recall at week 16 ($p < .001$) and delayed visual recall at weeks 6 ($p < .001$) and 16 ($p = .03$). Moreover, these same participants scored significantly higher on immediate and delayed verbal recall on the Logical Memory Subtest of the Wechsler Memory Scale (WMS) at week 12 ($p < .03$). Lastly, those in the PS condition scored significantly higher on the Clinical Global Rating Scale at week 12 for memory for names of persons after introductions ($p = .02$), ability to maintain concentration when reading, conversing or performing tasks ($p = .02$), whereas marginally significant improvements were found at week 12 for overall global change in cognitive status ($p = .05$) and visual analogue scale of global improvement ($p = .05$) in favour of PS supplementation, however, these benefits did not persist after ceasing supplementation for 4 weeks (i.e. at week 16).

3.4.4 Older adults (n=1)

Vakhapova et al. (2010) supplemented adults with memory complaints with PS conjugated to omega-3 LC PUFA (PS-DHA/EPA: 300 mg PS, 79 mg DHA+EPA, DHA/EPA ratio 3:1) or placebo for

15 weeks and tested participants using two separate cognitive measures and a cognitive battery at baseline and endpoint. In addition, participants were interviewed using the Clinical Global Impression of Change (CGI-C) at week 7 and endpoint. Performance by participants in the PS-DHA/EPA condition was found to be superior to that of the placebo condition for immediate verbal recall (trial 1; PP analysis: p = .041; ITT analysis: p = .069), and there was a trend for visuospatial ability, such that copy time was faster for the PS-DHA/EPA condition (PP: p = .079). Overall, on both measures, those in the PS-DHA/EPA condition tended to improve more than those in the placebo condition. Responder analysis based upon better performance in immediate verbal recall (trial 1) and overall improvement in the CGI-C was significantly associated with condition (PP analysis: p = .034; ITT analysis: p = .035), with the ratio of percentage of responders in the PS-DHA/EPA:placebo conditions being 22:8 (PP analysis) and 23:9 (ITT analysis), respectively. As with the study carried out by Crook and colleagues (1991), a subgroup of the sample (n=78) was identified as responding differentially to treatment. However, unlike Crook et al. (1991), the subgroup included participants with relatively good cognitive performance at baseline. The analysis of differences by condition within this subgroup revealed a benefit of PS-DHA/EPA over the placebo condition for immediate verbal recall trial 1 (p = .006), verbal total learning: sum of scores of trial 1-5 (p = .002) and delayed verbal recall (p = .045). Marginal significance was found for visuospatial ability, such that copy time was faster for the PS-DHA/EPA condition relative to the placebo condition (p = .055). In respect of the responder analysis, again a significant correlation was found (PP analysis: p = .016), with responders comprising 25% of the PS-DHA/EPA condition and 5% of the placebo condition. Although there were no significant differences reported for the CGI-C between the conditions both for the whole sample and the subgroup, in both cases, more participants in the PS-DHA/EPA condition were classified as clinically improved.

3.5 Discussion

A systematic review of GPL supplementation studies and their reported cognitive outcomes was conducted. Ten quantitative studies were eligible for inclusion in this review.

3.5.1 Summary of findings

Six of the reviewed studies reported benefits[4] in cognitive performance following supplementation compared to placebo (Boyle et al., 2019; Crook et al., 1991; Hellhammer et al., 2010; Parker et al., 2011; Schubert et al., 2011; Vakhapova et al., 2010). Across the six studies, cognitive performance enhancement was reported following PS and PS-DHA/EPA supplementation (Crook et al., 1991; Parker et al., 2011; Vakhapova et al., 2010). A composite GPL mixture included PL-20 bovine milk concentrate (Boyle et al., 2019; Hellhammer et al., 2010; Schubert et al., 2011) also resulted in improved cognitive performance. Moreover, benefits of supplementation to cognition were seen across the adult lifespan in individuals with and without memory complaints: young (Boyle et al., 2019; Hellhammer et al., 2010; Parker et al., 2011; Schubert et al., 2011), middle-aged (Crook et al., 1991) and older adults (Vakhapova et al., 2010). As infants were assessed in only one study, it is difficult to conclude that GPL supplementation does not have the potential to facilitate cognitive performance in this age group. Also, not all infants were breastfed for the expected 90 days (Cheatham et al., 2012), which may have affected the study findings. Memory (immediate, delayed, recognition and performance on neuropsychological measures sensitive to memory function) appeared to benefit more than any other cognitive domain (Crook et al., 1991; Hellhammer et al., 2010; Schubert et al., 2011; Vakhapova et al., 2010). However, of all cognitive domains, memory was predominantly assessed across the ten studies, therefore, this benefit for memory performance may reflect the extent to which memory measures were utilised, especially in two studies that recruited participants with memory complaints (Crook et al., 1991; Vakhapova et al., 2010). In addition to memory outcomes, PS and PS-DHA/EPA were also found to be beneficial for executive function (working memory; Parker et al., 2011) and visuospatial performance (Vakhapova et al., 2010) respectively, while PL-20 bovine milk concentrate facilitated performance on a measure assessing vigilance/focus (Boyle et al., 2019). Two studies that reported a main effect of treatment conducted post hoc subgroup analyses following identification of subsets within the sample that responded differentially to treatment (Crook et al., 1991; Vakhapova et al., 2010). Notably, Crook et al. (1991) identified a subgroup who demonstrated poor memory function at baseline, whilst conversely, Vakhapova et al. (2010) determined a subgroup who showed relatively good cognitive performance at baseline. Despite the differences in baseline performance, those who received the active supplement showed significantly or marginally

[4] Includes significant differences, marginally significant differences and trends.

significantly better performance vs. placebo, in line with the performance of the whole sample. In addition, Crook et al. (1991) also reported those who received PS scored significantly higher on a neuropsychological measure that assessed immediate and delayed verbal recall and were rated significantly or marginally significantly higher on a clinical global rating scale at endpoint; findings that were not evident from the analysis of the whole sample.

One acute study that supplemented PS at different doses (25 mg, 50 mg and 75 mg; Rosadini et al., 1990) and one chronic study that supplemented 200 mg soy-based PS as part of a nutritional bar for 6 weeks (Baumeister et al., 2008) did not find a benefit of PS. Importantly, both studies recruited small samples (n=8 and n=16, respectively). Studies with small samples have low statistical power (Suresh & Chandrashekara, 2012), contributing to a reduced likelihood of detecting true effects (Button et al., 2013). The notion that the small sample size contributed to the null findings reported by Baumeister and colleagues (2008) is further supported by the positive treatment effect observed following a 2 week intervention of equivalent quantity (200 mg) of soy-derived PS seen in healthy young males (crossover n=18; Parker et al., 2011); although as different outcome measures were employed by both studies, it may be that the measure used by Parker at al. (2011) was more sensitive to detect alterations in cognitive performance afforded by PS supplementation.

Of the two studies that used PC as the active supplement, neither study reported an advantage of supplementation for cognitive performance following acute (Harris et al., 1983) or chronic (Cheatham et al., 2012) administration. Notably, Harris et al. (1983) did observe that mean plasma choline levels were nearly double post-lecithin supplementation compared to placebo levels, however, this did not facilitate any improvement in cognitive performance. Cheatham et al. (2012) attributed the lack of a favourable influence of PC supplementation on cognitive performance to the use of a low dose and the location of the study, this being the United States, where most diets contain good sources of choline.

The benefit of PS supplementation for cognitive performance is likely to be the result of the many roles PS plays in facilitating cognitive function. PS readily crosses the blood-brain barrier (BBB) and is concentrated in the brain (Mozzi, Buratta, & Goracci, 2003; Starks, Starks, Kingsley, Purpura, & Jäger, 2008). In addition to its role in PKC activation, which can potentially promote synaptic remodelling and support neurotrophic activity in the hippocampus (see section 2.7.3), PS is also considered critical for the functioning of neuronal membranes, including secretory vesicle release, signal transduction, and cellular communication and growth (Vance & Steenbergen, 2005). In aged rats, PS supplementation led to an increase in the levels of PS in

the hippocampus, which was accompanied by the normalisation of cognitive function (Babenko & Semenova, 2011).

PS conjugated to omega-3 LC PUFA (DHA/EPA) was also found to confer a cognitive performance advantage following supplementation (Vakhapova et al., 2010). It is noteworthy that PS sourced from bovine cortex also has a high DHA content (Vakhapova et al., 2010), as supplemented by Crook and colleagues (1991). Sufficient quantities of DHA-enriched PS are necessary for fusion of intra-neuronal secretory granules with the pre-synaptic membrane, the subsequent release of neurotransmitter molecules into the synaptic cleft and postsynaptic neurotransmitter-receptor interactions (Kim, Akbar, & Kim, 2010; Tanaka, Farooqui, Siddiqi, Alhomida, & Ong, 2012). PS from squid has been reported to reduce choline acetyltransferase (AchE; the enzyme that synthesises ACh) reactive neuron loss in the hippocampal CA3 region and to raise glucose uptake in both the frontal lobe and hippocampus in rats with trimethyltin-induced memory deficits (Park et al., 2012). Similar findings have been reported by Lee et al. (2015) who noted oral squid derived PS supplementation restored AchE-reactive neurons in hippocampus CA1 and CA3 areas, hippocampal choline transporter mRNA and muscarinic acetylcholine receptor type 1 mRNA expression, enhanced hippocampal DHA concentration and improved memory ability in aged rats with age-related memory impairment. Importantly, PS can be converted to lysophosphatidylserine (lysoPS) by phospholipases (i.e. phospholipase A1 (PLA_1) and PLA_2) (Frasch & Bratton, 2012) and DHA-bound lysoPS has been suggested to be an effective substrate for foetal brain DHA accumulation (Tsushima, Ohkubo, Onoyama, Linder, & Takahashi, 2014). Additionally, the major facilitator superfamily domain-containing protein 2a (Mfsd2a) has been proposed as the major route of DHA uptake into the brain in the form of lysophospholipids including lysoPS but not unesterified FA (Nguyen et al., 2014). Therefore, DHA-bound lysoPS may have the potential to elevate levels of DHA availability in the brain, which in turn has consequences for neuronal membrane PS levels. That is, DHA in neural tissues is positively correlated with the accumulation of PS in neuronal membranes (Hamilton, Greiner, Salem, & Kim, 2000; Kim et al., 2010; Kim, Akbar, Lau, & Edsall, 2000). Moreover, there is some evidence that DHA by itself can facilitate cognition function across the lifespan (Ghasemi Fard, Wang, Sinclair, Elliott, & Turchini, 2019; Weiser, Butt, & Mohajeri, 2016), however, particular subpopulations may be more likely to benefit, such as those with low basal levels (Derbyshire, 2018; Ostermann & Schebb, 2017).

PL-20 bovine milk concentrate was also found to be favourable in supporting superior cognitive performance as reported in all three of the studies in which it was administered. Collectively, an advantage of PL-20 bovine milk concentrate was seen on an attention switch task assessing

vigilance/focus (Boyle et al., 2019), on a visual memory test that targeted immediate visual recall in a subgroup of the sample (Schubert et al., 2011) and on a measure of verbal recognition (Hellhammer et al., 2010). The milk concentrate used comprises PC, PE, PS, PI and SM (Hellhammer et al., 2010; Küllenberg et al., 2012; Schubert et al., 2011). In view of the physiological properties of GPLs, which may potentially protect and promote cognitive function (see section 2.7), there are a number of mechanisms that could underpin the performance advantages seen in these studies. That said, overall, the findings were not consistent. Boyle et al. (2019) did not find a significant effect of treatment on a measure of working memory, a marginal significant difference was found between the PL milk drink and placebo conditions after inter-individual variation in cortisol concentration had been controlled for (Hellhammer et al., 2010), and the benefit of treatment as reported by Schubert et al. (2011) was observed only after post-hoc analysis split by age.

3.5.2 Methodological issues

There are a number of methodological flaws in the studies reviewed that merit careful consideration. The first issue concerns the use of covariates. Entering a covariate into an analysis allows for the variance in performance associated with that covariate to be removed, thus enabling a more accurate estimate of the effects of the main factor (usually condition/treatment) of interest (Schneider, Avivi-Reich, & Mozuraitis, 2015). Age (Deary et al., 2009a; Murman, 2015; Nouchi & Kawashima, 2014; Pangelinan et al., 2011) and IQ (Diaz-Asper, Schretlen, & Pearlson, 2004; Mohn, Sundet, & Rund, 2014) have been found to significantly predict cognitive function. It is therefore desirable to enter these as covariates into any analysis of cognitive performance. Baseline performance, usually the biggest predictor of subsequent performance, can also be controlled for in intervention studies. There is some variation in the literature concerning the approach to take towards baseline performance, whether to include this as a covariate or to assess change from baseline. However, including baseline performance as a covariate reduces the extent to which experimental findings are affected by baseline performance differences (Vickers & Altman, 2001), which were evident in two studies in particular (Crook et al., 1991; Vakhapova et al., 2010). A comparison of ANOVA and ANCOVA (i.e. adjustment for baseline performance), with both change score and post-intervention score as the response variable, and linear mixed modelling for the estimation and testing of treatment effects found each approach provided unbiased estimates of treatment effects, however, ANCOVA was determined to be most effective based upon precision of estimates, 95% coverage probability and power (O' Connell et al., 2017). Out of the ten studies reviewed, eight studies included a baseline measure (all except Cheatham et al., 2012; Harris et al., 1983), three of which

were post-intervention but prior to acute stress exposure (Hellhammer et al., 2010; Parker et al., 2011; Schubert et al., 2011). Of these, baseline performance was entered into the analysis performed by Boyle et al. (2019), in the analysis of the neuropsychological tests and computerised psychometric battery undertaken by Crook and colleagues (1991), and in the analysis of the Rey Auditory Verbal Learning Test (RAVLT) and Rey Complex Figure Test (RCFT) by Vakhapova et al. (2010), although this was dichotimised (1 SD ± mean). In addition, age was also controlled for by Boyle et al. (2019), although this was subsequently removed, as it was not found to be a significant covariate. Vakhapova et al. (2010) also entered and removed participants' demographics, as these too were found to be nonsignificant. Of note, participants in the intervention study conducted by Hellhammer et al. (2010) were matched on age in order to reduce error variance. In addition, MMSE score (26/>26) at baseline was found to be a significant covariate and was therefore retained in the analysis of the RAVLT and RCFT by Vakhapova and colleagues (2010), whereas the inclusion of baseline performance in the analysis of the neuropsychological tests by Crook et al. (1991) controlled for baseline memory function.

A second methodological issue concerns the matching of study products and monitoring participant compliance in respect of supplementation consumption. Matching active and placebo supplements ensures allocation concealment and therefore researchers and participants remain blind, which reduces bias (Bhide, Shah, & Acharya, 2018). Keeping a record of compliance informs decisions taken on the inclusion of data in per protocol or intention-to-treat analysis, or even the removal of data collected from the analysis, depending upon how participant supplement consumption aligns with study requirements. Of the ten studies reviewed, seven studies reported the active and placebo supplements matched (Boyle et al., 2019; Cheatham et al., 2012; Crook et al., 1991; Harris et al., 1983; Parker et al., 2011; Rosadini et al., 1990; Vakhapova et al., 2010) and of the eight chronic interventions, three studies reported monitoring participant supplement consumption (Boyle et al., 2019; Cheatham et al., 2012; Vakhapova et al., 2010).

Other methodological issues that compromise the quality of the reviewed studies include the possible presence of practice effects and small sample sizes. Baumeister et al. (2008) observed cognitive performance improvements in both the PS and placebo conditions following the supplementation period, which they reason may have been due to familiarisation with the experimental situation and cognitive measures. Practice effects have been reported on measures assessing immediate and delayed recall of word lists and serial subtraction following repeated testing (Bell, Lamport, Field, Butler, & Williams, 2018). As such, it is possible that practice effects may have been a confounding variable in other studies reviewed, such as the

study by Vakhapova et al. (2010) who used the RAVLT or the study by Parker et al. (2011) who used a serial subtraction task (serial 7's). To minimise the influence of practice effects on participant performance, Boyle et al. (2019) had participants complete two practise versions of the cognitive measures during familiarisation. Sample sizes were particularly small in three studies in particular (Baumeister et al., 2008; Harris et al., 1983; Rosadini et al., 1990), which minimises the power of a study to detect any treatment effects. More generally, there was a lack of reported power calculations across the studies, however, as Boyle et al. (2019) report, this may be due to the lack of appropriate extant evidence concerning the protective effects of PL intake on cognitive performance.

A further methodological limitation concerns the selection of cognitive measures. Cognitive measures differ in their sensitivity to detect a benefit to cognitive function following nutritional intervention. A lack of treatment effect may represent a real absence of a nutrient-induced change to cognitive performance in a particular domain or it may be the result of a lack of sensitivity of a cognitive measure to detect that change (Lieberman, 2003). As such, the evaluation of cognitive measures as to their sensitivity to detect nutrient-induced change(s) to cognitive performance is critically important (Hoyland, Lawton, & Dye, 2008). None of the reviewed studies justified their utilisation of the cognitive measures employed in respect of their sensitivity to identify nutrient-induced changes in cognition. However, the selection of specific cognitive measures was justified in the context of them being sensitive to stress (Boyle et al., 2019) and in another study, selection of primary measures was based upon variables that showed the clearest pattern of decline in AAMI from normative data (Crook et al., 1991). On a somewhat related point, three of the ten studies used a cognitive test battery (Cheatham et al., 2012; Crook et al., 1991; Vakhapova et al., 2010), the use of which can substantially increase the number of measures used on a sample and therefore increase the risk of false positives i.e. Type 1 error rate.

3.5.3 Recommendations for future GPL supplementation studies

To address the above limitations and gaps in the research area, several recommendations can be made to support future research undertaken to explore the effects of GPL supplementation on cognitive performance. Importantly, there is a lack of studies to evaluate the effects of GPL supplementation in children and adolescents. Childhood and adolescence are characterised as periods of cognitive development (Bidzan-Bluma & Lipowska, 2018; Casey, Getz, & Galvan, 2008; Paus, 2005) and may therefore be particularly sensitive to GPL supplementation. Future research studies that supplement children and/or adolescents with GPLs would therefore address this gap in knowledge. Care should be taken in such circumstances to use age-

appropriate cognitive measures, for example, executive function is known to develop throughout childhood and adolescence (Best & Miller, 2010). Literature on omega-3 supplementation in children and adolescents, where there are numerous intervention studies, could be used as a basis for sample size estimates and in the selection of sensitive cognitive measures. Further, additional GPL intervention studies should be conducted using infants to build on the existing findings (Cheatham et al., 2012). To date, no longitudinal studies have been undertaken to examine the effects of GPL supplementation over an extended amount of time. In the context of cognitive ageing (adulthood), it has been recommended that intervention trials have long intervention periods of at least 18 months (Vauzour et al., 2017). However, long intervention periods will be expensive to run and potentially vulnerable to considerable attrition. Any supplementation studies that follow aged participants over a substantial period should plan for attrition bias and use cognitive measures that are sensitive to changes in cognitive function over time. Conversely, further research to investigate acute effects of GPL supplementation are also welcome to add to the current findings (Harris et al., 1983; Rosadini et al., 1990). Supplementing PS at higher doses in future acute supplementation studies may translate to improvements in cognitive performance not previously seen (Rosadini et al., 1990). This could inform understanding of the dose-response relationship, which is another avenue that should be further explored. Acute measurements can be built into chronic supplementation studies to minimise burden to participants and the resources required by research teams. Other methodological considerations for future work include assessing participants' cognitive performance prior to the commencement of supplementation (true baseline) to derive a clearer picture of whether supplementation aids cognitive function. By using an inert substance as the placebo and not combining this with other ingredients as part of the GPL supplement, a more accurate picture of treatment effects, which can confidently be attributed to GPLs might be obtained. Data analysis strategies should be stated prior to data collection (*a priori*) and the use of median splits should be avoided, as this may exaggerate or even produce effects that reflect artefacts of the reduction of continuous variables to categorical groups (Hamer et al., 2016). Moreover, analyses should include suitable covariates to control for confounding influences including baseline performance. This is especially important when there is recruitment bias, such as when the sample is highly educated, since education is known to correlate highly with cognitive performance. Studies should be sufficiently powered to detect statistical significance and therefore an *a priori* sample size calculation should be performed (Suresh & Chandrashekara, 2012). Lastly, the FA composition of the GPLs that comprise the supplement should be specified wherever possible. Vakhapova et al. (2010) was the only study

to detail the FA profile attached to the glycerol backbone of the GPL used, however, it is appreciated that lipidomics is a newly emerged discipline meaning that older studies were limited in the ability to provide such detail. Soy based GPL products are likely to have FAs that are derived from plants, whereas GPL products can now be formulated with marine derived FAs (as in the case of Vakhapova et al., 2010).

3.5.4 Conclusion

From the ten studies reviewed, it is difficult to draw any firm conclusions regarding the efficacy of GPLs on cognitive performance due to the lack of a consistent effect. However, there is some indication that PS may be particularly advantageous in facilitating cognitive performance. Memory was the most frequently assessed of all cognitive domains across the ten studies and GPL supplementation was observed to confer superior performance when immediate, delayed and recognition memory was tested. Given that GPLs and FAs have characteristics that promote and protect cognitive function, it is likely to be difficult to unpick the contribution that either one makes to improved cognitive performance, especially if they have synergistic effects, which has been suggested for PS-DHA (Zhang, Xu, Wang, & Xue, 2019). A risk of bias assessment was undertaken against each of the ten studies included within the review. An unclear risk of bias judgement was made most often against the 6 domains due to an insufficient provision of information within the corresponding research papers. A high risk of bias judgement was recorded against seven of the studies for the domain of other sources of bias. A small sample and a lack of supplement intake monitoring were most commonly identified across these studies and contributed to this judgement.

The aim of this systematic review was to address four key questions concerning GPL supplementation. Using the evidence based on the findings of the reviewed studies, it can be suggested that PS as a single supplement and PL-20 as a composite supplement may be promising in conferring a cognitive performance advantage, however, the advantage gained by PL-20 may be limited to individuals who are more vulnerable to experience stress (Boyle et al., 2019). Currently, there is no evidence to suggest a dose-response effect or the ideal duration over which supplementation should take place to promote cognitive performance. Both acute supplementation studies reviewed reported null findings (Harris et al., 1983; Rosadini et al., 1990), which may suggest that acute supplementation of GPLs does not offer any benefit. However, both studies used small samples, one study supplemented PC (Harris et al., 1983), which was not found to offer an advantage to cognitive performance in the only other study in which it was administered (Cheatham et al., 2012), and the dose of PS was potentially too low in the second study (Rosadini et al., 1990). Finally, as discussed, memory seems to benefit most

from GPL supplementation, however, evidence from other domains is suggestive of benefits, including vigilance/focus, working memory and visuospatial ability. The greater number of favourable effects reported for memory performance needs to be considered in context. Memory was tested most frequently of all cognitive domains across the ten studies; two studies that utilised multiple memory measures recruited samples with subjective memory decline and used a GPL supplement with a high concentration of DHA, both of which may increase the likelihood of finding a positive result of supplementation. Future research that heeds the above recommendations will further elucidate the potential of GPL supplementation to promote cognitive function.

Chapter 4 A randomised placebo-controlled trial examining the effects of chronic (six week) phospholipid intake on cognitive performance in children aged 6 – 8 years of age (Study 1).

4.1 Introduction

One of the recommendations that came out of the systematic review (Chapter 3) was for future GPL intervention studies to explore the potential benefit(s) of supplementing children, an area that has not received attention previously. To address this gap in the extant literature, this chapter reports on a 6 week intervention study that supplemented Lacprodan® PL-20 to a sample of children aged 6 – 8 years. As discussed in Chapter 1, the development of cognitive function is a protracted process that continues throughout childhood and adolescence through to young adulthood (Catherine Lebel, Treit, & Beaulieu, 2019; Nouchi & Kawashima, 2014). Childhood is therefore an optimal time in which to study the potential cognitive function enhancing properties of Lacprodan® PL-20.

4.2 Continuation of brain development during childhood

The frontal lobe has a prolonged developmental trajectory, however, there are developmental stages during which there is greater development from birth-2 years, 7-9 years and during the mid-teenage years (Bryan et al., 2004). The prefrontal cortex continues to mature through childhood and adolescence (Giedd, 2004), demonstrated by a reduction of neuronal and synaptic density and dendrite growth, but also gains in white matter volume, promoting the formation of distributed neural networks suitable for complex cognitive processing (Tsujimoto, 2008). This area of the frontal lobe facilitates higher cognitive functions by supporting memory consolidation and expression (Preston & Eichenbaum, 2013) and mediating social cognition (Bicks, Koike, Akbarian, & Morishita, 2015) and executive functions, such as planning, decision making and judgement (Funahashi & Andreau, 2013), working memory (Lara & Wallis, 2015) and attention, specifically attentional control (Rossi, Pessoa, Desimone, & Ungerleider, 2009). Development of subcortical structures, including the basal ganglia, amygdala, and hippocampus that are also involved in memory, executive function and emotion continues through to late adolescence (Nyaradi et al., 2013).

4.2.1 Region specific changes during development

The maturation of the brain is a complex process that reflects interactions between genetic, environmental and epigenetic factors (Kolb & Gibb, 2011; Lenroot & Giedd, 2008; Peterson,

2003; van Loo & Martens, 2007). By the age of 6, 95% of total cerebral volume has been achieved (Lenroot & Giedd, 2006); however, maximal growth is not reached until approximately age 14 years in males and 11 in females (Giedd et al., 1999). Importantly, individual subregions follow temporally unique developmental trajectories in which lower-order sensorimotor regions mature before higher-order association areas (Gogtay et al., 2004; Taki et al., 2011). The notion of regional specific development is supported by the findings that the prefrontal cortex is one of the last to mature (Casey, Giedd, & Thomas, 2000), and the development of areas with fronto-temporal connections are slower relative to other regions (Lebel, Walker, Leemans, Phillips, & Beaulieu, 2008). Fractional anisotropy, an indicator of white matter microstructural properties (Bathelt, Gathercole, Johnson, & Astle, 2017), is sensitive to white matter coherence, myelination and axonal organization (Alkonyi et al., 2011; Grieve, Williams, Paul, Clark, & Gordon, 2007), and has highlighted different white matter fibre tracts demonstrating different developmental patterns, possibly due to age-related reprogramming and myelination (Lebel et al., 2008).

4.2.2 Alterations in cortical thickness in development and associations with cognitive performance

A reduction in cortical thickness and an increase in white matter fractional anisotropy (a measure of white matter integrity) occurs during childhood and adolsecence (Moura et al., 2017). Cortical thickening takes place prior to cortical thinning (Kharitonova, Martin, Gabrieli, & Sheridan, 2013). Grey matter reduction is thought to reflect axonal myelination (Gogtay et al., 2004; Gogtay & Thompson, 2010; Paus, 2005; Sowell, Thompson, Leonard, et al., 2004), synaptic pruning (Gogtay et al., 2004; Sowell, Thompson, & Toga, 2004) and trophic glial and vascular changes (Morrison & Hof, 1997). Axonal myelination and synaptic proliferation and pruning are critical mechanisms that influence cognitive development (Luna et al., 2001; McGivern, Andersen, Byrd, Mutter, & Reilly, 2002). Widespread white matter anisotropy developmental changes have been reported in cognitively healthy children from age 6. Specifically, age-related increases have been found in prefrontal regions, in the pathways between the basal ganglia and the thalamus and those extending within the basal ganglia, in the internal capsule, in ventral visual streams and in the corpus callosum, as well as in intra-hemispheric tracts and areas corresponding to cortico-thalamic and cortico-spinal tracts extending from sensory-motor regions (Barnea-Goraly et al., 2005). Recent research using neurite orientation dispersion and density imaging (NODDI) has characterised increases in fractional anisotropy as being the result of increased myelination and/or axonal packing rather than alterations in axon coherence and geometry (Mah, Geeraert, & Lebel, 2017).

Cortical thinning has been related to cognitive performance in childhood and adolescence. For example, in a sample of 12-14 year olds, thinner parietal cortices were associated with better performance on measures of visuospatial planning abilities, problem solving, verbal learning and memory (Squeglia, Jacobus, Sorg, Jernigan, & Tapert, 2013). Frontal lobe grey matter thinning was predictive of better verbal memory retrieval independent of age (not mediated by) in children aged 7-16 years (Sowell, Delis, Stiles, & Jernigan, 2001). Moreover, frontal grey matter thinning in the left lateral dorsal frontal and the left lateral parietal regions related to improved vocabulary subtest scores, whereas grey matter thickening in the left medial occipital region was associated with improved block design subtest performance in a longitudinal study that assessed children on average 2.2 years apart between 5-11 years of age (Sowell, Thompson, Leonard, et al., 2004). The relationship between executive function and cortical thickness, independent of the effects of age, has been explored in children between 8-19 years of age. Thinner cortices in bilateral parietal and frontal regions were related to improved working memory updating; whereas for inhibition, better performance was associated with thinner cortices in bilateral occipital and parietal regions (Tamnes et al., 2010). Alternatively, better shifting performance was related to thinning in the central lateral region in the left hemisphere but also thicker cortex in the right occipital lobe (Tamnes et al., 2010). Mediation analysis has been used to consider whether cortical thinning can explain the relationship between age and cognitive performance on a context monitoring and inhibitory task in children aged 5-10 years. Cortical thinning of the right inferior frontal gyrus was found to mediate the relationship between age and faster reaction times on congruent and incongruent trials, whilst cortical thinning of the anterior cingulate cortex was found to mediate the relationship between age and reaction times on incongruent trials only (Kharitonova et al., 2013).

4.2.3 Increased activation and specialisation of connectivity between brain regions in development, and associations with cognitive performance

In addition to cortical thinning, other changes take place during development, which also have implications for cognition. Cortical activation patterns become more focal, with less reliance on regions uncorrelated with task performance, leading to improved efficiency (Durston et al., 2006). There is also evidence of increased neural activation with age being favourable for cognitive capacity (Klingberg, Forssberg, & Westerberg, 2002). Selective inhibitory control (Bedard et al., 2002; Luna et al., 2001) and visuo-spatial working memory (Kwon, Reiss, & Menon, 2002) are enhanced with age. Such behavioural improvements have been accompanied by increased brain activation in the prefrontal cortex and parietal cortex (Kwon et al., 2002; Luna et al., 2001) as well as the striatal and thalamic regions (Luna et al., 2001).

Functional magnetic resonance imaging (fMRI) during associative encoding of object face pairings shows a linear increase in the recruitment of visual processing regions with age, and activation within these regions mediated the relationship between age and memory performance to a greater extent than the mediating effect of hippocampal activation in 6-19 year olds. Moreover, greater activation during encoding in the bilateral fusiform gyrus and lateral occipital cortex was related to increased performance on a delayed recognition test (Rosen et al., 2018). On a task of verbal working memory, where executive function demands remained the same across task difficulty, children (9-15 years of age) demonstrated significantly less accurate and slower responses at greater difficulty levels (increased cognitive load) relative to young adults. Furthermore, children were also found to exhibit less of an increase in working memory load-dependent activation in multiple frontal and parietal cortical regions but also showed less of a decrease of activation in the default mode network (DMN) as a function of difficulty compared to young adults (20-25 years of age). Therefore, although the same neural networks were employed, clear differences were seen between the two age groups in terms of activation during increased cognitive demand (Vogan, Morgan, Powell, Smith, & Taylor, 2016).

A meta-analysis of functional neuroimaging data which examined activation associated with updating, switching and inhibition tasks reported that children aged 6-12 years were found to demonstrate no unique activation for updating tasks. Importantly, one of the conclusions drawn from the meta-analysis suggests that in the context of neural activation, updating and switching processes may be indistinguishable in children unlike in adolescence (McKenna, Rushe, & Woodcock, 2017). Indeed, executive function processes do follow different developmental trajectories and this notion of unity (indistinguishable) and diversity (distinguishable) is supported by the varying degree to which executive function unites or separates as a function of age (Best & Miller, 2010).

Improvements in episodic memory performance have been linked to different hippocampal connectivity patterns in 4 and 6 year olds, with 6 year olds having greater connectivity within the anterior/posterior hippocampal memory networks, which has been related to better episodic memory performance. In contrast, 4 year olds recruit more regions outside of the hippocampal memory network. Together, these findings suggest specialisation of hippocampal connectivity in which there is functional integration (Riggins, Geng, Blankenship, & Redcay, 2016). In another study considering developmental differences in white matter connectivity and episodic memory in children of the same age range, immediate recall and delayed recognition performance was predicted by white matter connectivity between the left hippocampus and the inferior parietal lobule. However, recall of contextual details was predicted by white matter

connectivity between the right hippocampus and the inferior parietal lobule (Ngo et al., 2017). Moreover, in a sample of 6 - 14 year olds and young adults, hippocampus maturity was related to better orthogonalization of highly similar representations on a Mnemonic Similarity Task (Keresztes et al., 2017).

4.2.4. Interim summary: Continuation of brain development during childhood

Brain imaging techniques and measures of brain activity have shown ongoing developmental changes throughout childhood. Specific regions of the brain show different developmental patterns, in which those associated with lower-order cognitive functions develop first. A reduction in grey matter reflects an increase in white matter connectivity enabling more focal cortical activation. Alongside these developmental changes, there is a sophistication of cognitive function demonstrated by improvements in performance on measures assessing visuospatial skills, executive functions and memory.

4.3 Nutrition and cognition in childhood

Nutrition plays a crucial role in brain development (van de Rest et al., 2012), learning and cognitive function of children (Frensham, Bryan, & Parletta, 2012). Optimal nutrition is crucial for the brain both in respect of healthy development and function (Bourre, 2006; Dani, Burrill, & Demmig-Adams, 2005; Singh, 2004). Indeed, prospective longitudinal studies have proposed that poor nutrition influences cognition and neurodevelopment (Liu, Raine, Venables, Dalais, & Mednick, 2003; Martins et al., 2011). Nutrients are available from bioactive natural compounds found in foods but also from fortified foods and dietary supplements that serve to enhance nutrient intake (Elliott & Ong, 2002). There is an extensive body of literature documenting micronutrient supplementation studies in children that consider the effects on cognitive performance of a range of micronutrients in healthy and unhealthy samples, and of different micronutrient doses and durations of administration (reviewed in Bellisle, 2004; Benton, 2010; Eilander et al., 2010; Lam & Lawlis, 2017; Spencer, Korosi, Layé, Shukitt-Hale, & Barrientos, 2017).

Omega-3 FAs are bioactive lipids, the benefits of which on cognitive function following supplementation has been studied and reviewed widely across the life span, particularly from marine sources. As discussed in Chapter 2, FAs are attached to GPLs at the *sn*-1 and *sn*-2 position of the glycerol backbone and the assimilation of DHA and EPA into membrane PLs affects cellular membrane composition, membrane fluidity and membrane raft formation (see Chapter 2). Moreover, the incorporation of DHA and EPA into the lipid membrane promotes pro-resolving

and anti-inflammatory properties (see sections 2.7.6.2.2.1 and 2.7.6.2.2.2). DHA is a principal omega-3 PUFA in the brain, contributing over 40% of total brain PUFA (Lacombe et al., 2018). High concentrations of DHA in neural tissues are associated with high levels of PS (Kim, 2011). Enrichment of DHA in neurons can promote efficient biosynthesis and accumulation of PS (Guo, Stockert, Akbar, & Kim, 2007). There is also evidence that PS and DHA have a synergistic effect in the promotion of cognitive function (Zhang et al., 2019). Although, as stated previously (see sections 2.7.6.2 and 3.5.1), DHA and EPA are known to support cognitive function separately also, for example, DHA regulates intracellular signalling, gene expression, myelination, neurogenesis, neurotransmission, synaptic plasticity, membrane receptor function (Calder, 2012; Kuratko, Barrett, Nelson, & Salem, 2013; Prado & Dewey, 2014; Weiser et al., 2016) and acts on ion channels (Elinder & Liin, 2017). Moreover, sufficient levels of DHA in neural membranes have been found to be critical for cortical glucose uptake (Pifferi et al., 2005; Ximenes da Silva et al., 2002). In the absence of GPL supplementation studies investigating the effects of GPL supplementation on cognitive performance in children, this next section will consider DHA and EPA supplementation studies that have been conducted in school-aged children, which are of interest.

4.3.1 Effects of nutritional intervention on cognitive performance in school-aged children: DHA and EPA

Numerous studies have considered the effect of omega-3 FAs, particularly DHA and EPA, on cognition in school-aged children; supplementing diet with varying doses, over different periods of time, in different populations (reviewed in Huffman, Harika, Eilander, & Osendarp, 2011; Kirby & Derbyshire, 2018; Kuratko, Barrett, Nelson, & Salem, 2013; Osendarp, 2011; Rangel-Huerta & Gil, 2018; Ryan et al., 2010; van de Rest et al., 2012). This interest arises from the known effects of omega-3 FAs in the brain, their prevalence in the brain, and the inadequacy of intake particularly in those consuming a western style diet. As discussed, DHA is quantitatively the most important omega-3 FA in the brain (Dyall, 2015). Dietary intake tends to be inadequate for children and adults (Nyaradi et al., 2013). A review of dietary intake of PUFAs in European countries reported intake to be inadequate when considered against the European Food Safety Authority (EFSA) recommendations. This was particularly pronounced for EPA and DHA in children aged 4 - 9 years, although there was large heterogeneity in intake assessment methods and data presentation across the included studies (Sioen et al., 2017). Earlier evidence suggests that this is a common finding. A systematic analysis of 226 country-specific nutrition surveys addressing consumption levels of dietary fats and oils between 1990 and 2010 reported increases in marine omega-3 were seen in countries that already demonstrated relatively high

consumption (e.g. Southeast Asia), and higher amounts tended to be consumed by older ages (Micha et al., 2014). Importantly, dietary intake data from local and national surveys has led to the suggestion that children who do not live close to a marine environment have diets low in DHA and EPA (Ryan et al., 2010). This is particularly problematic given that DHA and EPA are not efficiently synthesised in humans, rather they are more effectively obtained directly from diet (marine sources) or supplements (van de Rest et al., 2012).

Table 4.1 presents a summary of RCTs that have supplemented diet with marine-omega-3 FAs (DHA and EPA) in school-aged children to explore whether there is any benefit for cognitive performance. Only studies that recruited and supplemented the diet of school-aged children (4-12 years; Cooper, Tye, Kuntsi, Vassos, & Asherson, 2015) without cognitive impairment, and assessed cognitive performance objectively were included. Studies that supplemented marine-omega-3 FAs in combination with micronutrients (where there was no separate omega-3 condition) were excluded. Follow-up studies of prenatal or infant interventions were also not considered. All studies meeting these eligibility criteria were RCTs. All children that participated in the trials were cognitively healthy, however, the DOLAB studies (I and II) recruited children underperforming in reading – DOLAB I: ≤ 33rd centile (Richardson, Burton, Sewell, Spreckelsen, & Montgomery, 2012); DOLAB II: < 20th centile (Montgomery, Spreckelsen, Burton, Burton, & Richardson, 2018); these being the target populations. Measures other than those that assessed cognitive performance, for example, behavioural questionnaires (Montgomery et al., 2018; Richardson et al., 2012), are not reported. Notably, one study assessed changes in functional activation whilst also measuring performance on a continuous performance task (McNamara et al., 2010), whilst another included a measure of academic performance, reflecting the average score in six subjects (Portillo-Reyes, Pérez-García, Loya-Méndez, & Puente, 2014). Where not obvious, a statement concerning the cognitive domain assessed by each measure listed within Table 4.1 is provided according to the classification made by the original paper.

The shortest duration of supplementation was 8 weeks (Kennedy et al., 2009; McNamara et al., 2010) and the longest was 12 months in the NEMO study (Osendarp et al., 2007). All trials supplemented DHA, although 4/9 studies supplemented EPA in addition to DHA. However, one of these used a negligible dose (~4mg; Kennedy et al., 2009). The smallest amount supplied was 88mg DHA per day (Osendarp et al., 2007) and the largest was 1200mg DHA per day in a dose-ranging study (McNamara et al., 2010). Children recruited to the trials included healthy (Kennedy et al., 2009; McNamara et al., 2010; Montgomery et al., 2018; Osendarp et al., 2007; Richardson et al., 2012; Ryan & Nelson, 2008), marginally nourished (Osendarp et al., 2007), mild-moderately malnourished (Portillo-Reyes et al., 2014) and children with poor omega-3

intake and iron deficiency (Baumgartner et al., 2012) or poor omega-3 intake, of which 26% were stunted and ~30% were iron deficient (Dalton et al., 2009). The youngest children that participated were 4 years of age (Ryan & Nelson, 2008), whilst the oldest were 12 years of age (Kennedy et al., 2009; Portillo-Reyes et al., 2014). All studies recruited both boys and girls, except one that included boys only (McNamara et al., 2010); and seven out of the nine trials used tablets or capsules to deliver either the active or placebo ingredients, whilst one used a powder dissolved in a fruit-flavoured drink (Osendarp et al., 2007) and another used a spread that was provided on two slices of bread (Dalton et al., 2009).

Table 4.1 Summary of Randomised Control Trials of marine-omega-3 FA supplementation on outcomes of cognitive performance in school-aged children.

Author (year)	Country, setting	Intervention	Sample demographics	Measures	Findings
Baumgartner et al. (2012)	South Africa – 4 primary schools serving low-income rural villages.	Randomised, placebo-controlled, double-blind study. 8.5 month duration - 2-by-2 factorial design. 1. 1 x Iron tablet 50 mg + non-iron placebo tablet per day; 2. 2 x 420 mg DHA + 80 mg EPA oral gelatin-coated fish-oil capsules + non-DHA/EPA placebo capsules (containing medium-chain triglycerides) per day; 3. 1 x Iron (50 mg) tablet + 2 x 420 mg DHA + 80 mg EPA capsules per day; 4. Placebo + placebo per day. Supplemented 4 d/wk during school days. Iron or non-iron placebo tablets were given before 08.00 and swallowed with a 200ml fruit-flavoured beverage containing ~10mg vitamin C per serving. DHA + EPA or non-DHA/EPA placebo capsules were given between 10.30 – 11.00 during midmorning break.	n=321 iron deficient children aged 6 – 11 years of age with a poor iron and ω-3 FA intake – dietary assessment indicated ω-6:ω-3 ratio of ~60:1 randomly allocated. 1. n=81 of which N=70 completed cognitive assessment follow-up. Male:female ratio % - 48:52; age (average ± SD): 8.9 ± 1.4 years. 2. n=81 of which 72 completed cognitive assessment follow-up. Male:female ratio % - 57:43; age: 8.9 ± 1.3 years. 3. n=79 of which N=73 completed cognitive assessment follow-up. Male:female ratio % - 53:47; age: 8.8 ± 1.3 years. 4. n=80 of which N=73 completed cognitive assessment follow-up. Male:female ratio % - 45:55; age: 9.1 ± 1.4 years. No difference in the prevalence of anaemia between boys and girls (nearly 1 in 5 children were anaemic). DHA + EPA supplementation significantly increased relative composition of each in total phospholipid fraction of erythrocyte membranes and reduced AA and ω-6:ω-3 ratio in membranes ($p < .05$).	Tested and baseline and endpoint. a) Kaufman Assessment Battery for children (KABC-II) x 4 subtests - working memory, short-term memory, long-term (delayed) memory and visuospatial copy task; b) Hopkins Verbal Learning Test (HVLT). Measures short-term and recognition memory.	No significant main treatment effects. *Subgroup analysis*: - Anaemic children (at baseline) given DHA + EPA performed significantly worse in the working memory subtest of KABC-II ($p < .05$). - Girls with iron deficiency performed significantly worse following consuming DHA + EPA in the long-term memory subtest of KABC-II ($p < .05$), whereas boys with iron deficiency tended to demonstrate better performance following DHA + EPA ($p = .09$). - Girls recalled significantly more words on HVLT recall 2 after receiving DHA + EPA (or iron alone) relative to those supplemented with iron + DHA + EPA or placebo + placebo ($p > .05$).

Author (year)	Country, setting	Intervention	Sample demographics	Measures	Findings
Dalton et al. (2009)	South Africa – single primary school serving a community with low socioeconomic status and of mixed ancestry (African–European–Malay).	Randomised, placebo-controlled, single-blind study. 6 month duration (104 days). Intervention group: 25 g specially formulated bread spread containing ω-3 PUFA-rich fish flour p/school day. Contained fish flour (42.0% wt/wt), red palm oil fat (41.6% wt/wt), canola oil, lecithin, flavourings, sugar syrup, citric acid, and ascorbic acid per school-day. Control group: 25 g analogous spread with no fish flour (commercial superfine rusk (94% wt/wt) bread flour) p/school-day. Both types of spread were given on two slices of bread. Spread containing ω-3 PUFA-rich fish flour was equivalent to ~892mg of DHA per week.	n=183 7 - 9 year old children randomly allocated. Dietary assessment determined the children had virtually no intake of fatty fish and a very low intake of lean fish. Intervention group: N=91 of which n=77 completed cognitive assessment follow-up. Male:female ratio % - 48:52; age (average ± SD): 8.2 ± 0.7 years. Control group: n=92 of which N=78 completed cognitive assessment follow-up. Male:female ratio % - 45:55; age: 8.1 ± 0.6 years. 26% of sample were stunted and ~30% were iron deficient. Very few were anaemic. Significant increase of DHA and EPA concentrations in plasma PC ($p < .001$), RBC membrane PC ($p < .001$) and RBC membrane PE ($p < 0.005$) fractions in those in the intervention group but not placebo group.	Tested and baseline and endpoint in local language. a) Hopkins Verbal Learning Test (HVLT); b) Reading test- naming / association test; c) Spelling test; Reading and spelling test scores were converted to a standardized score (T-score).	**PP analysis** - Marginally significant effect of treatment for HVLT recall 1 ($p = .063$) and a trend for recall 3 ($p = .075$). A significant treatment effect for HVLT recognition ($p = .019$) and discrimination index scores ($p = .008$). - Marginally significant effect of treatment for the reading test ($p = .065$). - Performance by the intervention group remained the same at pre- and post-intervention on the spelling test, however, performance significantly declined in the control group ($p = .013$). **ITT analysis** - Significant treatment effects for HVLT recognition ($p = .015$) and HVLT discrimination index scores ($p = .005$). - A significant treatment effect was also found for the spelling test ($p = .016$) (see above). RBC PE membrane FA composition: - Associations found between DHA, total ω-3, and ω-6:ω-3 ratio respectively with HVLT total score ($p = .042; .058; .013$), HVLT recognition ($p = .013; .035; .085$)

Author (year)	Country, setting	Intervention	Sample demographics	Measures	Findings
					and HVLT discrimination index scores (p = .015; .027; .007). - Marginal inverse correlation between total ω-6 and spelling test score (p = .055). Neither iron deficiency nor stunting confounded the findings.
Kennedy et al. (2009)	UK, Newcastle-upon-Tyne area – home-based intervention.	Randomised, double-blind, placebo-controlled, dose-ranging pilot study. 8 week duration. 1.1000 mg DHA per day; 2.400 mg DHA per day; 3.Placebo. Parents of the children received 2 x bottles, each containing 100 soft-gel capsules for consumption in the morning and 2 x bottles, each containing 100 soft-gel capsules for consumption in the evening. Capsules in each bottle contained either 500 mg DHASCO-S containing 200 mg DHA + ~4 mg EPA and vegetable oil as the remainder or placebo capsules – 500 mg commercially available vegetable oil + ~15 mg ALA + 250 mg LA. Capsule consumption was under parental supervision where 2 x capsules were consumed in the morning and 3 x capsules were consumed in the evening.	n=90 10 – 12 year old children (healthy as per self- and parental-ratings) randomly allocated. 1. n=30 (male: 15); age (average ± SD): 10.70 ± 0.79 years; 2. n=28 (male: 17); age: 11.11 ± 0.79 years; 3. n=30 (male: 12); age: 10.87 ± 1.01 years.	Tested and baseline and endpoint. Assessed pre and post-8-week period intervention: pre-breakfast (08.00), +1 hr (09.45) and +3 hrs (11.45) after a standard breakfast. a) Internet battery comprising word presentation, picture presentation, arrow reaction time test, arrow flanker test, paired associate learning, sentence verification, delayed word recognition, delayed picture recognition. This was administered +1 hr post the standard breakfast. b) Cognitive Drug Research (CDR) battery comprising picture presentation; word presentation/immediate word recall; simple reaction time; digit vigilance task: choice reaction time; spatial working memory; numeric working memory; delayed word recall; delayed word	Data capture errors meant n=86 provided a full set of data for inclusion in the analysis of the Internet Battery assessments +1 hr following breakfast pre and post-intervention. a) No significant main treatment effects. b) Significant effect of treatment on the speed of performing the delayed word recognition task (p < .05), whereby, in comparison to placebo, the 400 mg DHA group were significantly faster prior to breakfast (p < .05) and +3 hrs after breakfast (p < .01). The 1000 mg DHA group performed significantly slower prior to breakfast (p < .05). Overall, the pattern of results strongly suggests that this effect was due to chance fluctuations in performance and that the treatments had no consistent or interpretable effect on performance.

Author (year)	Country, setting	Intervention	Sample demographics	Measures	Findings
				recognition; delayed picture recognition. This was administered pre- and +3 hrs post the standard breakfast.	
McNamara et al. (2010)	USA	Randomised, placebo-controlled, double-blind, dose-ranging functional magnetic resonance imaging study. 8 week duration. 1. 1200 mg DHA (algal triglyceride DHASCO) per day; 2. 400 mg DHA (algal triglyceride DHASCO) per day; 3. Placebo (corn oil). All children took 6 x 500 mg capsules daily – 3 with breakfast and 3 with dinner. Each DHA capsule contained ~200 mg DHA and no EPA. Children in the 1200 mg DHA condition received 600 mg twice per day, whilst those in the 400 mg DHA condition received 200 mg twice per day. All children were asked to maintain their normal diet throughout. Mean erythrocyte DHA composition at baseline did not differ significantly between the treatment and placebo groups.	n=38 8 – 10 year old boys (healthy) randomly allocated. 1. n=14 of which n=13 completed; age (average ± SD): 9.5 ± 0.7 years; 2. n=12 of which n=10 completed; age: 9.2 ± 1.0 years; 3. n=12 or which n=10 completed; age 8.8 ± 0.8 years. Erythrocyte DHA composition increased significantly following 8 weeks in 400 mg DHA condition (p < .001), and in 1200 mg DHA condition (p < .001). This was not seen in the placebo condition.	Tested and baseline and endpoint. Assessed functional activation of the dorsolateral prefrontal cortex *during* a sustained attention task: - Identical-pairs continuous performance task – series of 1-digit numbers presented. Respondents required to press a button with their right index finger for all instances where the same number is presented twice sequentially. Outcomes included: - Percentage of correct selections; - Errors of commission; - Discriminability; - Reaction time. The task was alternated with a control task to control for finger movement.	No significant main treatment effect for Identical-pairs continuous performance task. DHA supplementation dose-dependently raised erythrocyte DHA composition, which was positively correlated with functional cortical activation and inversely correlated with reaction time at baseline (p = .01) and endpoint (p = .02) during performance of the task. fMRI summary of findings (all p < .05): - Supplementation either with 1200 mg or 400 mg of DHA for 8 weeks significantly increased functional activation in the dorsolateral prefrontal cortex during performance of an attention task relative to placebo, which could not be attributed to performance differences on the task. - Lower activation was seen in the occipital cortex in the 400mg DHA group and cerebellar cortex in the 1200 mg DHA group relative to placebo. - Greater decrease in activation of the bilateral cerebellum was seen in the 1200 mg DHA group

Author (year)	Country, setting	Intervention	Sample demographics	Measures	Findings
					relative to the 400 mg DHA group.
Montgomery et al. (2018) DOLAB II	UK, across 5 counties proximate to Oxfordshire – 84 x primary schools and academies.	Fixed-dose, randomized (minimization, 30% random element), double-blind, placebo-controlled trial. 16 week duration. Intervention group: 3 x 200 mg DHA (from algal oil) in 500 mg capsules = 600 mg DHA per day. Control group: 3 x 500 mg capsules containing corn/soybean oil per day. Schools received 16 week supply of capsules (labelled with participant's name) and were required to give 3 x capsules once a day at lunchtime. Parents also received 16 week supply and were required to provide these to their children at weekends, during school holidays and any other times when their child was not in school.	n=376 7 - 9 year old children (healthy) randomly allocated. A recalibrated version of the British Ability Scales (New BAS II) and the new BAS 3 (for comparison) were used to assess children's reading ability. Recruited those who were underperforming in reading (< 20th centile) on either measure. Baseline data indicated mean reading performance of the children was 1.3 SD below normal, equating to a reading performance around 27 months below chronological age. Baseline working memory scores indicated children were around 0.8 SD (digits forwards) and 0.7 SD (digits backwards) below population norms. Intervention group: N=187 of which n=185 completed the follow-up. Male:female ratio % - 64.2:35.8; age (average ± SD): 105.6 ± 10.2 months. Control group: n=189 of which N=187 completed the follow-up. Male:female ratio % - 63.2:40.7; age: 105.3 ± 10.1 months. ~20% eligible for free-school meals in each group. Blood DHA levels significantly increased across the 16 weeks in the intervention group relative to the	Tested and baseline and endpoint. Measures of reading ability: a) Word Reading Achievement sub-tests from the New BAS II and BAS 3 – single word reading tests. Measures working memory: a) Digits forward; b) Digits backwards. Measures were age-standardised and measures of working memory and reading ability use T-scores (working memory: $\bar{x}$ 50, SD 10; reading ability $\bar{x}$ 100, SD 15).	Group comparisons carried out for the whole sample, with subgroup analysis on those children whose baseline reading scores ≤ 10th centile (n=231). No significant main effect of treatment for all measures. Post-hoc multivariate regressions reached the same conclusion.

Author (year)	Country, setting	Intervention	Sample demographics	Measures	Findings
			control group, where there was no change ($p < .001$).		
Osendarp et al. (2007) – NEMO study	South Australia - home-based intervention and Indonesia - 6 x primary schools	Randomised, placebo-controlled, double-blind studies. 12 month duration - 2-by-2 factorial design. 1. Micronutrient mix – 10 mg iron, 5 mg zinc, and vitamins – 150 µg folate, 400 µg A, 1 mg B-6, 1.5 µg B-12, and 45 mg C per day; 2. 88 mg DHA + 22 mg EPA per day; 3. Micronutrient mix + 88 mg DHA + 22 mg EPA per day; 4. Placebo. 4 x supplement products were in powder form that were added to a base powder containing 8 g protein, 12 g sugar, 4 g maltodextrin. The supplement powders were dissolved in a 100 ml fruit-flavoured drink (soy 0.6%). Powders and the fruit-flavoured drink were mixed using a plastic shaker with a screw top, which were shaken for ≥ 20 seconds. In Indonesia only, in addition to the drinks, children received 3 x biscuits at the same time providing ~100 kcal as a protein-energy supplement.	6 – 10 year old children either well-nourished (Australia) or marginally nourished (Indonesia) school-aged children. Australian sample (n=396 randomly allocated): 1. n=106 of which n=67 completed cognitive assessment follow-up (male: 36); age (average ± SD): 8.5 ± 1.0 years. 2. n=96 of which n=67 completed cognitive assessment follow-up (male: 37); age: 8.8 ± 1.0 years. 3. n=92 of which n=71 completed cognitive assessment follow-up (male: 43); age: 8.8 ± 0.9 years. 4. n=102 of which n=71 completed cognitive assessment follow-up (male: 43); age: 8.8 ± 0.9 years. Effect of treatment of DHA + EPA on change in plasma EPA, plasma DHA, and total plasma ω-3 FAs ($p < .05$). Indonesian sample (n=384 randomly allocated): 1. n=94 of which n=92 completed cognitive assessment follow-up (male: 52); age: 8.2 ± 0.9 years. 2. n=97 of which n=94 completed cognitive assessment follow-up (male: 45); age: 8.1 ± 1.1 years.	Tested and baseline, midpoint (+6 months) and endpoint. Indonesian sample - English language assessment battery was translated and back-translated into the local language, Bahasa Indonesia, for verification. Some items were changed according to cultural sensitivity. Both samples: a) Digits backwards; b) Visual attention 2 –Measures visual selective attention; c) Coding. Measures visual-motor processing speed and coordination, short-term memory, visual perception, visual scanning, cognitive flexibility, attention; d) Block design. Measures visuospatial problem solving, visual nonverbal reasoning, visual perception and organization; e) Fluency structured and random. Measures executive function; f) Rey Auditory Verbal Learning Test (RAVLT). Measures immediate and long-term verbal memory;	69/384 Indonesian children performed at floor on the RAVLT. The scores of these children were removed from the analysis performed using the data from this measure. To reduce the chances of obtaining a false positive when analysing multiple outcomes, the analysis of the effect of the treatment was conducted on clusters of the individual cognitive tests by means of a factor analysis using oblique rotation – 3 factors emerged for each country: Australian sample:- 1. Fluid intelligence - coding, fluency structured and random, vocabulary, digits backwards, mathematical reasoning and block design. 2. Verbal learning and memory - RAVLT-A3, RAVLT-slope and RAVLT delayed recall. 3. Attention - visual attention 2. Following 6 and 12 months of the intervention, no significant treatment effects were found. Indonesian sample:- 1. Fluid intelligence - design fluency and block design.

Author (year)	Country, setting	Intervention	Sample demographics	Measures	Findings
		Given the lack of detail concerning the placebo, assumed this included the base powder and fruit-flavoured drink only. Supplemented 6 days per week – powdered based drinks consumed.	3. n=98 of which n=94 completed cognitive assessment follow-up (male: 49); age: 8.2 ± 1.1 years. 4. n=95 of which n=88 completed cognitive assessment follow-up (male: 44); age: 8.1 ± 1.1 years. Effect of treatment of DHA + EPA on change in plasma DHA, and total plasma ω-3 FAs ($p < .05$). Children from Southern Australia were in schools of higher socioeconomic status, whilst those from Indonesia were in schools of middle to low socioeconomic status in an urban poor area. No child was severely malnourished or severely anaemic.	g) Vocabulary. Measures acquired knowledge and verbal concept formation; h) Mathematical reasoning.	2. Verbal learning and memory - RAVLT-A3, RAVLT-slope, delayed recall, and vocabulary. 3. Attention and concentration - visual attention, coding, digits backwards, and mathematical reasoning. Following 12 months of the intervention, no significant treatment effects were found.
Portillo-Reyes et al. (2014)	Mexico - 2 schools of low socioeconomic status.	Randomised, placebo-controlled, double-blind study. 3 month duration. Intervention group: 3 x oral gelatin-coated ω-3 capsules (each containing 60 mg of DHA and 90 mg of EPA) per day. Control group: 3 x soybean oil capsules per day. Capsules given by teachers in the early morning and at lunchtime. Capsules given in the afternoon, at weekends and during school holidays were provided by parents.	n=55 8 – 12 year old, mild to moderately malnourished children randomly allocated. Dietary assessment revealed: 8% of the children consumed one portion of fish two or more times a week, 39% one ration a week, 19% a ration every two weeks, and 34% a ration of once a month. Intervention group: n=30 - all completed cognitive assessment follow-up (male: 26); age (average ± SD): 9.37 ± 1.17 years. Control group: n=25 of which N=20 completed cognitive assessment	Tested and baseline and endpoint. Measure of processing speed: a) Symbol search; Measures of visuoperceptual integration: a) Embedding figures test; b) Visual closure; Measures of visuoconstructive integration: a) Block design; b) TMT A; Measure of attention: a) Letter cancellation;	Effect of treatment for symbol search ($p = .006$), embedded figures ($p = .020$), visual closure ($p = .035$). block design ($p = .001$), Stroop colour ($p = .007$), Stroop colour-word ($p = .039$) and matrix reasoning ($p = .045$). No significant effect of treatment for academic performance outcomes.

Author (year)	Country, setting	Intervention	Sample demographics	Measures	Findings
		Supplemented 7 days a week.	follow-up (male: 16); age: 9.08 ± .99 years.	Measures of memory: a) Rey complex figure; b) Word list – immediate free recall and delayed recall; Measures of language: a) Semantic fluency; b) Comprehension instruction; Measures of executive function (EF): a) Matrix reasoning; b) Letter-number sequencing; c) Stroop colour and word test; d) TMT-B. Also assessed academic performance – average score achieved by children in Spanish, Mathematics, History, Geography, Science and Civic Education.	
Richardson et al. (2012) – DOLAB I study	UK, Oxfordshire – 74 x primary schools	Randomised, placebo-controlled, double-blind study. 16 week duration. Intervention group: 3 x 200 mg DHA (from algal oil) in 500 mg capsules = 600 mg DHA per day. Control group: 3 x 500 mg capsules containing corn/soybean oil per day. Schools received 16 week supply of capsules (labelled with participant's name) and were required to give 3 x capsules once	n=362, 7 - 9 year old children (healthy) randomly allocated. Underperforming in reading (≤ 33rd centile - equivalent to reading at around 18 months behind chronological age). Baseline data indicated mean reading performance of the children was 1.5 SD below normal, equating to a reading performance around 18 months below chronological age. Intervention group: n=180 of which n=179 completed the follow-up (male:	Tested and baseline and endpoint. Measure of reading ability: a) Word Reading Achievement sub-tests of the British Ability Scales (BAS II) – single word test of reading ability; Measures working memory: a) Digits forwards; b) Digits backwards. Measures of working memory and reading ability use *T*-scores	Additional planned group comparisons were conducted as follows: - Children whose baseline reading scores ≤ 20th centile (n=224). - Children whose baseline reading scores ≤ 10th centile (n=105). No significant main effect of treatment for reading ability. *Subgroup analysis:* - Significant treatment effect for children whose baseline reading scores ≤ 20th centile (*p* = .041)

Author (year)	Country, setting	Intervention	Sample demographics	Measures	Findings
		a day at lunchtime. Parents also received 16 week supply and were required to provide these to their children at weekends, during school holidays and any other times when their child was not in school.	96); age (average ± SD): 103.7 ± 10.0 months. Control group: n=182 of which n=180 completed the follow-up (male: 96); age: 104.8 ± 10.1 months.	(working memory: x̄ 50, SD 10; reading ability x̄ 100, SD 15).	and for children whose baseline reading scores ≤ 10th centile (p = .011). No significant main effect of treatment for measures of working memory. *Subgroup analysis:* - Marginal significance for better performance by those in the intervention group for digits forward out of those children whose baseline reading scores ≤ 20th centile (p = .069). Based on the gains in reading ability, in children with an initial reading performance ≤ 20th centile, active treatment was associated with an additional 0.8 months mean increase in reading age change scores vs. placebo, while those whose baseline reading scores ≤ 10th centile, the additional reading age gain from treatment was 1.9 months.
Ryan and Nelson (2008)	Multicentre (11 sites) across USA	Randomised, placebo-controlled, double-blind study. 4 month duration. Intervention group: 400 mg DHA (DHASCO-S) as a triglyceride supplied as 2 x 200 mg bubble gum-flavoured softgel chewable capsules per day.	n=202 4.0 – 4.8 year old children (healthy) randomly allocated. **PP analysis** Intervention group: n=77 (male: 39), age (average ± SD): 52.0 ± 2.3 months. Control group: n=86 (male: 43); age: 51.4 ± 2.4 months.	Tested and baseline and endpoint. a) Leiter-R Test of Sustained Attention; b) Peabody Picture Vocabulary Test (PPVT)- listening comprehension and receptive vocabulary;	No significant treatment effects (for either PP or ITT analysis). Regression analysis revealed a statistically significant positive association between level of DHA in capillary whole blood and better performance on PPVT for the ITT group (p = .018). Removal of Hispanic/Latino participants from

Author (year)	Country, setting	Intervention	Sample demographics	Measures	Findings
		Control group: high-oleic sunflower oil supplied as 2 x softgel capsules per day.	**ITT analysis** Intervention group: n=85 (male: 45); age: 51.9 ± 2.3 months. Control group: n=90 (male: 47); age: 51.6 ± 2.4 months. **DHA blood analysis** Intervention group: n=46 (male: 28); age: 51.7 ± 2.4 months. Control group: n=47 (male: 25); age: 51.3 ± 2.4 months. Mean capillary whole blood content of DHA in those in the intervention group increased by more than 300% (p < .001) post supplementation. This remained low in the control group.	c) Day-Night Stroop Test. Measures executive function; d) Conners' Kiddie Continuous Performance Test (kCPT). Measures attention deficits.	the analysis meant this finding increased in magnitude (ITT analysis, p = .008 / PP analysis, p = .04).

Notes. AA: Arachidonic acid (C20:4); ALA: Alpha-Linolenic acid (C18:3); BAS II: British Ability Scales (BAS II); CDR: Cognitive Drug Research; CPRS-L / CTRS-L: Conners' Rating Scales; DHA: Docosahexaenoic acid (C22:6); EPA: Eicosapentaenoic acid (C20:5); FA: Fatty acid; fMRI: Functional Magnetic Resonance Imaging; HVLT: Hopkins Verbal Learning Test; ITT: intention to treat; KABC-II: Kaufman Assessment Battery for children; kCPT: Conners' Kiddie Continuous Performance Test; LA: Linoleic acid (C18:2); LC-PUFA: Long-chain polyunsaturated fatty acid; ω-3: omega-3 fatty acids; ω-6: omega-6 fatty acids; x̄: mean; PC: phosphatidylcholine; PE: Phosphatidylethanolamine; PP: Per protocol; PPVT: Peabody Picture Vocabulary Test; PUFA: Polyunsaturated fatty acid; RAVLT: Rey Auditory Verbal Learning Test; RBC: Red blood cell.

Collectively, the findings of the studies are inconsistent making it difficult to reliably draw a conclusion regarding the effects of marine-omega-3 FA supplementation on cognitive performance in school-aged children. Comparing the studies is made difficult by the heterogeneity that exists across the nine studies in terms of the populations assessed (including differences in inclusion criteria), dosage, treatment types and combinations, sample size, routes of omega-3 administration, assessment of cognitive performance, study duration, method of assessment of nutrient intake and the presence of confounding variables (Rangel-Huerta & Gil, 2018; van de Rest et al., 2012). Of those to report a benefit (5/9), two studies included healthy children (McNamara et al., 2010; Richardson et al., 2012), whilst three studies recruited children with poor omega-3 intake and iron deficiency (Baumgartner et al., 2012), children who were classed as mild-moderately malnourished (Portillo-Reyes et al., 2014) or children with poor omega-3 intake, a quarter of these being stunted and approximately a third being iron deficient (Dalton et al., 2009). The effect of treatment was most clearly seen across a range of cognitive domains following 3 months of treatment (Portillo-Reyes et al., 2014), yet the quality of this study was poor.

Importantly, as the focus of this Chapter concerns whether cognitive performance of healthy school-aged children can be improved following GPL plus SM supplementation, trials that recruited healthy children are of central interest. In trials with suboptimal nutrient status, there is likely to be a more readily detectable impact following supplementation. However, conversely, the effects of supplementation may not be evident when there is inadequate intake of other nutrients (other than those supplemented, which may be likely to be the case in undernourished samples, since only one deficiency is addressed) (Stonehouse, 2014; van de Rest et al., 2012). In the two studies that recruited healthy children and reported an effect of treatment, participants received 600 mg DHA per day over 16 weeks (DOLAB I; Richardson et al., 2012), or 400 mg (low-dose) or 1200 mg (high-dose) of DHA per day over 8 weeks (McNamara et al., 2010). Reading scores were found to improve significantly in those with a baseline reading score of ≤ 20th centile and separately, ≤ 10th centile, and marginally better performance was seen on a measure of working memory in those whose baseline reading score was ≤ 20th centile (Richardson et al., 2012). Therefore, the significant benefit of treatment on reading ability was only evident in those whose reading performance fell within the lowest 20 per cent of the normal distribution (Richardson et al., 2012). However, in an RCT (DOLAB II) that replicated the original DOLAB I study (same eligibility criteria, dosage and intervention period), no benefit of supplementation was found (Montgomery et al., 2018). It was reasoned that several differences may have contributed to this disparity, including the use of a recalibrated

version of the British Ability Scales II (BAS II) due to the change in the UK National Curriculum, which may be less sensitive to detect change, multiple recruitment issues, and lower omega-3 uptake in blood DHA levels. However, the authors note that there is a lack of clear relationship between blood DHA levels and changes in performance (Montgomery et al., 2018). In the second study, supplementation of DHA (at 400 mg or 1200 mg doses) for 8 weeks did not lead to an improvement on a task of sustained attention, yet functional activation in the prefrontal cortex increased significantly relative to placebo in the absence of differences in performance (McNamara et al., 2010). In addition, lower activation was identified in the occipital cortex following 400 mg DHA supplementation and in the cerebellar cortex after 1200 mg DHA supplementation for 8 weeks (McNamara et al., 2010). It was concluded that DHA regulates functional activity in cortical attention networks, which may be mediated by augmented dopamine receptor-mediated activity, amplification of astrocyte-mediated neurovascular metabolic coupling, neurotrophic effects and/or decreases in central inflammatory signalling cascades (McNamara et al., 2010). Consistent with the lack of improvement in cognitive performance after 8 weeks of taking 400 mg DHA per day, Kennedy and colleagues (2009) concluded that their findings were due to chance (type 1 error), and that DHA supplementation had no significant effect. The remaining trials that recruited healthy children found no treatment effect after receiving 88 mg DHA and 22 mg EPA per day, for 6 days per week over 12 months in a well-nourished Australian sample (Osendarp et al., 2007) or of 400 mg DHA per day over 4 months in an American sample (A. S. Ryan & Nelson, 2008). In spite of the lack of consistent evidence to support an effect of DHA supplementation on cognitive performance, DHA supplementation raised erythrocyte DHA composition in a dose-dependent manner and was positively associated with functional cortical activation. DHA was, however negatively correlated with reaction time on a sustained attention task both at baseline and endpoint (McNamara et al., 2010).

4.3.2 Interim summary: Nutrition and cognition in childhood

Dietary intake data suggests suboptimal intake of omega-3 FAs from marine sources in children. Studies that have considered the role played by these FAs have reported their involvement in signal transduction and intracellular signalling and the facilitative effects they have on neurotransmitter communication and the amelioration of inflammation. Nine RCTs have explored supplementing the diet of school-aged children with preformed DHA; four of these administered DHA in combination with EPA. Of those that recruited healthy children, no consistent benefit of supplementation was found. Across the nine studies, there were a number of methodological limitations including small sample sizes, inappropriate intervention lengths

and doses administered, use of control products with bioactive constituents and multiple measurement issues. Such limitations are likely to compromise the possibility of finding an effect of treatment.

4.4 Aims of Study 1 and related hypothesis

The primary aim of the study presented in this Chapter was to examine whether supplementing the diet of 6 – 8 year old school-children over a period of 6 weeks with a GPL and SM supplement (Lacprodan® PL-20) would improve cognitive performance on measures assessing memory, motor skills, working memory and processing speed (cognitive battery). Based upon the empirical evidence presented in Chapter 2, it was hypothesised that supplementation with Lacprodan® PL-20 would facilitate cognitive function, in turn promoting better performance on the cognitive battery relative to that shown by the control group. It was further hypothesised that age (see section 4.2 for a discussion on brain maturation, this being correlated with sophistication of cognitive function) and IQ (Checa & Fernández-Berrocal, 2015; Haier, 2014) would be positively associated with performance on the cognitive battery. Moreover, females were expected to demonstrate an advantage on a verbal memory measure (Andreano & Cahill, 2009; Asperholm, Nagar, Dekhytar & Herlitz, 2019; Pauls, Petermann & Lepach, 2013) relative to males, whilst males were expected to perform faster on the processing speed measures compared to females (Roivainen, 2011). No further hypotheses could be proposed regarding gender performance differences, either because such differences have not been identified in the previous literature or there are inconsistent findings. Data from the Choice Reaction Time measure of the Cambridge Neuropsychological Test Automated Battery (CANTAB) obtained following an acute study on the effects of breakfast compared with no breakfast in 11 – 13 year olds was used to calculate the sample size (Adolphus et al., under review). A secondary aim was to explore whether Lacprodan® PL-20 supplementation had any effect on subjective evaluations of appetite, mood, motivation and mental alertness, as measured by a Likert scale.

4.5 Methods

4.5.1 Participants

The study sample comprised healthy males and females, aged 6-8 years, from two primary schools in Leeds (UK). Both schools are geographically close (within the same village) and comparable in size and type. Study participants were recruited from a pool of children (n=193) in two academic years across the two schools. Ages 6-8 years correspond to compulsory primary school Years 2 and 3 in the British School System. Year 2 corresponds to Key Stage 1 and Year 3 corresponds to Key Stage 2 in the education system. Following screening, a total of 133 children met the inclusion criteria. This was reduced to 132 due to one child being absent from baseline testing. Of these, 108 (80%) children completed the intervention. A CONSORT diagram showing the flow of participants through each phase of the trial is provided in Figure 4.1.

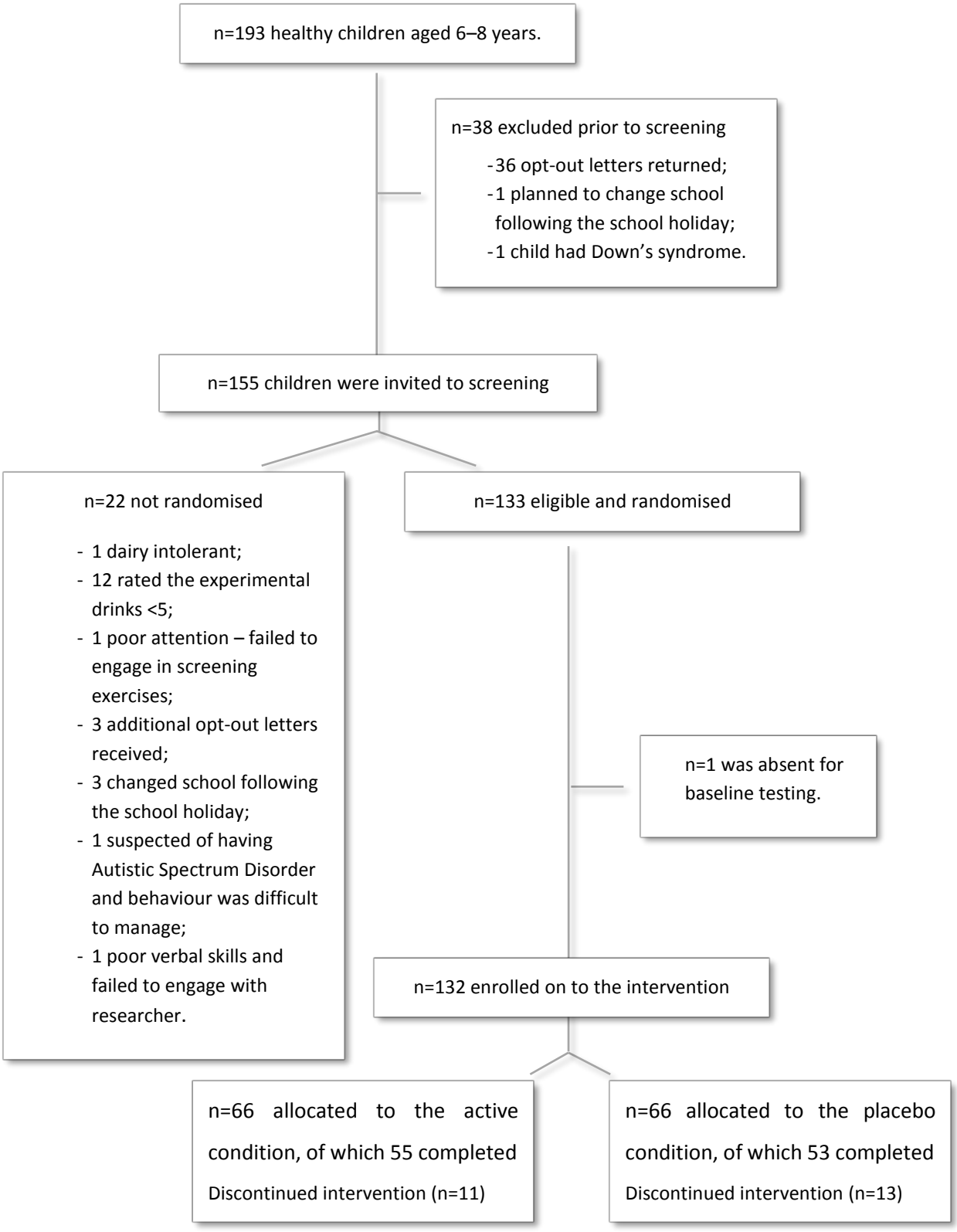


Figure 4.1 CONSORT diagram showing the flow of participants through each phase of the trial (pre-screening, screening, randomisation and intervention).

4.5.1.1 Eligibility criteria

A full list of the study inclusion and exclusion criteria is presented in Table 4.2. A child was enrolled into the study if they met these criteria and if their parent/guardian had provided written consent.

Table 4.2 Inclusion and exclusion criteria for study sample

Inclusion criteria	Exclusion criteria
- Aged 6-8 years; - Rated the experimental drinks >5 on a Likert scale for at least one flavour (out of three). A score of >5 indicated the child found the drink palatable and expressed they were happy to consume it over the intervention period; - Willingness to consume the experimental drinks and participate in all test sessions across the intervention period (6 weeks); - Able to follow verbal and simple written instructions in English; - Normal vision, with appropriate corrective lenses, if required; - Able to understand cognitive testing instructions and responding requirements.	- Poor general health; - Inability to perform or impaired performance on the cognitive measures due to red-green colour vision deficiency. Deficiency tested using a colour perception test (Ishihara, 1951) during screening; - Behavioural difficulties or attention disorders (e.g. Attention Deficit Hyperactivity Disorder); - A learning disability that interferes with the ability to understand written or verbal communication; - Inability to understand the objective(s) of the cognitive measures or carry out the measures; - Any food allergies or intolerances (e.g. lactose intolerance); - Acute illness within the week prior to testing; - Current administration of any psychotropic medication or supplementation in the month prior to testing, or during testing.

4.5.1.2 Recruitment

Opt-out permission letters (Appendix 4) were sent by the participating schools to parents/guardians to gain permission for their child/children to participate. Children were not enrolled into the study in all cases where parents/guardians did not give their permission. Prior to each study cohort commencing the study, open sessions were held at the schools to which all parents/guardians were invited to attend should they want to find out more about the study. Staff at the schools were provided with an information sheet detailing the study requirements and a study timetable.

4.5.2 Experimental design

The study conformed to a randomised, double-blind, placebo-controlled, parallel groups study design. A breakdown of the allocation by condition is provided in the CONSORT diagram (Figure 4.1).

4.5.2.1 Experimental procedure

Allocation to condition using a pre-prepared randomisation list took place prior to screening. This avoided the risk of unblinding a child during screening in the case of any minor differences between the active and placebo drink supplements (colour/taste) when they sampled them. Participants were seen in school, Monday-Friday, 25 minutes before breaktime during their usual school day for supplement consumption. Participants were also seen for screening and cognitive testing sessions in school, which took place alongside participants' usual school routine (between 09.00 – 15.00). Cognitive performance was assessed on three occasions: at baseline (week 0) prior to starting the intervention, mid-intervention (+ 3 weeks/21 days; midpoint) and after consuming the experimental drinks for 6 weeks (+ 6 weeks/42 days; endpoint), as shown in Figure 4.2.

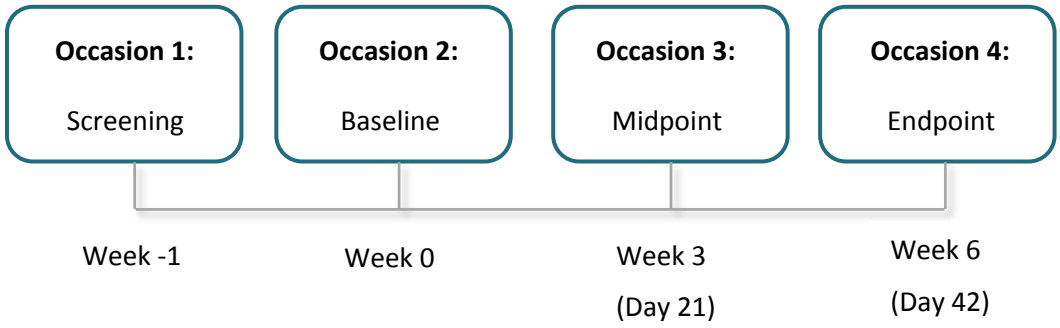


Figure 4.2 Study test schedule

4.5.2.2 Sample size calculation

To estimate the number of participants required to detect a statistically significant difference between conditions, power was calculated based upon the effect observed in Adolphus et al. (under review) for the CANTAB Choice Reaction Time (movement time) outcome measure. The calculation was undertaken by an independent statistician (Quadt Consultancy BV) using SAS (SAS Institute Inc, NC, USA) and indicated that a sample of 58 participants per arm (n=116) would be sufficient to detect a difference of means (mean difference) of 0.05 with an alpha of 0.05 and 80% power.

4.5.2.3 Ethical approval

This study received ethical approval from the Institute of Psychological Sciences Research Ethics Committee (Ref 14-0101) on 17/05/2014. Parents/guardians of the participants were informed as to the nature and aims of the study via opt-out permission letters. Return of opt-out letter slips were collected by the schools and provided to the research team. In all cases where letter slips were not returned, it was assumed that informed consent had been given by the parents/guardians. Ongoing oral assent was obtained from the participants throughout the trial.

4.5.3 Intervention

4.5.3.1 Supplements

Either an active or placebo supplement (isovolumetric drinks) was consumed by participants over 6 weeks:

a) Active: Containing Lacprodan®PL-20 (22.5 g PL-20 within a 250 ml drink);

b) Placebo: Matched for taste and appearance against the active supplement (250 ml).

The ingredients within the active and placebo supplements (%) are provided in Table 4.3 by flavour and a breakdown of the PL constituents of Lacprodan® PL-20 is listed in Table 4.4.

Table 4.3 Composition of active and placebo supplements by flavour (%).

Ingredient	Strawberry active	Raspberry active	Banana active	Strawberry placebo	Raspberry placebo	Banana placebo
Lacprodan® PL-20	9	9	9	0	0	0
Skim Milk Powder	0	0	0	13,2	13,2	13,2
Butter oil	0	0	0	2,4	2,4	2,4
Lactose	6,1	6,1	6,1	0	0	0
Sucrose	1,1	1,1	1,1	1,1	1,1	1,1
Sucralose	0,02	0,02	0,02	0,02	0,02	0,02
Stabilizer	0,25	0,25	0,25	0,25	0,25	0,25
Potassium chloride	0,17	0	0	0,17	0,17	0,17
Flavour - Strawberry	0,25	0	0	0,25	0	0
Flavour - Vanilla	0,1	0,1	0	0,1	0,1	0
Flavour-Raspberry	0	0,22	0	0	0,22	0
Flavour-BananaCream	0	0	0,2	0	0	0,2
Water	83	83	83,1	82,5	82,5	82,7

Table 4.4 Composition of molecular PL species in Lacprodan® PL-20 (Sokol et al., 2015)

Lipid classes	Molar abundance (mol%)
Phosphatidylethanolamine	35
Phosphatidylcholine	18
Phosphatidylserine	9
Phosphatidylinositol	5
Sphingomyelin	8

Experimental drinks were available in three flavours - banana, strawberry or raspberry - and contained in TetraBrik® cartons. Only the colour and fruit character appearing on the front of each carton (to identify flavour) and a condition code differed between cartons. Experimental drinks were stored in a refrigerator onsite. Experimental drinks were consumed in a controlled manner in the classroom under the supervision of the research team and teachers.

4.5.3.2 Assessment of compliance

The weight of each experimental drink carton was recorded prior to and following consumption to determine how much of the supplement had been consumed on each occasion. Names of the intended recipients were written on each carton prior to weighing and drink dissemination was monitored to ensure the correct participant received the correct drink. Experimental drinks were not consumed at weekends or during holidays.

The inclusion of a participant in the PP analyses was determined by their consumption of the experimental drinks across the intervention period. Consumption across this period had to meet a recommended threshold (~85% of the prescribed dose and taking into account the regularity of consumption). Consumption by each participant was plotted and subsequently inspected by the research team and Arla Food Ingredients P/S, Denmark, in order to decide which participants could be included in the PP analysis (n=70) based on the amount consumed and pattern of intake.

4.5.3.3 Known drug reactions and interaction with other therapies

An expert panel approved Lacprodan®PL-20 as part of nutrition bars and milk-based nutritional beverages as safe, suitable and GRAS in July 2014. A copy of this is provided in Appendix 5. As both supplements contain ingredients available from / used in regular food products only, it is not anticipated that the supplements will interact with other therapies or lead to a drug reaction.

4.5.3.4 Study restrictions

Participants were asked to maintain their usual diet throughout the 6 week study period.

4.5.3.5 Adverse events

Participants were asked to inform a teacher or any member of the research team if they felt unwell at the time of or following consuming experimental drinks. On such occasions, an adverse event form was required to be completed. Any adverse event was to be discussed with Arla Food Ingredients P/S, Denmark, enabling a decision to be made as to whether the situation should be monitored or whether the participant should be withdrawn from the study.

4.5.3.6 Blinding

Parents, teachers, participants and researchers were blind to condition allocation (active/placebo) throughout the study and data analysis. Both experimental drinks were matched for appearance and taste as far as possible.

4.5.4 Screening

Each screening session lasted ~45 minutes and took place in a quiet environment (school library). During screening, children tried a sample of the experimental drink in each flavour (either the active or placebo supplement pre-determined by condition allocation), rated each one for palatability (out of 10), and undertook screening measures (see section 4.5.4.1). Following completing the screening measures, children competed a 10-point Likert scale to measure subjective evaluation of appetite, mood, motivation and mental alertness (Appendix 6), cognitive measures (practice version; see Table 4.5), followed by a 10-point Likert scale asking recipients to provide cognitive measure evaluation ratings (Appendix 7). Completing a practice version of the cognitive measures allowed each child to become familiar with all test requirements and provided an opportunity to identify any child who failed to comprehend these. All children demonstrated that they were able to understand all test instructions and requirements.

4.5.4.1 Screening measures

4.5.4.1.1 Wechsler Abbreviated Scale of Intelligence (WASI)

Administering the WASI (Wechsler, 1999) enabled the statistical analysis to be adjusted for individual differences in current levels of intellectual functioning in the analyses. This measure covers the age range of 6-89 years and has four subtests, however, two can be used to establish a measure of intellectual functioning, specifically, the vocabulary and matrix reasoning subtests

(Wechsler, 1999). Split-half reliabilities for the subtest are between 0.8-0.9 (Axelrod, 2002). The abbreviated form of WASI has a high reliability coefficient of 0.93 for children (Wechsler, 1999). Factor analysis has corroborated the two-factor model specified in the WASI manual (The Psychological Corporation, 1999) of Verbal Comprehension and Perceptual Organisation, thereby evidencing construct validity (Ryan et al., 2003).

4.5.4.1.2 Ishihara colour perception test

This test screens for 90-95% of congenital red-green colour deficiency. Single and double digits are shown on plates, 1 plate per page. Plates 1-25 were administered. For scoring, ≥ 6 errors on plates 2-17 suggest a colour deficiency (HSE, 2005).

4.5.4.1.3 Socio-demographic information

In addition to age, gender, and ethnicity, it was anticipated that eligibility for free school meals would be obtained as a proxy for socioeconomic status. However, school meal status records kept by the schools were inaccurate and therefore not recorded.

4.5.5 Testing procedure

4.5.5.1 Test session

Each experimenter referred to a test schedule on each test occasion. Consequently, as far as possible, participants were tested at roughly the same time of day at baseline, midpoint and endpoint. Any participant absent from school (and therefore not consuming supplement drinks) was tested on return to school providing this was within 3 days of the date of the original planned test session. Participants were tested in a quiet environment (school library). At the start of each test session, participants completed the 10-point Likert scale assessing subjective state (Appendix 6), then the cognitive measures (see Table 4.5), followed by the 10-point Likert scale concerning cognitive measure evaluation (Appendix 7). Four participants were tested at any one time and test sessions took ~25 minutes. Test session duration varied owing to response latencies and the number of attempts participants took at a set number of trials (by stage) for one of the measures. All teaching staff were aware of when test sessions were taking place and school timetables were altered, as far as possible, to accommodate where necessary, however, few disruptions were caused. All researchers followed a standardised instruction sheet, ensuring consistency in the administration of the measures. Figure 4.3 provides a flow diagram of the study procedure from screening to endpoint.

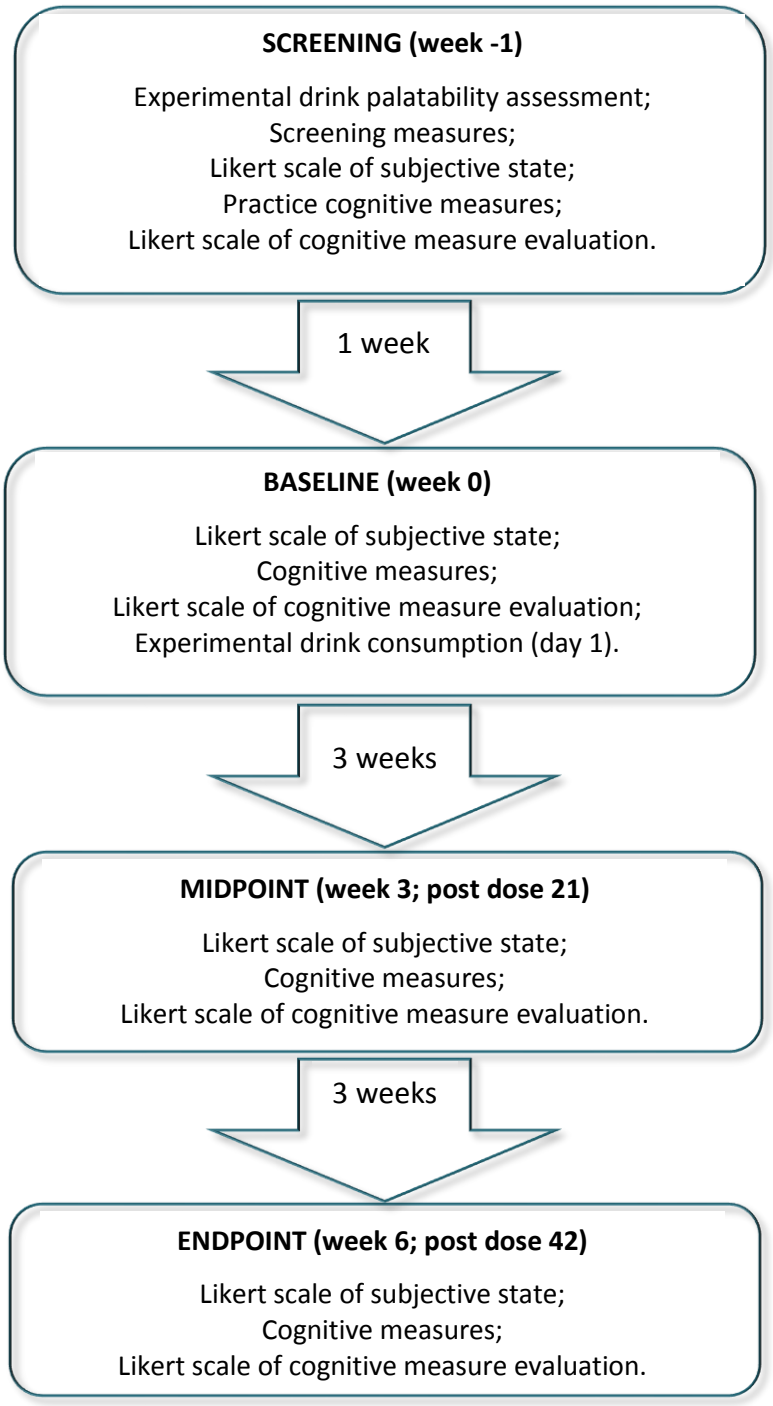


Figure 4.3 Study protocol flow diagram

4.5.5.1.1 Self-report measures completed during test sessions

4.5.5.1.1.1 Self-report measure of subjective state

Prior to the administration of the cognitive measures, participants completed a 10-point Likert scale to assess subjective evaluation of appetite, mood, motivation and mental alertness. Eight subjective states were measured including hunger, cheerfulness, bad temperedness, energy levels, keenness to try hard (on the cognitive measures), ease of distraction, ease of focusing and wakefulness.

Originally it was planned to utilise a visual analogue scale (VAS) i.e. a 10 cm line anchored at both ends with opposing descriptors defining the bounds of each subjective state being measured e.g. hunger. Respondents were required to place a vertical line against each scale to indicate the level of intensity they felt. However, after trialling this in a pilot study with similarly aged children (6-8 years), it was determined that the children had trouble in deciding where to place the line against each scale according to how they felt. Following this, 10-star shapes, as an alternative to a horizontal line were trialled, requiring recipients to colour in the appropriate number of stars to represent how they felt according to the scale. This alteration in measurement ensured that respondents had no difficulty in completing the scales.

4.5.5.1.1.2 Cognitive measure evaluation

Following the administration of the cognitive measures, a 10-point Likert scale was administered to obtain ratings evaluating the cognitive tests. Participants were asked about their perceptions concerning test battery difficulty, how much they had concentrated during the test battery, how they felt they had performed and how frustrated they had felt whilst completing the cognitive measures.

4.5.5.1.2 Assessment of cognitive performance

The order and nature of the cognitive measures that were administered along with respective completion times, the cognitive domains assessed, and outcome variables generated are listed in Table 4.5.

Table 4.5 Cognitive measures presentation during test sessions

Cognitive measure	Measure duration (minutes)	Cognitive domain(s) assessed	Outcome variables
Rivermead Behavioural Memory Test for Children: story subtest (RBMT-C)	3	Immediate verbal memory	Number of details correctly recalled out of 31.
CANTAB: Motor Screening (MOT)	2[a]	Motor skills	Distance from the cross; reaction time.
CANTAB: Spatial Recognition Memory (SRM)	5[a]	Visuospatial recognition memory	Number of correct trials; reaction time.
CANTAB: Spatial Span (SSP)	5[a]	Working memory capacity (executive function)	Highest span achieved; number of correct trials; reaction time (correct trials).
CANTAB: Simple and Choice Reaction Time (SRT/CRT)	3[a]	Processing speed	Number of correct trials; reaction time and movement time (correct trials).
Rivermead Behavioural Memory Test for Children: story subtest	1	Delayed verbal memory	Number of correctly answered story-based questions out of 10.

Note. CANTAB: Cambridge Neuropsychological Test Automated Battery.
[a]Reported by Cambridge Cognition (2006).

4.5.5.1.2.1 Rivermead Behavioural Memory Test for children (RBMT-C)

The Rivermead Behavioural Memory Test for Children (RBMT-C; Wilson, Adrich, & Ivani-Chalian, 1991) assesses memory capacity (in children) by attempting to simulate everyday situations (Anderson, Catroppa, Morse, & Haritou, 1999). The measure is suitable for use with children aged 5-10 years and includes 12 separate subtests that can be combined to give an overall numeric memory score or administered and scored individually. This study used the story subtest to assess immediate and delayed verbal memory. Parallel versions were used for each test occasion and version allocation followed a Latin square design. Performance on this measure is not affected by previous experience of the test (no learning effect) and the correlation coefficients between test and retest have been reported as r=0.44 - 0.73 (Spearman's rank correlation coefficient) across the age groups 5 – 9 year olds, all $p < .05$ (Aldrich & Wilson, 1991).

The story subtest comprises an immediate recall stage and a delayed recall stage. Prior to the administration of this measure, each participant was asked if they were willing to be recorded using audio recording equipment. No participant refused the request. Story cards are available as part of the measure, which depict specific scenes from each story (1 story card per parallel version). A story card was presented to participants during the immediate recall stage, shortly before a story was read aloud by a researcher.

To assess immediate verbal memory, respondents are read a story aloud, which they are asked to listen carefully to. Each story communicates 31 separate details. Immediately following story presentation, respondents are asked to recall the story they have just heard in as much detail as possible. Responses are scored out of a total of 31 using a marking schedule, representing the total number of unique story details recalled. To assess delayed verbal memory, respondents are asked 10 story-based questions successively. Responses for this section are scored out of a total of 10. Any corrections made by participants either during story recall or when answering the 10 questions were accepted.

4.5.5.1.2.2 Cambridge Neuropsychological Test Automated Battery (CANTAB)

CANTAB is a neuropsychological test battery that has been used in numerous research studies in school-aged children without impairment (Evans, 2006; Luciana & Nelson, 1998; Sheppard and & Cheatham, 2013), as well as in those with Down Syndrome (Edgin et al., 2010; Pennington, Moon, Edgin, Stedron, & Nadel, 2003; Visu-Petra, Benga, Ţincaş, & Miclea, 2007), attention deficit hyperactivity disorder (Coghill, Banaschewski, Bliss, Robertson, & Zuddas, 2018; Fried, Hirshfeld-Becker, Petty, Batchelder, & Biederman, 2015), autism spectrum disorder (Corbett, Constantine, Hendren, Rocke, & Ozonoff, 2009) and epilepsy (Palade & Benga, 2007). CANTAB is validated and standardised based on a normative database from healthy populations aged 4-90 years (Cambridge Cognition, 2006) and comprises a suite of neuropsychological tests that assess executive function, memory, attention and social cognition (Cacciamani et al., 2018; Teixeira, Zachi, Roque, Taub, & Ventura, 2011). It is ideal for use with children, as responses are made via touchscreen and there are minimal instructions, so language ability does not confound results (Sheppard and & Cheatham, 2013). Moreover, with many of the measures being error-based, this reduces the risk of floor effects and the availability of parallel versions helps to minimise practice effects (Edgin et al., 2010).

Parallel versions of each measure were employed on each test occasion and version allocation was carried out using a Latin square design. Four nonverbal cognitive measures available from the CANTAB suite were administered using a touch-screen portable computer, which was

acceptable to all participants given their experience with touch-screen items available at home. All responses were automatically recorded during performance. Participants were asked to respond as quickly and as accurately as they could and were able to use their preferred hand for responding.

4.5.5.1.2.2.1 Motor Screening Task (MOT)

Principally, this measure assesses whether sensorimotor or other difficulties of the respondent will impair data collection (Soares et al., 2015). Respondents are required to press a coloured cross following its presentation on screen. There are 10 trials (1 cross per trial). The location of each cross is random. The maximum number of attempts taken to press the cross by some participants in this study was 3. Crosses were not missed consistently on trials by any participant, meaning where multiple attempts to press the cross were taken, this was not due to movement or dexterity problems.

4.5.5.1.2.2.2 Spatial Recognition Memory (SRM)

Five boxes appear on the screen in succession, the location of each is to be remembered. Following five separate box presentations, two boxes appear simultaneously. Of these, one is positioned in a novel location (distractor) and the other is positioned in the same location that was occupied previously by one of five boxes (target). Respondents are required to press the target box. There are 20 trials in four blocks of five. The location of the target boxes is tested in the reverse order to their original presentation order. A review of the neuropsychological, behavioural, and neuroimaging studies concluded that spatial memory is supported by the hippocampus (Burgess, Maguire, & O'Keefe, 2002). This has also been reported in hippocampal lesion studies using rats (Barker & Warburton, 2011; Broadbent, Squire, & Clark, 2004).

4.5.5.1.2.2.3 Spatial Span (SSP)

Nine randomly positioned white boxes are presented on screen and change colour in a sequence that alters for each trial. The position of the nine boxes remain the same throughout the trials. Respondents are required to recall the same sequence by pressing the same boxes that changed colour in the same order. Trials start with 2 boxes changing colour and increase to 9 boxes in a sequence (spans 2-9) depending on performance i.e. better performance results in a higher span being reached. Respondents are allowed three attempts at each span; if all three attempts are failed, the test ends automatically. The sequences presented for the three trials within each span are always different. Essentially this measure assesses the ability to recall a series of discrete stimuli immediately following their presentation and is considered to be a nonverbal

analogue of the Digit Span Test (Teixeira et al., 2011). The measure is based upon the Corsi Block Tapping Test (Cambridge Cognition, 2012), which taps visuospatial working memory (Brunetti, Del Gatto, & Delogu, 2014), requiring a respondent to keep a visuospatial pattern and a movement sequence in mind (Guariglia, 2007). The prefrontal lobe has been implicated in Spatial Span performance in both cognitively healthy participants and patients with frontal lobe damage (Bor, Duncan, Lee, Parr, & Owen, 2006).

4.5.5.1.2.2.4 Simple and Choice Reaction Time (SRT/CRT)

This measure was presented in two parts. Firstly, Simple Reaction Time involves a yellow flash presented inside a circle located in the centre of the screen. Respondents are required to press within the circle as quickly as possible post-onset of the flash. For the final part, Choice Reaction Time, a yellow flash is presented in one of five smaller circles and respondents are required to press within the same circle that the flash was presented in as quickly as possible post-onset. A press-pad is used for this measure and respondents are required to hold a button down (on the press-pad) at all times unless they are responding. The same hand is used to hold the button down and respond with. Each part comprises 15 trials. Reaction time is measured as the time between stimulus onset and release of the button, whilst movement time is the time between release of the button and contact with the screen. Simple and Choice Reaction Time measures are sensitive to processing speed, with the former engaging stimulus detection and response production states and the latter involving additional processing stages concerning stimulus discrimination and response selection (Woods, Wyma, Yund, Herron, & Reed, 2015a, 2015b).

4.6 Statistical approaches common across both studies

Cognitive measure data were extracted and entered in Excel and checked for accuracy. Participant responses from the RBMT-C measure were audio recorded, transcribed using a template and scored according to the marking scheme. Subjective data was also scored. Scores were tallied by participant and week, entered and checked for accuracy in Excel.

The analytical approach for this study and the study of middle-aged and older adults with a subjective memory complaint (SMC) presented in Chapter 5 was reviewed by an independent statistician (Quadt Consultancy BV, personal communication). In the present study, Choice Reaction Time was the primary outcome variable, whilst other cognitive outcomes and subjective measures were secondary outcome variables. A *p*-value of <.05 was considered statistically significant, between .05 and .07 marginally significant, and between >.07 and <.10 as a trend. For all analyses, the significance level was set at $\alpha = 5\%$. All data were analysed using

SAS 9.4 (SAS Institute Inc, NC, USA). Plotted data represent individual observations and means (± standard error; SE) unless otherwise stated.

Baseline participant characteristics were plotted and checked for outliers and skewness and compared using independent groups t-tests or the Mann-Whitney U test for continuous variables and Pearson's Chi-Squared test or Fisher's Exact test (when the expected cell counts were below 5) for frequency data. The assumptions of each test were checked as appropriate.

Cognitive and subjective data were analysed using the SAS® mixed procedure (PROC MIXED). The mixed procedure fits linear mixed models to data to model means, variance and covariance and permits data to show nonconstant variability (SAS Institute Inc. 2008). By computing Restricted Maximum Likelihood (REML) to estimate covariance parameters, the mixed procedure can accommodate data that is missing at random (Rubin, 1976). This method of estimation is preferred especially with unbalanced data (Yang, 2010). Within the model, condition (active or placebo) was a fixed between-subjects factor and week (midpoint and endpoint) was a fixed within-subjects factor. As age (Deary et al., 2009a; Murman, 2015; Nouchi & Kawashima, 2014; Pangelinan et al., 2011), gender (Maitland, Intrieri, Schaie, & Willis, 2000; Palejwala & Fine, 2015; Pangelinan et al., 2011; Upadhayay & Guragain, 2014; Weiss, Kemmler, Deisenhammer, Fleischhacker, & Delazer, 2003) and IQ (Diaz-Asper et al., 2004; Mohn et al., 2014) all correlate with cognitive function, these were controlled for in the analysis of the cognitive data. Baseline cognitive performance was also included as a covariate within the same analyses. For tests where the number of correct trials achieved exceeded performance at baseline, a corresponding baseline was not available to use as a varying covariate. Therefore, the average of the available baseline trials (i.e. RT) was used. Adjusting for baseline performance is preferred to analysis of change from baseline (Senn, 2014; Vickers & Altman, 2001). Importantly, even when there is no significant difference between experimental groups (due to randomisation), adjusting the analysis by including baseline as a covariate can reduce the within-group variance thereby improving the accuracy of the estimates and the power of the statistical test (Tabachnick & Fidell, 2013). Importantly, variance explained by the component being controlled for is removed from the error variance when estimating the difference in the outcome between treatment conditions (Fleiss, 1999). Trial also featured as a covariate in analyses where time (reaction time / movement time) or distance was the outcome of interest following independent statistical advice (Quadt Consultancy BV, personal communication). The total number of attempts at each span to the highest span achieved and the highest span achieved (both inclusive) on each test occasion for the SSP measure was also entered into the model as a covariate for all outcome measures of SSP where this led to an improvement in model fit.

All cognitive and subjective data were plotted, and skewness and kurtosis were checked. Extreme negative skew was corrected by reflecting the data. Transformations (logarithmic or square root) were applied to normalise the distribution of residuals. Data was modelled with and without outliers to explore whether exclusion resulted in a better model. Data were considered outliers where studentized residuals exceeded ± 3.0. On all occasions, removal of outliers led to an improvement in model fit. Specifically, an improvement in model fit was determined by the corrected Akaike Information Criterion (AIC_C; Hurvich & Tsai, 1989), which indicates the amount of remaining unexplained variance after a model has been fitted. Model fitting is an iterative process, with a smaller AIC_C value indicating an improvement in the fit of a model (smaller-is-better; SAS Institute Inc. 2008). The AIC_C protects against overfitting (Quadt Consultancy BV, personal communication). For the initial model for each outcome, all main effects, covariates and interactions were requested, following which, terms with a *p* value ≥.10 were removed one at a time, starting with the higher order interactions to improve model fit. On each occasion of model generation, the AIC_C, *F* values and corresponding significance of the main effects, covariates and interactions were inspected. After all higher order interactions that did not contribute to the variance explained by the model had been removed, two-way interactions were then examined and removed accordingly and so on. The order of removal of each term was determined by its *F* and significance value: the term with the smallest *F* value and therefore the highest *p* value was removed first. If the removal of a term led to a poorer fit (higher AIC_C value), it was added back into the model. For each model, the AIC_C, table of fixed effects and residual plots were inspected (to identify deviations from normality), and final models were chosen based on 'best fit' i.e. the model that explained the greatest proportion of variance.

F values and corresponding significance values for main effects, covariates and interactions that were retained in the final model and therefore contributed to the variance explained by that model for each outcome variable are presented in the respective Chapters or corresponding appendices 8-28. Significant covariates were plotted to determine direction of relationship with the dependent variable. The main effect of condition and interactions featuring condition where *p* <.10 are plotted. Bar charts present main effects of condition and trial, or condition*week interactions, whilst line charts represent gender*condition*week interactions and scatter plots show all other higher order interactions. All *p* values are rounded to three decimal places unless *p* <.001 (reported as *p*<.001). All differences between nominal variables (gender, condition and week) where *p* <.10 are reported by reference to least squares means (± SE). Least squares means (LS-means) are adjusted means for unbalanced data and covariates (SAS Institute Inc.

2014). Trial was also included in a model as a nominal variable for outcomes where this led to an improvement in the model (Quadt Consultancy BV, personal communication). The Tukey method was employed for multiple comparison adjustment of LS-means differences. Heterogeneity of regression slopes was identified by the presence of significant baseline*condition, baseline*week and baseline*condition*week interactions. In such cases, adjusted (Tukey method) LS-means differences were reported (at different levels of the corresponding covariate for higher order interactions), as these give the most appropriate test of the effect in question for the entire sample (Quadt Consultancy BV, personal communication). Only meaningful comparisons between main effects and a covariate i.e. differences within a condition at midpoint and endpoint or differences between conditions at either midpoint or endpoint are reported.

4.7 Results

4.7.1 Participant characteristics

The characteristics of the 70 school-aged children that met the experimental drink consumption threshold (per protocol analysis) across the 6 weeks are presented in Table 4.6.

Table 4.6 Participant characteristics of those included in the analyses from both conditions

	Active (n=31) Median (Range) / Mean ± SE	**Placebo (n=39) Median (Range) / Mean ± SE**	**Active vs. placebo condition**
Age (months)	89.0 (*76, 104*)	89.0 (*78, 104*)	U = 598, p = .939
IQ	89.0 *(75, 102)*	90.0 *(79, 114)*	U = 441.5, p = .054
WASI vocabulary	15.71 ± 0.83	19.08 ± 0.95	$t(68) = 2.59$, $p = .012$[a]
WASI matrix reasoning	13.16 ± 1.05	14.67 ± 0.91	$t(68) = 1.09$, $p = .281$[a]
Gender			
M	15 (48%)	21 (54%)	$X^2(1, N = 70) = .21$, $p = .650$
F	16 (52%)	18 (46%)	
Ethnicity			$X^2(2, N = 70) = 1.92$, $p = .693$[b]
WB	30 (97%)	38 (97%)	
Mixed W&BC	1 (3%)		
AOMB		1 (3%)	
Colour deficiency	2 (6%)	1 (3%)	$X^2(1, N = 70) = .64$, $p = .580$[b]

	Active (n=31) Median (Range) / Mean ± SE	Placebo (n=39) Median (Range) / Mean ± SE	Active vs. placebo condition
Status			$X^2(1, N = 70) = .00$, $p = .961$
None	24 (77%)	30 (77%)	
High functioning autism	1 (3%)		
Gifted and Talented - reading and maths		1 (3%)	
Special Educational Need - motor skill impairment		1 (3%)	
Special Educational Need – behaviour		1 (3%)	
On a behaviour plan	1 (3%)		
Intermittent hearing loss		1 (3%)	
Poor gross/fine motor skills	1 (3%)		
Behavioural characteristics identified during testing[c]	4 (13%)	5 (13%)	

Notes. Values in order of appearance: Median (range), Mean ± SE and number of participants (percentages rounded to nearest whole number). AOMB: Any other mixed background, F: Female, M: Male, Mixed W&BC: Mixed White and Black Caribbean, WASI: Wechsler Abbreviated Scale of Intelligence, WB: White British.

[a]Equal variances assumed. [b]Exact test. [c]Apathetic, distractible, reserved, unmotivated.

There were no significant differences between conditions for age or gender. There was a marginally significant difference between the IQ of participants, with those in the placebo condition having a higher IQ score (*Median* = 90) compared to those in the active condition (*Median* = 89), $U = 441.5$, $p = .054$ (Table 4.6). It is also noteworthy that the range of IQ scores is greater in the placebo condition relative to the active condition. Figure 4.4 presents the distribution of IQ by condition and gender and indicates that females in the placebo condition tended to score more highly in respect of current levels of intellectual functioning compared to females in the active condition and males per se.

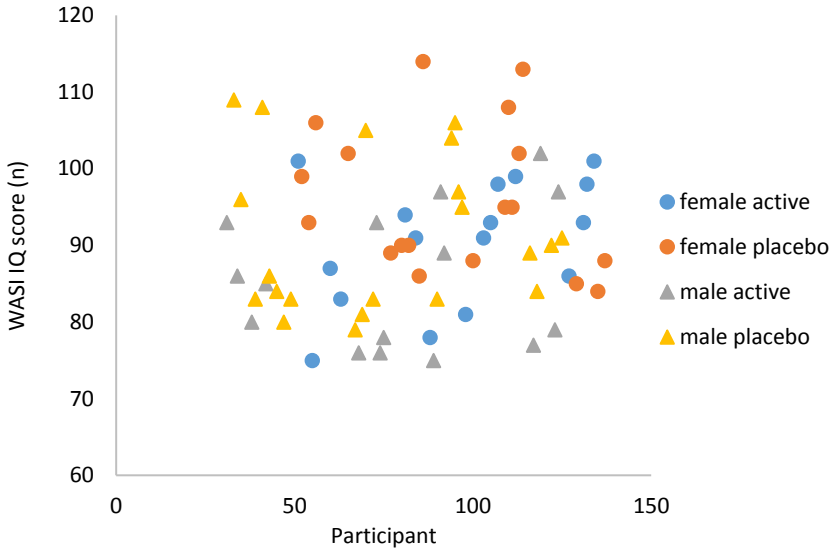


Figure 4.4 WASI IQ score of each participant included in the per protocol analysis by gender and condition.

Further exploration of this difference indicated that this is driven by a significant difference in WASI vocabulary subcomponent scores (Wechsler, 1999), with those in the placebo condition (M = 19.08) scoring significantly higher than those in the active condition (M = 15.71), $t(68)$ = 2.59, p = .012 (Table 4.6). This difference is shown in Figure 4.5, where both males and females in the placebo condition tended to score more highly comparatively to males and females in the active condition.

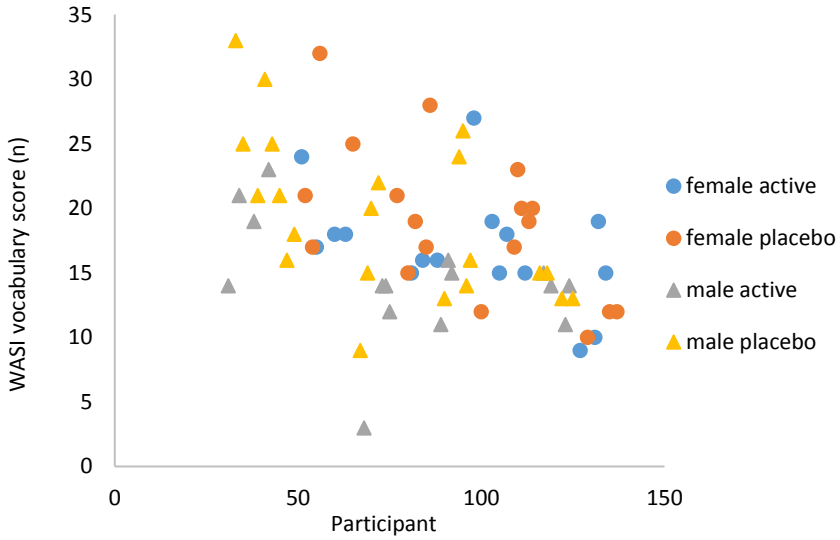


Figure 4.5 WASI vocabulary score of each participant included in the per protocol analysis by gender and condition.

4.7.2 Effect of intervention on cognitive performance on the RBMT-C and CANTAB measures

4.7.2.1 Measures of processing speed

Table 4.7 provides a summary of the means (± SE) across measures of processing speed for each condition.

Table 4.7 Mean (± SE) on measures of processing speed (Simple and Choice Reaction Time) by condition and week

Outcome	Baseline *Week 0* Mean ± SE	Midpoint *Week 3* Mean ± SE	Endpoint *Week 6* Mean ± SE	Active vs. placebo
CANTAB CRT movement time for correct trials (ms)				
Active	416.84 ± 9.64	429.12 ± 10.05	420.88 ± 11.99	*F*(1,65) = 0.54, *p* = .467
Placebo	400.86 ± 7.95	425.91 ± 12.66	421.23 ± 9.70	
CANTAB CRT number of correct trials (n)				
Active	14.03 ± 0.20	14.13 ± 0.16	13.97 ± 0.25	*F*(1,67) = 0.61, *p* = .437
Placebo	14.18 ± 0.15	14.00 ± 0.20	13.72 ± 0.21	
CANTAB CRT reaction time for correct trials (ms)				
Active	494.54 ± 10.49	505.13 ± 10.01	506.65 ± 11.91	*F*(1,65) = 7.25, *p* = .009
Placebo	478.71 ± 8.00	502.28 ± 8.50	491.83 ± 9.29	
CANTAB SRT number of correct trials (n)				
Active	13.52 ± 0.26	13.61 ± 0.27	13.45 ± 0.27	*F*(1,65) = 2.37, *p* = .129
Placebo	13.26 ± 0.24	13.74 ± 0.26	13.41 ± 0.25	
CANTAB SRT reaction time for correct trials (ms)				
Active	485.70 ± 13.98	458.59 ± 8.99	496.18 ± 17.71	*F*(1,65) = 2.20, *p* = .143
Placebo	458.35 ± 10.09	478.71 ± 11.92	478.40 ± 10.04	
CANTAB SRT movement time for correct trials (ms)				
Active	460.67 ± 14.50	476.32 ± 14.49	465.77 ± 16.73	*F*(1,64) = 2.45, *p* = .122
Placebo	496.43 ± 18.58	447.49 ± 16.49	470.03 ± 15.21	

4.7.2.1.1 Choice Reaction Time (CRT)

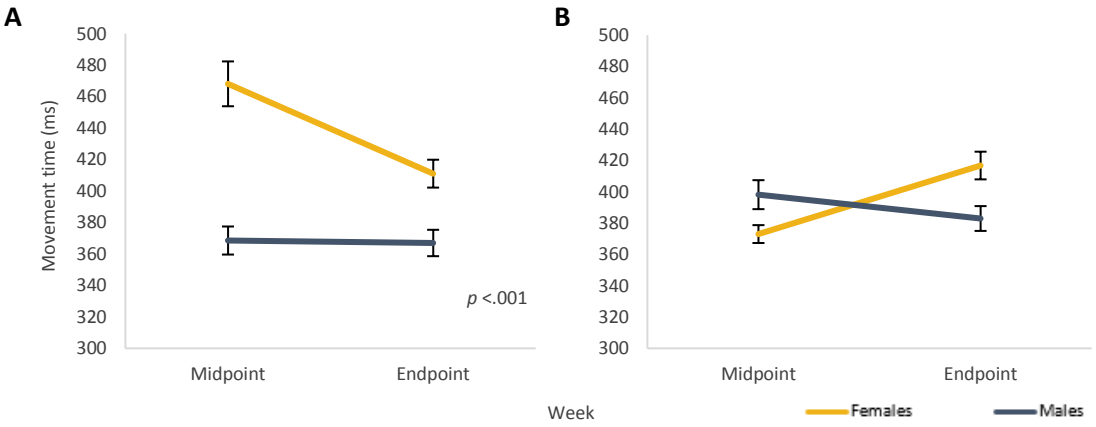
4.7.2.1.1.1 Movement time for correct trials

In the final model, 46 outlying observations were removed to normalise residuals. Baseline movement time was a significant covariate, $F(1,65) = 82.72$, $p = <.001$, with baseline performance being positively correlated with movement time at midpoint and endpoint. Trial was marginally significant, $F(14,922) = 1.70$, $p = .050$, performance over which fluctuated across the 15 trials. Gender was also marginally significant, $F(1,65) = 4.00$, $p = .050$, with females demonstrating slower movement times (2.59 ± 0.01) than males (2.56 ± 0.01). IQ was not a significant covariate, $F(1,65) = 2.40$, $p = .126$. Age was also not significant and removed from the final model. There was no significant main effect of condition, $F(1,65) = 0.54$, $p = .467$, and baseline*condition interaction was found not to be significant and was removed from the final model. There was a significant baseline*condition*week interaction, $F(3,1768) = 2.79$, $p = .039$, however, inspection of post hoc comparisons did not reveal a significant difference. One other higher order interaction was found to be significant, gender*condition*week, $F(3,65) = 8.55$, $p <.001$ (Figure 4.6).

The significant interaction between gender*condition*week is shown in Figure 4.6(A) and 4.6(B). Figure 4.6(A) shows females in the active condition illustrated faster movement times at endpoint compared to midpoint, whereas males performed similarly across both weeks in the same condition. Figure 4.6(B) indicates that females in the placebo condition performed better at midpoint relative to endpoint, but the opposite was shown by males in the same condition. In line with this, inspection of post hoc analysis revealed females in the active condition were significantly slower at midpoint (2.61 ± 0.01) than at endpoint (2.58 ± 0.01; $t(65) = 3.69$, $p = .010$). In addition, females in the placebo condition were significantly faster at midpoint (2.57 ± 0.01) than at endpoint (2.61 ± 0.01; $t(65) = -4.58$, $p <.001$).

A significant interaction between condition*week was also found, $F(1,65) = 9.67$, $p = .003$, shown in Figure 4.6(C). At midpoint, the placebo condition demonstrated superior performance to the active condition, however, this was not maintained at endpoint, as the active condition illustrated faster movement speeds on this test occasion. Inspection of post hoc comparisons revealed those in the active condition at midpoint (2.58 ± 0.01) performed significantly slower than at endpoint (2.56 ± 0.01; $t(65) = 3.07$, $p = .016$).

Interaction between gender*condition*week on Choice Reaction Time (CRT): Movement time for correct trials



Interaction between condition*week on Choice Reaction Time (CRT): Movement time for correct trials

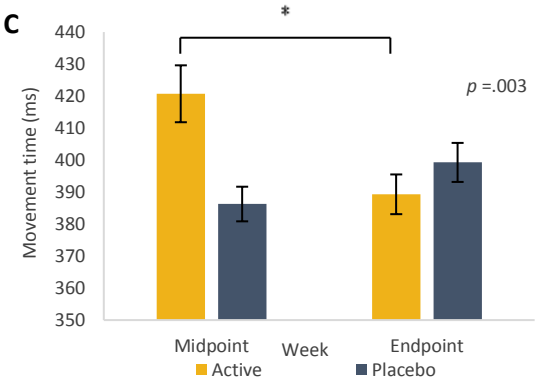


Figure 4.6 Movement time for correct trials on the Choice Reaction Time (CRT). (A-B) The x axis represents week and the y axis is movement time (ms) over midpoint and endpoint. Lines denote the mean. Error bars denote the SE of the mean. (C) The x axis is week and the y axis is movement time (ms) over midpoint and endpoint. Columns denote the mean. Error bars denote the SE of the mean. * p <.05. (A) Is the active condition, (B) is the placebo condition.

4.7.2.1.1.2 Number of correct trials

No outlying observations were removed from the final model. Baseline performance was a significant covariate, $F(1,67) = 8.05$, $p = .006$, with baseline performance positively associated with the number of correct trials at midpoint and endpoint. Age, IQ and gender were not significant covariates and were removed from the final model. There was no significant main effect of condition, $F(1,67) = 0.61$, $p = .437$. Moreover, baseline*condition interaction was not significant and was removed from the final model. Of the higher order interactions, only gender*condition*week was significant, $F(4,63) = 3.16$, $p = .020$.

The significant interaction between gender*condition*week is presented in Figure 4.7(A) and 4.7(B). Figure 4.7(A) indicates that both females and males tended to be more accurate at midpoint relative to endpoint in the active condition. Figure 4.7(B) shows that females performed similarly across both weeks, however, males illustrated superior performance at endpoint compared to midpoint in the placebo condition. Inspection of post hoc comparisons revealed a marginally significant difference between females in the placebo condition at midpoint (0.62 ± 0.05) performing more accurately than males in the same condition at endpoint (0.40 ± 0.05; $t(63) = 3.09$, $p = .0.57$). Also, there was a trend towards males in the placebo condition (0.40 ± 0.05) completing less trials correctly compared to males in the active condition at endpoint (0.63 ± 0.06; $t(63) = -2.97$, $p = .076$).

Interaction between gender*condition*week on Choice Reaction Time (CRT): Number of correct trials

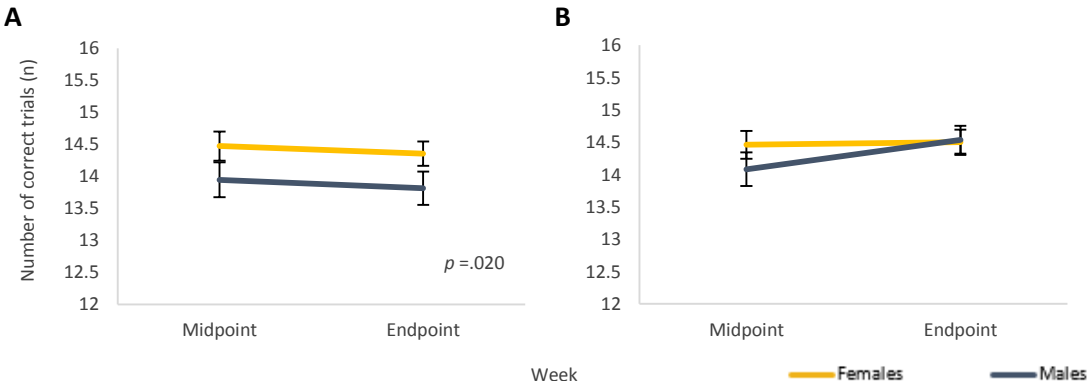


Figure 4.7 Number of correct trials on the Choice Reaction Time (CRT). The x axis represents week and the y axis is the number of correct trials (n) over midpoint and endpoint. Lines denote the mean. Error bars denote the SE of the mean. (A) Is the active condition, (B) is the placebo condition.

4.7.2.1.1.3 Reaction time for correct trials

In the final model, 29 outlying observations were removed to normalise residuals. Baseline reaction time was a significant covariate, $F(1,65) = 89.32$, $p < .001$, with reaction time at baseline positively correlating with performance at subsequent test points. Trial was also found to be a significant covariate $F(14,923) = 6.44$, $p < .001$, performance over which fluctuated across the 15 trials. IQ was not a significant covariate, $F(1,65) = 0.30$, $p = .584$. Age and gender were also not significant covariates and were removed from the final model. Of the higher order interactions, IQ*condition*week and gender*condition*week interactions were found to be significant.

The significant interaction between IQ*condition*week, $F(3,1830) = 4.12$, $p = .006$, is shown in Figure 4.8(A) and 4.8(B). Inspection of post hoc comparisons revealed a marginally significant

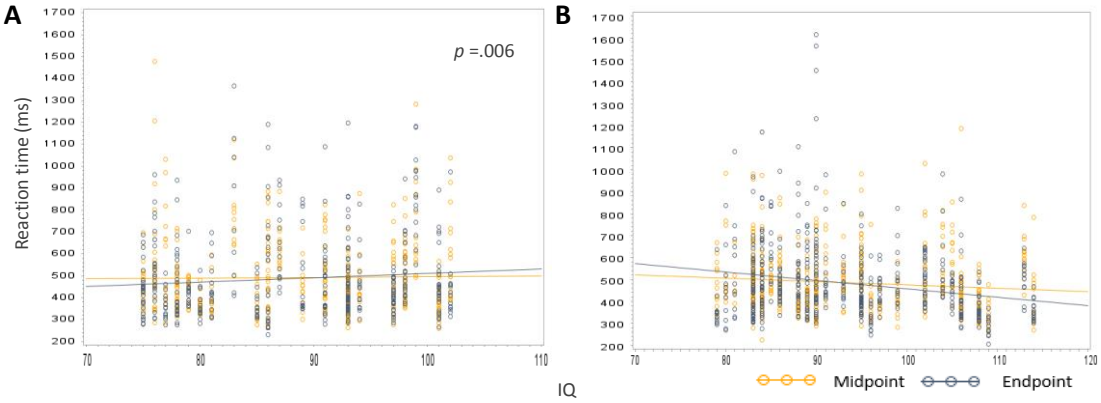
difference at an IQ score of 100 between reaction time shown by those in the placebo condition at midpoint (2.67 ± 0.01) relative to their performance at endpoint (2.65 ± 0.01; $t(64) = 2.63$, $p = .052$), with better performance demonstrated at endpoint. This became significant at an IQ score of 110 (2.66 ± 0.02 vs. 2.62 ± 0.02; $t(64) = 3.23$, $p = .010$).

Figure 4.8(C) and 4.8(D) depict the significant gender*condition*week interaction, $F(4,64) = 7.08$, $p < .001$. In both conditions, females demonstrated faster reaction speeds at midpoint compared to endpoint, whereas males responded more quickly at endpoint relative to midpoint. Post hoc comparisons revealed a marginally significant difference between females in the placebo condition (2.70 ± 0.01) demonstrating slower reaction time to that of males in the same condition at endpoint (2.64 ± 0.01; $t(64) = 3.02$, $p = .067$). Further, there was a marginally significant difference between the performance of males in the placebo condition at midpoint (2.67 ± 0.01) compared to endpoint (2.64 ± 0.01; $t(64) = 3.07$, $p = .059$).

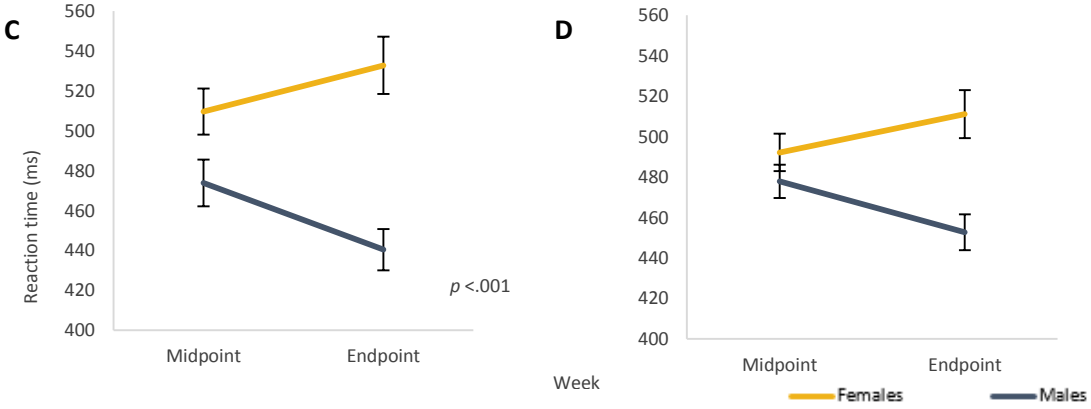
A significant baseline*condition interaction was also found, $F(1,65) = 5.94$, $p = .018$, shown in Figure 4.8(E). Inspection of post hoc comparisons revealed that at a log baseline reaction time of 2.55, those in the placebo condition (2.62 ± 0.01) were significantly slower than those in the active condition (2.58 ± 0.01; $t(65) = 2.03$, $p = .046$). There was a marginally significant difference in performance between the conditions at a log baseline reaction time of 2.80, such that those in the placebo condition (2.73 ± 0.01) performed more quickly than those in the active condition (2.77 ± 0.02; $t(65) = -1.95$, $p = .055$). This became significant at a log baseline reaction time of 2.85 (2.76 ± 0.02 vs. 2.82 ± 0.02; $t(65) = -2.13$, $p = .037$) and greater (slower reaction time).

A significant main effect of condition and a significant condition*week interaction were also found, $F(1,65) = 7.25$, $p = .009$ and $F(2,64) = 4.42$, $p = .016$, respectively. In respect of the main effect of condition, those in the active condition (2.667 ± 0.01) demonstrated better performance compared to those in the placebo condition (2.670 ± 0.01), however, post hoc comparisons did not reveal a significant difference. The condition*week interaction indicated that performance was similar across both conditions over time. Specifically, those in the active condition performed similarly to those in the placebo condition at midpoint (2.67 ± 0.01 vs. 2.67 ± 0.01) and at endpoint (2.66 ± 0.01 vs. 2.67 ± 0.01). Inspection of post hoc comparisons did not reveal a significant difference.

Interaction between IQ*condition*week on Choice Reaction Time (CRT): Reaction time for correct trials



Interaction between gender*condition*week on Choice Reaction Time (CRT): Reaction time for correct trials



Interaction between baseline*condition on Choice Reaction Time (CRT): Reaction time for correct trials

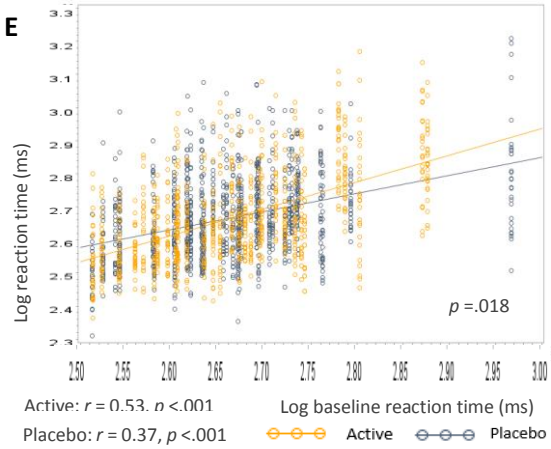


Figure 4.8 Reaction time for correct trials on the Choice Reaction Time (CRT). (A-B) the x axis represents IQ score and the y axis is reaction time (ms) over midpoint and endpoint. Regression lines show relationship between x and y by week. (C-D) the x axis represents week and the y axis is reaction time (ms) over midpoint and endpoint. Lines denote the mean. Error bars denote the SE of the mean. (E) The x axis is the log baseline reaction time (ms) and the y axis is log reaction

time (ms) over subsequent test points. Regression lines show relationship between x and y by condition. (A & C) Is the active condition, (B & D) is the placebo condition.

4.7.2.1.2 Simple Reaction Time (SRT)

4.7.2.1.2.1 Number of correct trials

In the final model, 3 outlying observations were removed to normalise residuals. Number of correct trials at baseline was a significant covariate, $F(1,65) = 9.61$, $p = .003$, with this being positively related to number of correct trials at subsequent test points. There was a trend towards gender as a significant covariate, $F(1,65) = 3.09$, $p = .084$, with females (13.95 ± 0.18) being more accurate than males (13.49 ± 0.19). IQ was not a significant covariate, $F(1,65) = 0.23$, $p = .632$. Age as a covariate and baseline*condition, baseline*week and baseline*condition*week interactions were also nonsignificant and were removed from the final model. There was no significant main effect of condition, $F(1,65) = 2.37$, $p = .129$.

A significant interaction between gender*condition*week was found, $F(3,59) = 5.95$, $p = .001$. Figure 4.9(A) indicates females in the active condition illustrated superior performance at endpoint, however, males in the same condition were more accurate at midpoint compared to endpoint. In the placebo condition, Figure 4.9(B), females performed similarly over both weeks, whereas males again performed better at midpoint. Inspection of post hoc comparisons revealed that males in the active condition were significantly more accurate at midpoint (14.32 ± 0.37) than at endpoint (12.78 ± 0.36; $t(59) = 3.58$, $p = .015$). Furthermore, males in the placebo condition at midpoint (13.96 ± 0.29) were marginally significantly more accurate relative to their performance at endpoint (12.92 ± 0.28; $F(59) = 3.14$, $p = .050$).

There was also a marginally significant IQ*condition*week interaction, $F(3,59) = 2.57$, $p = .063$, and a significant condition*week interaction, $F(1,59) = 5.81$, $p = .019$. Inspection of post hoc comparisons revealed a marginally significant difference between the performance of those in the placebo condition at midpoint (14.16 ± 0.22) compared to endpoint (13.51 ± 0.21; $t(59) = 2.62$, $p = .053$). No other post hoc comparisons were significant.

Interaction between gender*condition*week on Simple Reaction Time (SRT): Number of correct trials

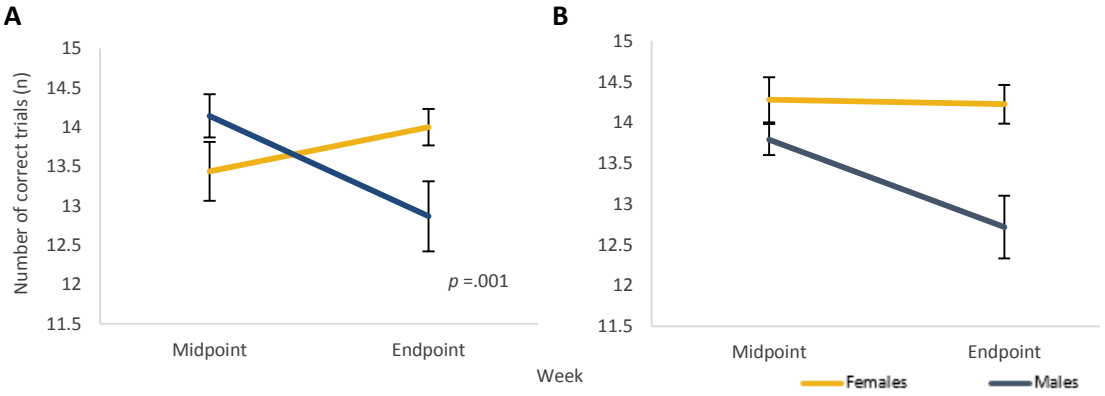


Figure 4.9 Number of correct trials on the Simple Reaction Time (SRT). The x axis represents week and the y axis is the number of correct trials (n) over midpoint and endpoint. Lines denote the mean. Error bars denote the SE of the mean. (A) Is the active condition, (B) is the placebo condition.

4.7.2.1.2.2 Reaction time for correct trials

In the final model, 33 outlying observations were removed to normalise residuals. Baseline reaction time was a significant covariate, $F(1,65) = 108.03$, $p < .001$, with baseline reaction time showing a positive relationship with reaction time at midpoint and endpoint. Trial and gender were also found to be significant covariates, $F(14,900) = 2.49$, $p = .002$ and $F(1,65) = 4.87$, $p = .031$, respectively. Performance fluctuated across trial. Females demonstrated poorer performance (2.65 ± 0.01) compared to that of males (2.62 ± 0.01). IQ was not a significant covariate, $F(1,65) = 0.95$, $p = .334$, and age was also not significant and removed from the final model. There was no significant main effect of condition, $F(1,65) = 2.20$, $p = .143$. Baseline*condition*week and baseline*condition were also found not to be significant and were removed from the final model. There was a significant baseline*week interaction, $F(1,1775) = 5.32$, $p = .021$, and a significant main effect of week, $F(1,65) = 5.25$, $p = .025$, however, post hoc comparisons did not reveal a significant difference

A significant interaction between gender*condition*week was also found, $F(3,65) = 5.67$, $p = .002$, shown in Figure 4.10. Irrespective of condition, females performed better at midpoint whilst males performed better at endpoint. Inspection of post hoc comparisons revealed that females in the placebo condition reacted significantly faster at midpoint (2.63 ± 0.01) than at endpoint (2.66 ± 0.01; $t(65) = -3.18$, $p = .044$). Also, females in the placebo condition (2.66 ± 0.01) reacted significantly slower than males in the active condition at endpoint (2.59 ± 0.01; $t(65) = 3.57$, $p = .015$). There was a marginally significant difference between females in the

active condition (2.65 ± 0.01) responding more slowly than males in the same condition at endpoint (2.59 ± 0.01; $t(65) = 3.06$, $p = .060$).

Interaction between gender*condition*week on Simple Reaction Time (SRT): Reaction time for correct trials

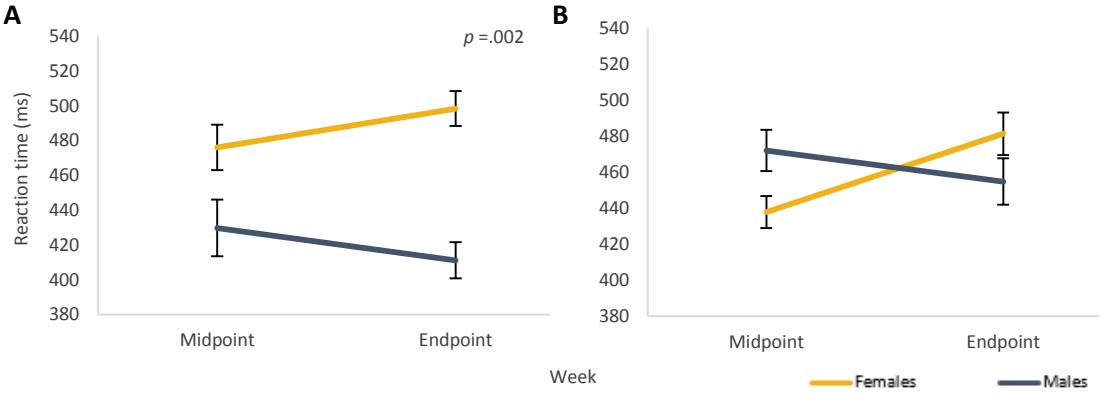


Figure 4.10 Reaction time for correct trials on the Simple Reaction Time (SRT). The x axis represents week and the y axis is reaction time (ms) over midpoint and endpoint. Lines denote the mean. Error bars denote the SE of the mean. (A) Is the active condition, (B) is the placebo condition.

4.7.2.1.2.3 Movement time for correct trials

In the final model, 29 outlying observations were removed to normalise residuals. Baseline movement time was a significant covariate, $F(1,64) = 54.69$, $p < .001$, which showed a positive association with movement time at later test points. Age was marginally significant, $F(1,64) = 3.74$, $p = .058$. IQ was not a significant covariate, $F(1,64) = 0.31$, $p = .582$, and gender and trial were also not significant and removed from the final model. Performance by week was marginally significant, $F(1,64) = 3.54$, $p = .064$, such that movement times were slower at midpoint (2.61 ± 0.01) compared to endpoint (2.59 ± 0.01). There was no significant main effect of condition, $F(1,64) = 2.45$, $p = .122$ and no significant interaction between baseline*condition*week and this was removed from the final model. Of the higher order interactions, the following were found to be significant; trial*condition*week, $F(56,1673) = 1.37$, $p = .038$, IQ*condition*week, $F(3,1731) = 5.19$, $p = .001$, age*condition*week, $F(3,1731) = 3.80$, $p = .010$, and gender*condition*week, $F(4,64) = 2.98$, $p = .025$. Two way interactions including baseline*condition and condition*week were also significant, $F(1,64) = 4.83$, $p = .032$, and $F(1,64) = 8.37$, $p = .005$, respectively.

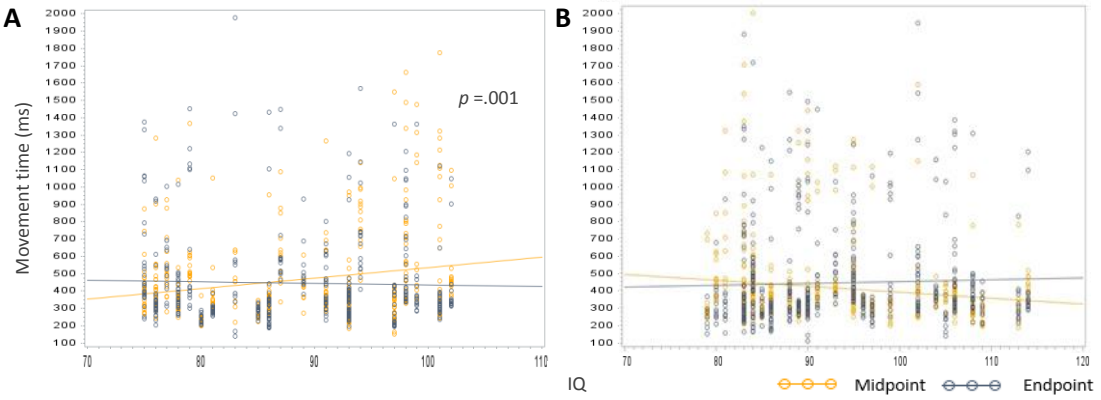
Although performance by trial, condition and week appeared to be similar, post hoc comparisons revealed a marginally significant difference between those in the placebo condition

at midpoint moving slower for trial 13 (2.67 ± 0.03) than at endpoint for trial 10 (2.52 ± 0.03; $t(1673) = 4.03$, $p = .062$). No other post hoc comparisons were significant.

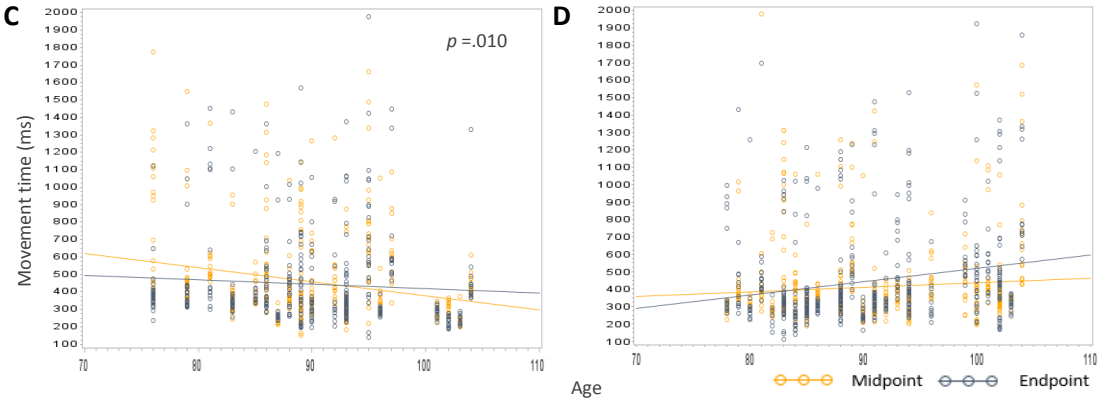
Figure 4.11(A) and 4.11(B) show the significant interaction between IQ*condition*week, $F(3,1731) = 5.19$, $p = .001$. Figure 4.11(A) indicates that at lower IQ scores, those in the active condition demonstrated faster movement time at midpoint, but at higher IQ scores, faster movement time was displayed at endpoint. Participants in the placebo condition, Figure 4.11(B), demonstrated the opposite pattern of performance by week. Inspection of post hoc comparisons revealed at an IQ score of 90 and greater, there was a marginally significant difference at midpoint between those in the placebo condition (2.58 ± 0.01) and those in the active condition (2.63 ± 0.02; $t(64) = -2.52$, $p = .067$), with faster movement time for those in the placebo condition. This became significant at an IQ score of 100 (2.55 ± 0.02 vs. 2.68 ± 0.03; $t(64) = -3.50$, $p = .005$) and 110 (2.53 ± 0.03 vs. 2.72 ± 0.05; $t(64) = -3.29$, $p = .009$). Within condition, at an IQ score of 90 and greater, those in the active condition were significantly slower at midpoint (2.63 ± 0.02) than at endpoint (2.60 ± 0.02; $t(64) = 2.98$, $p = .021$). Whilst, those in the placebo condition were significantly faster at midpoint (2.55 ± 0.02) compared to their performance at endpoint (2.59 ± 0.02; $t(64) = -3.33$, $p = .008$) at an IQ score of 100 and greater.

The significant interaction between age*condition*week, $F(3,1731) = 3.80$, $p = .010$, is shown in Figure 4.11(C) and 4.11(D). Irrespective of condition, younger participants demonstrated slower movement time at midpoint, whereas older participants showed slower movement time at endpoint. Inspection of post hoc comparisons revealed at 90 months of age, participants in the placebo condition (2.58 ± 0.01) were significantly faster than those in the active condition at midpoint (2.64 ± 0.02; $t(64) = -2.83$, $p = .030$). Within condition, those in the active condition were significantly slower at midpoint (2.63 ± 0.03) than at endpoint (2.57 ± 0.03; $t(64) = 3.13$, $p = .014$) at 80 and 90 months of age (2.64 ± 0.02 vs. 2.60 ± 0.02; $t(64) = 3.19$, $p = .012$). Whereas, at 100 months of age, those in the placebo condition were significantly faster at midpoint (2.59 ± 0.02) than at endpoint (2.65 ± 0.02; $t(64) = -3.38$, $p = .007$).

Interaction between IQ*condition*week on Simple Reaction Time (SRT): Movement time for correct trials



Interaction between age*condition*week on Simple Reaction Time (SRT): Movement time for correct trials



Interaction between gender*condition*week on Simple Reaction Time (SRT): Movement time for correct trials

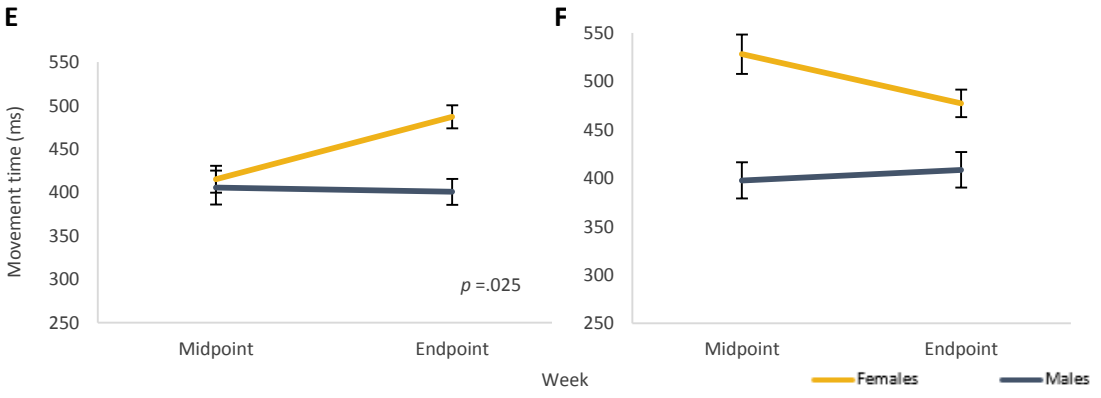


Figure 4.11 Movement time for correct trials on the Simple Reaction Time (SRT).

Interaction between baseline*condition on Simple Reaction Time (SRT): Movement time for correct trials

Interaction between condition*week on Simple Reaction Time (SRT): Movement time for correct trials

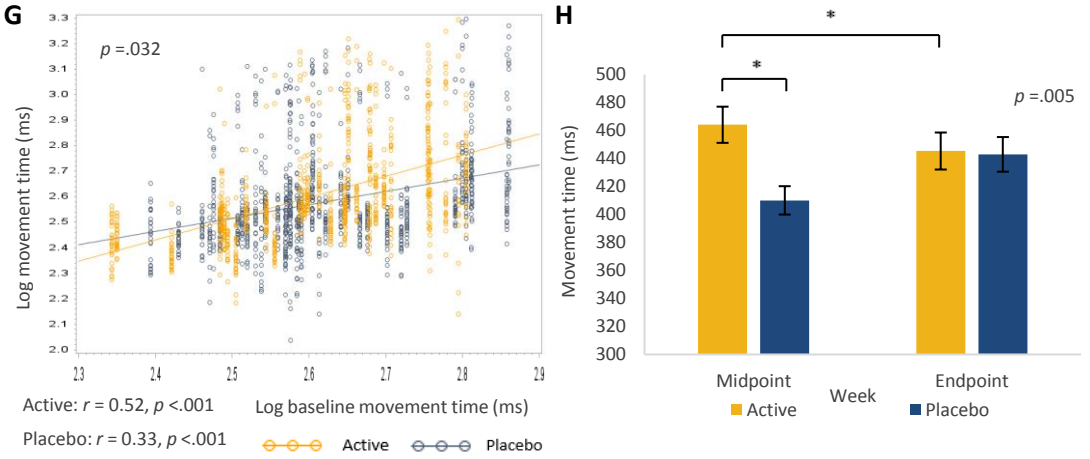


Figure 4.11 continued. (A-B) The x axis represents IQ score and the y axis is movement time (ms) over midpoint and endpoint. (C-D) The x axis is age (months) and the y axis is movement time (ms) over midpoint and endpoint. (A-D) Regression lines show relationship between x and y by week. (E-F) The x axis represents week and the y axis is movement time (ms) over midpoint and endpoint. Lines denote the mean. Error bars denote the SE of the mean. (G) The x axis is the log baseline movement time (ms) and the y axis is the log movement time (ms) over subsequent test points. Regression lines show relationship between x and y by condition. (H) The x axis is week and the y axis is movement time (ms) over midpoint and endpoint. Columns denote the mean. Error bars denote the SE of the mean. * p <.05. (A, C & E) Is the active condition, (B, D & F) is the placebo condition.

Figure 4.11(E) and 4.11(F) present the significant gender*condition*week interaction, $F(4,64) = 2.98$, $p = .025$. Figure 4.11(E) indicates females in the active condition moved more slowly at endpoint relative to midpoint, however, males tended to perform similarly across both test occasions in the same condition. Figure 4.11(F) on the other hand shows females in the placebo condition demonstrated greater movement latencies at midpoint relative to endpoint, whilst males tended to perform better at midpoint. In general, females tended to move more slowly compared to males irrespective of condition. Inspection of post hoc comparisons revealed a marginally significant difference between females in the placebo condition moving more quickly at midpoint (2.58 ± 0.02) than at endpoint (2.62 ± 0.02; $t(64) = -3.02$, $p = .067$). Further, males in the placebo condition (2.57 ± 0.02) demonstrated marginally significantly faster movement time relative to females in the active condition at midpoint (2.66 ± 0.02; $t(64) = -3.03$, $p = .065$). Also, females in the active condition were marginally significantly slower at midpoint (2.66 ± 0.02) than at endpoint (2.61 ± 0.02; $t(64) = 3.06$, $p = .061$).

The significant baseline*condition interaction, $F(1,64) = 4.83$, $p = .032$, is shown in Figure 4.11(G). Inspection of post hoc comparisons revealed that at a log baseline movement time of 2.7 and greater (slower movement time), those in the placebo condition illustrated significantly faster movement time (2.62 ± 0.02) relative to participants in the active condition (2.69 ± 0.02; $t(64) = -2.51$, $p = .015$).

Lastly, the significant condition*week interaction, $F(1,64) = 8.37$, $p = .005$, is shown in Figure 4.11(H). This indicates that at midpoint, those in the placebo condition illustrated faster movement time relative to participants in the active condition, however, at endpoint, performance was similar across both conditions. Inspection of post hoc comparisons revealed at midpoint, those in the placebo condition (2.58 ± 0.01) were significantly faster than those the active condition (2.64 ± 0.02; $t(64) = -2.83$, $p = .031$). Further, those in the active condition were significantly slower at midpoint (2.64 ± 0.02) than at endpoint (2.60 ± 0.02; $t(64) = 3.19$, $p = .012$).

4.7.2.2 Measures of memory performance

Table 4.8 provides a summary of the means (± SE) across measures of memory performance for each condition.

Table 4.8 Mean (± SE) on measures of memory performance (Rivermead Behavioural Memory Test for Children and Spatial Recognition Memory) by condition and week

Outcome	Baseline *Week 0* Mean ± SE	Midpoint *Week 3* Mean ± SE	Endpoint *Week 6* Mean ± SE	Active vs. placebo
RBMT-C immediate verbal recall (n)				
Active	13.02 ± 1.23	11.94 ± 1.33	13.60 ± .98	$F(1,65) = 2.06$, $p = .156$
Placebo	15.91 ± .99	16.66 ± .88	17.21 ± .89[a]	
RBMT-C delayed verbal recall (n)				
Active	5.32 ± .36	5.15 ± .32	5.24 ± .24	$F(1,64) = 3.82$, $p = .055$
Placebo	5.67 ± .23	5.88 ± .24	5.90 ± .26[a]	
CANTAB SRM number of correct trials (n)				
Active	10.63 ± 0.39[b]	11.77 ± 0.40[b]	12.03 ± 0.59[b]	$F(1,66) = 0.11$, $p = .739$
Placebo	11.72 ± 0.37	12.44 ± 0.34	12.41 ± 0.39	

Outcome	Baseline *Week 0* Mean ± SE	Midpoint *Week 3* Mean ± SE	Endpoint *Week 6* Mean ± SE	Active vs. placebo
CANTAB SRM reaction time for correct trials (ms)				
Active	2491.38 ± 98.14[b]	2660.73 ± 110.64[b]	2785.46 ± 138.81[b]	$F(1,64) = 4.10$, $p = .047$
Placebo	2591.59 ± 89.12	2428.96 ± 92.07	2357.50 ± 84.64	

Note: [a]n=38 [b]n=30

4.7.2.2.1 Rivermead Behavioural Memory Test for Children (RBMT-C)

4.7.2.2.1.1 Immediate recall

No outlying observations were removed from the final model (see section 4.6). Baseline performance was a significant covariate, $F(1,65) = 47.77$, $p < .001$, such that this showed a positive relationship with the number of items recalled at midpoint and endpoint. Gender was also a significant covariate, $F(1,65) = 4.10$, $p = .047$, with females (15.87 ± 0.63) recalling significantly more items correctly than males (14.08 ± 0.62). Age showed a trend when included as a covariate, $F(1,65) = 3.31$, $p = .073$, with more items being recalled by older participants. IQ was not a significant covariate and was removed from the final model. There was no significant main effect of condition, $F(1,65) = 2.06$, $p = .156$. No higher order interactions nor baseline*condition and baseline*week interactions were found to be significant. There was a marginally significant condition*week interaction, $F(1,61) = 3.54$, $p = .065$, and this interaction is driven by the performance by those in the active condition (12.76 ± 0.86), which is significantly poorer to that of participants in the placebo condition at midpoint (16.14 ± 0.76; $t(61) = 2.93$, $p = .024$).

4.7.2.2.1.2 Delayed recall

No outlying observations were removed from the final model. Age was marginally significant, and IQ showed a trend when included as covariates, $F(1,64) = 3.52$, $p = .065$ and $F(1,64) = 3.23$, $p = .077$, respectively. Both were positively associated with midpoint and endpoint i.e. those who were older or who had a higher IQ performed better on subsequent test occasions. Gender was not a significant covariate, $F(1,64) = 0.03$, $p = .859$. Baseline was not a significant covariate also and was removed from the final model. No higher order interactions nor baseline*condition and baseline*week interactions were found to be significant. Condition was marginally significant, $F(1,64) = 3.82$, $p = .055$, such that those in the placebo condition (5.80 ±

0.19) recalled more items correctly following a delay compared to those in the active condition (5.33 ± 0.21).

4.7.2.2.2 Spatial Recognition Memory (SRM)

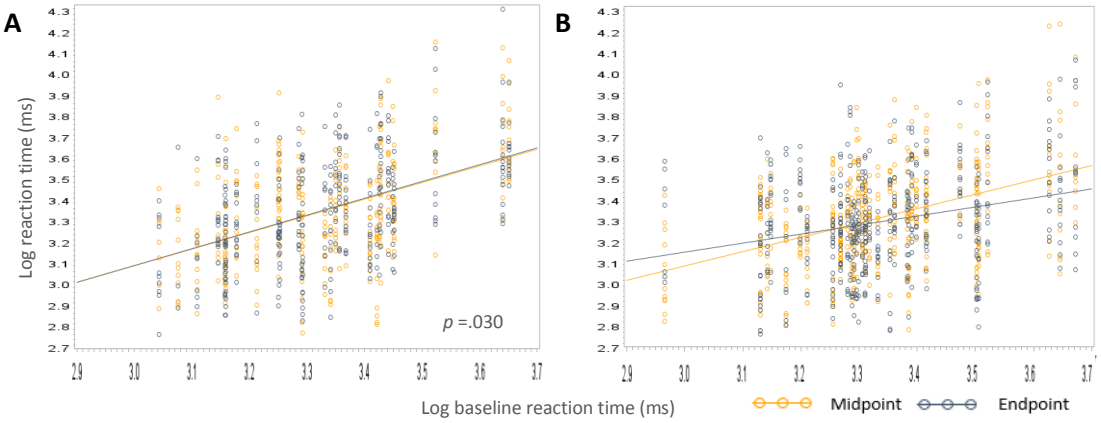
4.7.2.2.2.1 Number of correct trials

No outlying observations were removed from the final model. There was a trend towards baseline being a significant covariate, $F(1,66) = 3.31$, $p = .074$, such that the total number of correct trials at baseline was positively correlated with the total number achieved at midpoint and endpoint. Age, IQ and gender were not significant covariates and were removed from the final model. There was no significant main effect of condition, $F(1,66) = 0.11$, $p = .739$. No higher order interactions nor baseline*condition and baseline*week interactions were found to be significant. There was a significant main effect of week, $F(1,60) = 4.12$, $p = .047$, such that performance was better at endpoint (1.58 ± 0.07) relative to midpoint (1.53 ± 0.07).

4.7.2.2.2.2 Reaction time for correct trials

In the final model, 13 outlying observations were removed to normalise the residuals. Baseline reaction time was a significant covariate, $F(1,64) = 77.07$, $p < .001$, being positively correlated with reaction time at subsequent test points. Trial was also significant covariate, $F(16,851) = 3.63$, $p < .001$, such that reaction time varied by trial. IQ was marginally significant, $F(1,64) = 3.96$, $p = .051$ and was positively associated with reaction time at midpoint and endpoint. Age and gender were not significant covariates and were removed from the final model. There were significant baseline*condition*week and baseline*condition interactions, $F(1,1570) = 4.74$, $p = .030$ and $F(1,64) = 4.17$, $p = .045$, respectively (Figure 4.12).

Interaction between baseline*condition*week on Spatial Recognition Memory (SRM): Reaction time for correct trials



Interaction between baseline*condition on Spatial Recognition Memory (SRM): Reaction time for correct trials

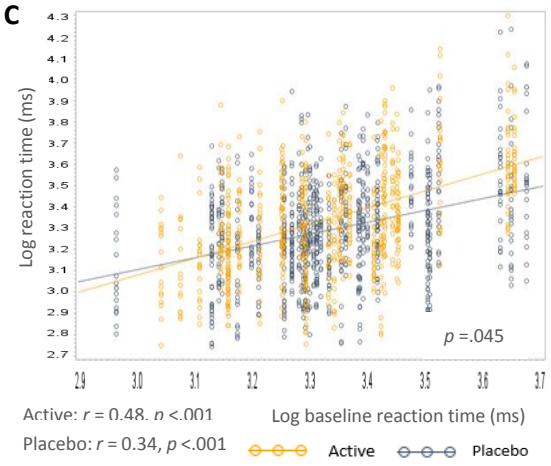


Figure 4.12 Reaction time for correct trials on the Spatial Recognition Memory (SRM). (A-C) The x axis is log baseline reaction time (ms) and the y axis is log reaction time (ms) over subsequent test points (A-B) Regression lines show relationship between x and y by week. (C) Regression lines show relationship between x and y by condition. (A) Is the active condition, (B) is the placebo condition.

The significant interaction between baseline*condition*week is presented in Figure 4.12(A) and 4.12(B). Inspection of post hoc comparisons indicated that those in the placebo condition (3.30 ± 0.02) were significantly faster compared to participants in the active condition at endpoint (3.40 ± 0.02; $t(63) = -3.57$, $p = .004$) at a log baseline reaction time of 3.4 and greater (slower baseline reaction time). Within condition, those in the placebo condition were significantly slower at midpoint (3.34 ± 0.02) compared to endpoint (3.30 ± 0.02; $t(63) = 3.18$, $p = .012$) at a log baseline reaction time of 3.4 and greater.

Figure 4.12(C) represents how performance by condition differed as a function of baseline performance. Inspection of post hoc comparisons revealed that those in the placebo condition (3.27 ± 0.02) responded significantly faster than those in the active condition (3.32 ± 0.02; $t(64) = -2.22$, $p = .030$) at a log baseline reaction time of 3.3 and above (slower baseline performance).

A significant main effect of condition was found, $F(1,64) = 4.10$, $p = .047$, such that participants in the active condition performed more slowly (3.35 ± 0.02) relative to their counterparts in the placebo condition (3.28 ± 0.02), however, post hoc comparisons did not reveal a significant difference. There was a trend towards a main effect of week, $F(1,63) = 3.37$, $p = .071$, with midpoint performance being slower (3.33 ± 0.02) than endpoint (3.31 ± 0.02). The analysis also revealed a marginally significant interaction between baseline*week, $F(1,1570) = 3.49$, $p = .062$.

4.7.2.3 Measure of motor skills

Table 4.9 provides a summary of the means (± SE) for each condition.

Table 4.9 Mean (± SE) on Motor Screening Task (MOT) outcomes by condition and week

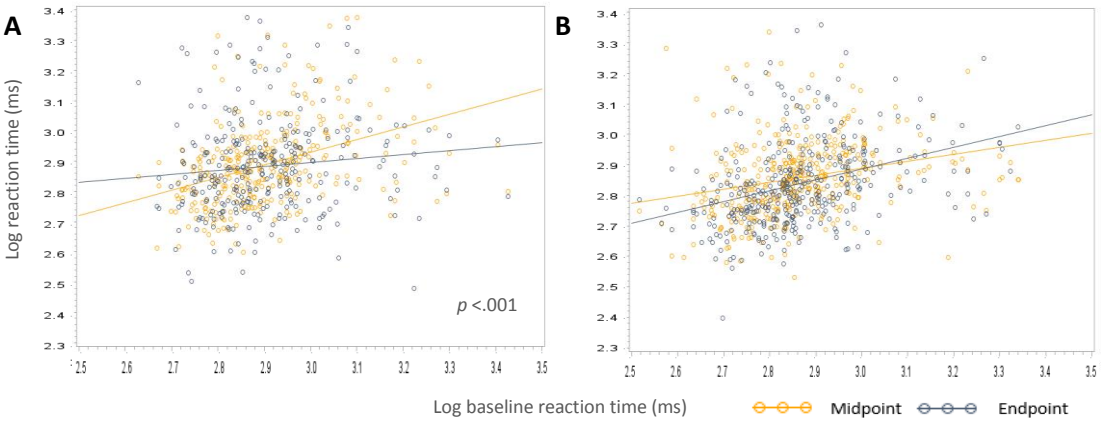
Outcome	Baseline *Week 0* Mean ± SE	Midpoint *Week 3* Mean ± SE	Endpoint *Week 6* Mean ± SE	Active vs. placebo
CANTAB Motor reaction time (ms)				
Active	844.36 ± 17.42	834.05 ± 19.78	865.70 ± 28.23	$F(1,66) = 0.91$, $p = .344$
Placebo	775.86 ± 14.86	778.18 ± 17.47	751.90 ± 19.01	
CANTAB Motor distance (mm)				
Active	31.51 ± 1.09	32.73 ± 1.01	30.88 ± 1.03	$F(1,68) = 0.00$, $p = .995$
Placebo	32.66 ± .95	30.47 ± .89	33.42 ± .88	

4.7.2.3.1 Motor Screening Task (MOT)

4.7.2.3.1.1 Reaction time

In the final model, 22 outlying observations were removed to normalise the residuals. Baseline reaction time was a significant covariate, $F(1,1260) = 6.28$, $p = .012$, with baseline performance positively correlated with reaction time at midpoint and endpoint. Trial was also found to be a significant covariate, $F(9,621) = 7.32$, $p < .001$, with performance fluctuating across the ten trials. Age and IQ were not significant covariates, $F(1,66) = 0.16$, $p = .692$ and $F(1,66) = 0.11$, $p = .739$, respectively. Gender was also not a significant covariate and was removed from the final model. There was no significant main effect of condition, $F(1,66) = 0.91$, $p = .344$. There were significant baseline*condition*week and IQ*condition*week interactions, $F(2,1260) = 9.64$, $p < .001$ and $F(3,1260) = 3.70$, $p = .011$, respectively (Figure 4.13).

Interaction between baseline*condition*week on Motor Screening Task (MOT): Reaction time



Interaction between IQ*condition*week on Motor Screening Task (MOT): Reaction time

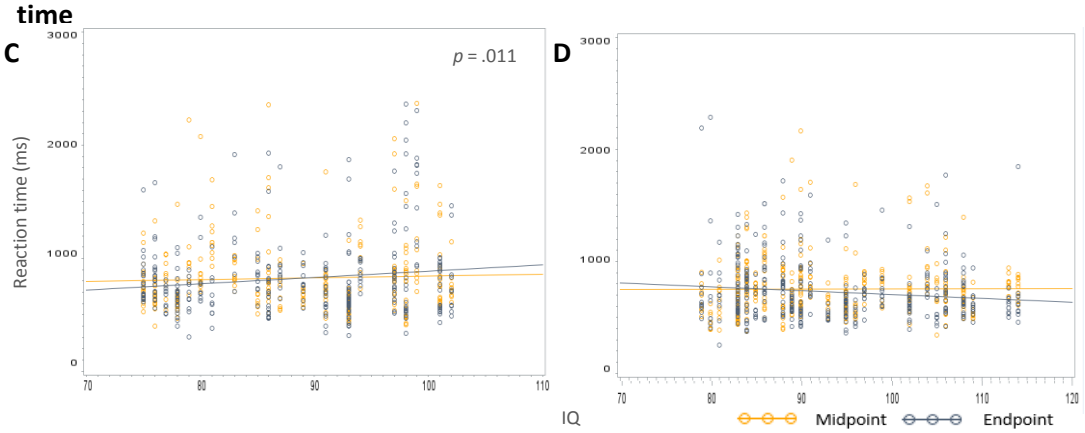


Figure 4.13 Reaction time on the Motor Screening Task (MOT). (A-B) The x axis is the log baseline reaction time (ms), and the y axis is the log reaction time (ms) over subsequent test points. (C-D) The x axis represents IQ scores, and the y axis is reaction time (ms) over midpoint and endpoint. Regression lines show relationship between x and y by week. (A & C) Is the active condition, (B & D) is the placebo condition.

Figure 4.13(A) and 4.13(B) illustrate how performance differed by week and condition as a function of baseline performance. Inspection of post hoc comparisons revealed at a log baseline reaction time between 2.6 to 2.8 (inclusive), those in the placebo condition were significantly faster (2.81 ± 0.02) than those in the active condition at endpoint (2.91 ± 0.02; $t(68) = -3.16$, $p = .012$). Whereas, at a log baseline reaction time of 3.0, there was a marginally significant difference, such that particiapants in the placebo condition (2.86 ± 0.02) demonstrated faster reaction time relative to those in the active condition at midpoint (2.92 ± 0.02; $t(68) = -2.56$, $p = .060$). This became significant at a log baseline reaction time of 3.1 and greater (slower baseline reaction time: 2.86 ± 0.02 vs. 2.94 ± 0.02; $t(68) = -3.13$, $p = .014$). Within condition, at a log baseline reaction time of 2.6 to 2.8 (inclusive), those in the active condition were significantly faster at midpoint (2.81 ± 0.02) than at endpoint (2.91 ± 0.02; $t(68) = -3.87$, $p = .001$). Further, there was also a trend towards those in the placebo condition responding more slowly at midpoint (2.86 ± 0.02) relative to their performance at endpoint (2.81 ± 0.02; $t(68) = 2.43$, $p = .082$) at a log baseline reaction time of 2.6; this became marginally significant at a log baseline reaction time of 2.7 (2.86 ± 0.02 vs. 2.82 ± 0.02; $t(68) = 2.55$, $p = .061$). At a log baseline reaction time of 3.1 and greater (slower baseline reaction time), participants in the active condition were significantly slower at midpoint (2.94 ± 0.02) compared with endpoint (2.88 ± 0.02, $t(68) = 3.35$, $p = .007$).

The significant IQ*condition*week interaction is shown in Figure 4.13(C) and 4.13(D). At a lower IQ score, those in the active condition (Figure 4.13(C)) showed poorer performance (slower reaction time) at midpoint, this being the opposite to that shown by those with lower IQ scores in the placebo condition (Figure 4.13(D)). Conversely, at higher IQ scores, those in the active condition showed faster reaction speeds at midpoint, whilst those in the placebo condition performed better at endpoint. Inspection of post hoc comparisons revealed at an IQ score of 100, participants in the placebo condition (2.83 ± 0.02) were significantly faster than the active condition at endpoint (2.91 ± 0.03; $t(68) = -2.77$, $p = .036$). Within condition, at an IQ score of 100 and above (higher IQ), those in the placebo condition were significantly slower at midpoint (2.86 ± 0.02) than at endpoint (2.83 ± 0.02; $t(68) = 3.38$, $p = .006$).

There was also a marginally significant age*condition*week interaction, $F(3,1260) = 2.47$, $p = .060$, a significant condition*week interaction $F(1,68) = 7.01$, $p = .010$, and a significant baseline*week interaction, $F(1,1260) = 4.21$, $p = .041$. Inspection of post hoc comparisons in respect to the significant condition*week interaction revealed a trend towards faster reaction times at endpoint shown by those in the placebo condition (2.84 ± 0.01) compared to those in the active condition (2.89 ± 0.02; $t(68) = -2.38$, $p = .092$).

4.7.2.3.1.2 Distance

In the final model, 2 outlying observations were removed to normalise the residuals. Baseline performance was a significant covariate, $F(1,1282) = 4.52$, $p = .034$, such that baseline performance was positively correlated with performance at midpoint and endpoint. Gender was also a significant covariate, $F(1,68) = 9.09$, $p = .004$, with females (5.22 ± 0.09) making responses significantly closer to the location of the stimulus than males (5.60 ± 0.09). A trend for trial was also revealed $F(9,621) = 1.67$, $p = .094$, where again, performance varied across the 10 trials. Age and IQ were not significant covariates and were removed from the final model. There was no significant main effect of condition, $F(1,68) = 0.00$, $p = .995$. No higher order interactions nor baseline*condition and baseline*week interactions were found to be significant. There was a significant main effect of week, $F(1,69) = 5.57$, $p = .021$, such that responses were closer to the stimulus at midpoint (5.38 ± 0.07) than at endpoint (5.44 ± 0.07).

4.7.2.4 Measure of executive function performance (working memory)

Table 4.10 provides a summary of the means (± SE) for each condition.

Table 4.10 Mean (± SE) on Spatial Span outcomes by condition and week

Outcome	Baseline *Week 0* Mean ± SE	Midpoint *Week 3* Mean ± SE	Endpoint *Week 6* Mean ± SE	Active vs. placebo
CANTAB SSP reaction time for correct trials (ms)				
Active	4149.09 ± 81.24[a]	4144.87 ± 73.76[a]	4254.14 ± 81.35[a]	$F(1,62) = 2.39$, $p = .127$
Placebo	4241.16 ± 73.48[b]	4319.63 ± 79.46[b]	4014.94 ± 60.95[b]	
CANTAB SSP number of correct trials (n)				
Active	11.97 ± 0.95[a]	13.63 ± 0.99[a]	14.60 ± 1.04[a]	$F(1,61) = 3.04$, $p = .086$
Placebo	13.19 ± 0.96[b]	13.95 ± 0.90[b]	13.65 ± 0.90[b]	
CANTAB SSP highest span achieved (n)				
Active	4.50 ± 0.20[a]	4.83 ±0.19[a]	5.00 ± 0.20[a]	$F(1,63) = 3.33$, $p = .073$
Placebo	4.73 ± 0.19[b]	4.89 ± 0.17[b]	4.84 ± 0.17[b]	

Note: [a]n=30 [b]n=37

4.7.2.4.1 Spatial Span (SSP)

4.7.2.4.1.1 Number of correct trials

In the final model, 3 outlying observations were removed to normalise residuals. Baseline was not a significant covariate, $F(1,61) = 1.85$, $p = .179$. Age and IQ were significant covariates, $F(1,61) = 6.42$, $p = .014$ and $F(1,61) = 7.95$, $p = .007$ respectively, with both being positively associated with performance at midpoint and endpoint i.e. those who were older or had higher IQ performed better on subsequent test sessions. Gender was also a significant covariate, $F(1,61) = 7.71$, $p = .007$, with males (15.01 ± 0.05) responding correctly on significantly more trials than females (14.89 ± 0.05). The total number of attempts made to the highest span achieved and the highest span achieved both by week were also significant covariates, $F(10,38) = 84.85$, $p < .001$ and $F(1,45) = 14646.3$, $p < .001$, respectively. Both were positively associated with improvement at midpoint and endpoint. No higher order interactions nor baseline*condition and baseline*week interactions were found to be significant. There was a trend for condition (Figure 4.14), $F(1,61) = 3.04$, $p = .086$, with those in the active condition (14.96 ± 0.05) correctly responding to marginally more trials compared to participants in the placebo condition (14.93 ± 0.05).

Main effect of condition on Spatial Span (SSP): Number of correct trials

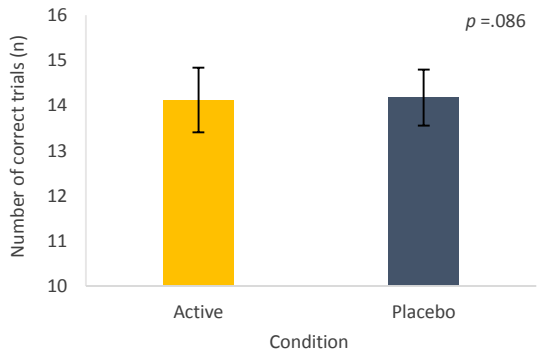


Figure 4.14 Number of correct trials on the Spatial Span (SSP). The x axis is condition and the y axis is the number of correct trials (n) over midpoint and endpoint. Columns denote the least squares means. Error bars denote the SE of the least squares means.

4.7.2.4.1.2 Reaction time for correct trials

In the final model, 23 outlying observations were removed to normalise residuals. Baseline reaction time was a significant covariate, $F(1,62) = 21.82$, $p < .001$, with baseline reaction time showing a positive relationship with reaction time demonstrated at midpoint and endpoint. Trial and the total number of attempts at each span to the highest span achieved were also significant covariates, $F(26,983) = 240.90$, $p < .001$ and $F(10,41) = 3.31$, $p = .003$, respectively. Both of which were positively correlated with performance at midpoint and endpoint. IQ and

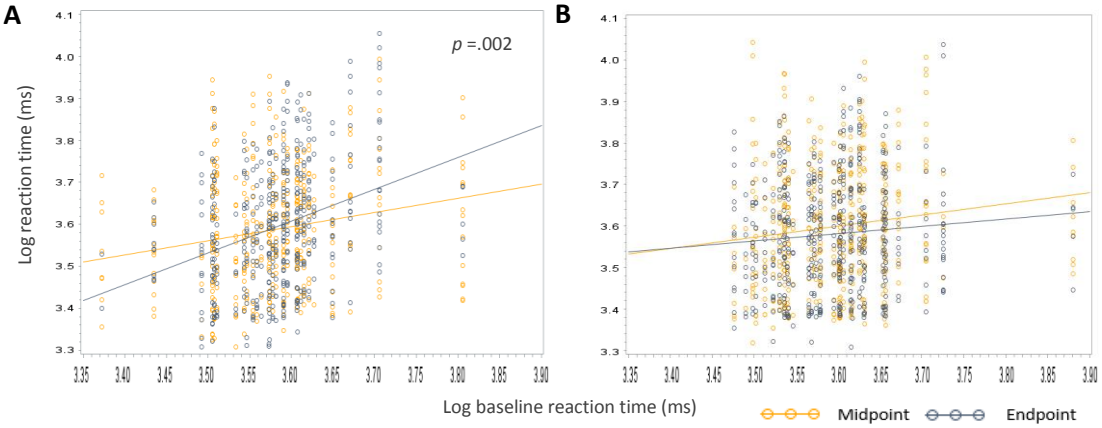
gender were not significant covariates, $F(1,62) = 0.20$, $p = .653$, and $F(1,62) = 0.75$, $p = .391$, respectively. Age was also not a significant covariate and was removed from the final model. There was no significant main effect of condition, $F(1,62) = 2.39$, $p = .127$. There were significant higher order interactions as follows, baseline*condition*week interaction, $F(3,1727) = 4.86$, $p = .002$, gender*condition*week interaction, $F(3,62) = 6.14$, $p = .001$, age*condition*week, $F(4,1727) = 4.59$, $p = .001$, and IQ*condition*week, $F(3,1727) = 4.37$, $p = .005$.

The significant baseline*condition*week interaction, $F(3,1727) = 4.86$, $p = .002$, is displayed in Figure 4.15(A) and 4.15(B). Inspection of post hoc comparisons revealed at a log baseline reaction time of 3.65 and slower (poorer baseline performance), those in the placebo condition (3.64 ± 0.01) were significantly faster compared to participants in the active condition at endpoint (3.68 ± 0.01; $t(62) = -2.68$, $p = .045$). Within condition, those in the placebo condition were significantly slower at midpoint (3.66 ± 0.01) compared to endpoint (3.64 ± 0.01; $t(62) = 4.85$, $p < .001$) at a log baseline reaction time of 3.60 and slower.

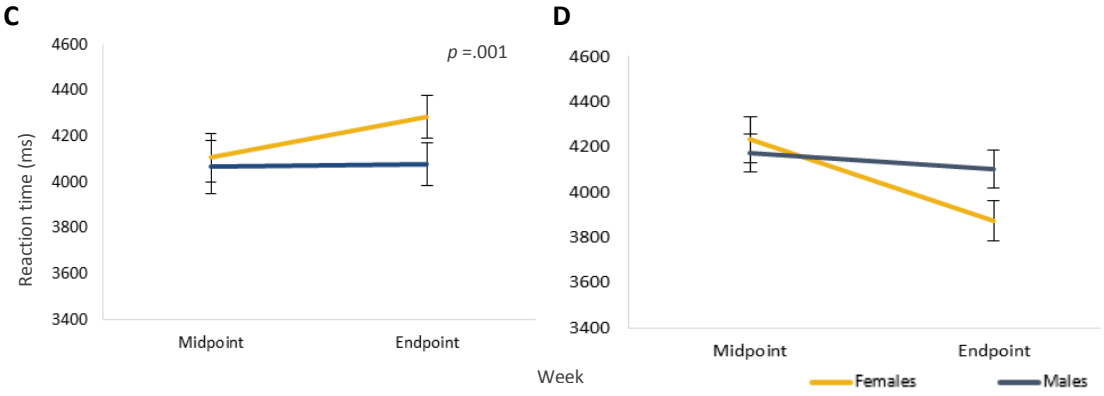
The significant gender*condition*week interaction, $F(3,62) = 6.14$, $p = .001$, is displayed in Figure 4.15(C) and 4.15(D). Figure 4.15(C) represents performance within the active condition and shows that females within this condition demonstrated faster reaction time at midpoint relative to endpoint. However, males in the active condition performed similarly across both weeks. In the placebo condition (Figure 4.15(D)), both females and males performed better at endpoint relative to midpoint. Inspection of post hoc comparisons revealed that only the females in the placebo condition were significantly slower at midpoint (3.66 ± 0.01) than at endpoint (3.63 ± 0.01; $t(62) = 5.72$, $p < .001$).

The presence of significant interactions between age*condition*week, $F(4,1727) = 4.59$, $p = .001$ and IQ*condition*week, $F(3,1727) = 4.37$, $p = .005$ also indicate that performance also varied across condition and week depending upon the age and IQ of the participants. The interaction between condition, week and age is shown by Figure 4.15(E) and 4.15(F). Both indicate that younger participants performed better at endpoint, whilst older participants illustrated faster reaction time at midpoint, irrespective of condition. Inspection of post hoc comparisons revealed that those in the placebo condition demonstrated significantly slower reaction times at midpoint (3.66 ± 0.01) relative to endpoint (3.62 ± 0.01; $t(62) = 5.83$, $p < .001$) at 80 and 90 months of age.

Interaction between baseline*condition*week on Spatial Span (SSP): Reaction time for correct trials



Interaction between gender*condition*week on Spatial Span (SSP): Reaction time for correct trials



Interaction between age*condition*week on Spatial Span (SSP): Reaction time for correct trials

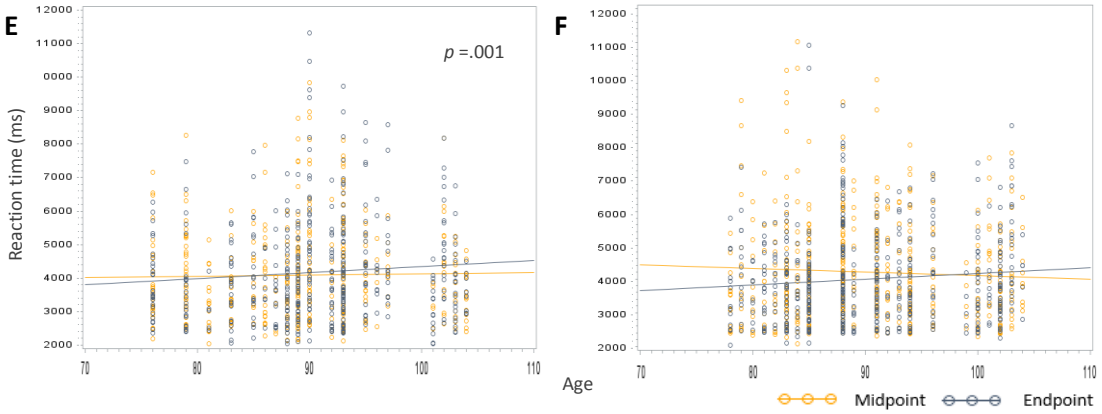
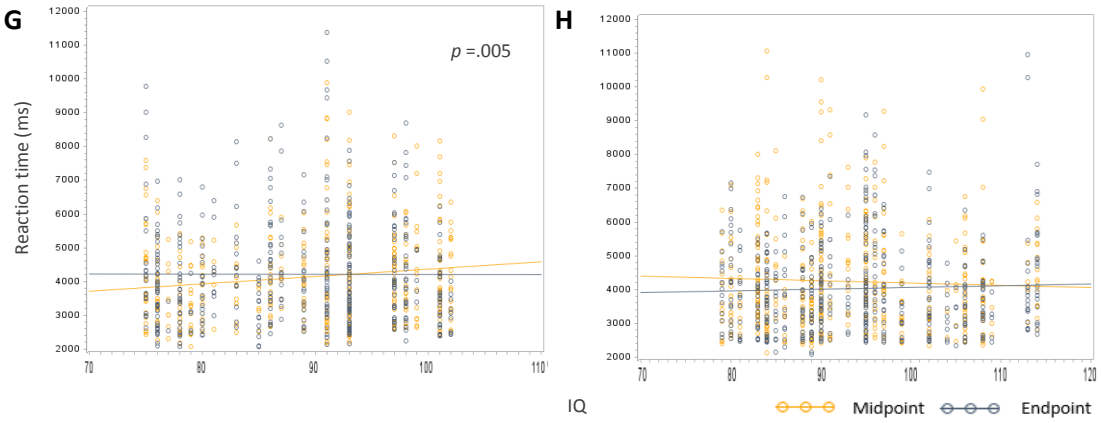


Figure 4.15 Reaction time for correct trials on the Spatial Span (SSP).

Interaction between IQ*condition*week on Spatial Span (SSP): Reaction time for correct trials



Interaction between condition*week on on Spatial Span (SSP): Reaction time for correct trials

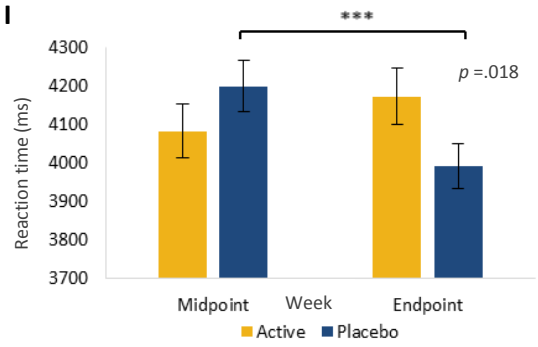


Figure 4.15 continued. (A-B) The x axis is the log baseline reaction time (ms) and the y axis is the log reaction time (ms) over subsequent test points. (C-D) The x axis represents week and the y axis is reaction time (ms) over midpoint and endpoint. Lines denote the mean. Error bars denote the SE of the mean. (E-F) The x axis is age (months) and the y axis is the reaction time (ms) over midpoint and endpoint. (G-H) The x axis represents IQ score and the y axis is reaction time (ms) over midpoint and endpoint. (I) the x axis is week and the y axis is the reaction time (ms) over midpoint and endpoint. Columns denote the mean. Error bars denote the SE of the mean. *** p <.001. (A-B, E-F & G-H) regression lines show relationship between x and y by week. (A, C, E & G) Is the active condition, (B, D, F & H) is the placebo condition.

Figures 4.15(G) and 4.15(H) present the relationship between condition, week and IQ, $F(3,1727) = 4.37$, $p = .005$, for the active and placebo conditions, respectively. The former illustrates at lower IQ scores, the active condition demonstrated the slowest reaction time at endpoint, however, at higher IQ scores, performance was better at endpoint. Figure 4.15(H) indicates that faster reaction time was shown at endpoint by those with lower IQ scores in the placebo condition, whereas performance appeared to be similar in both test sessions at higher IQ scores. Inspection of post hoc comparisons revealed a trend towards those in the placebo condition

responding more slowly at midpoint (3.66 ± 0.01) compared to their performance at endpoint (3.64 ± 0.01; $t(62) = 2.46$, $p = .076$) and those in the active condition responding significantly faster at midpoint (3.64 ± 0.01) than at endpoint (3.66 ± 0.01; $t(62) = -3.10$, $p = .015$) at an IQ score of 80. Further, at an IQ score of 90 and 100, those in the placebo condition demonstrated significantly slower reaction times at midpoint (3.65 ± 0.01) than at endpoint (3.64 ± 0.01; $t(62) = 4.15$, $p < .001$). Lastly, at an IQ score of 100, those in the active condition were marginally significantly slower at midpoint (3.66 ± 0.02) than at endpoint (3.64 ± 0.02; $t(62) = 2.59$, $p = .056$). This became significant at an IQ score of 110 (3.68 ± 0.02 vs. 3.63 ± 0.03; $t(62) = 3.13$, $p = .014$).

There was a significant condition*week interaction, $F(1,62) = 5.87$, $p = .018$ (Figure 4.15(I)). Inspection of post hoc comparisons revealed that those in the placebo condition were significantly slower at midpoint (3.65 ± 0.01) relative to endpoint (3.63 ± 0.01; $t(62) = 4.32$, $p < .001$).

4.7.2.4.1.3 Highest span achieved

No outlying observations were removed from the final model. Baseline performance and the total number of attempts made to the highest span achieved were both significant covariates, $F(1,63) = 21.98$, $p < .001$ and $F(10,41) = 36.41$, $p < .001$, respectively. Both covariates were positively related to performance at midpoint and endpoint. No higher order interactions nor baseline*condition and baseline*week interactions were found to be significant. There was a trend towards a main effect of condition, $F(1,63) = 3.33$, $p = .073$, shown in Figure 4.16. Those in the active condition (5.19 ± 0.12) demonstrated better performance compared to those in the placebo condition (5.07 ± 0.11).

Main effect of condition on Spatial Span (SSP): Highest span achieved

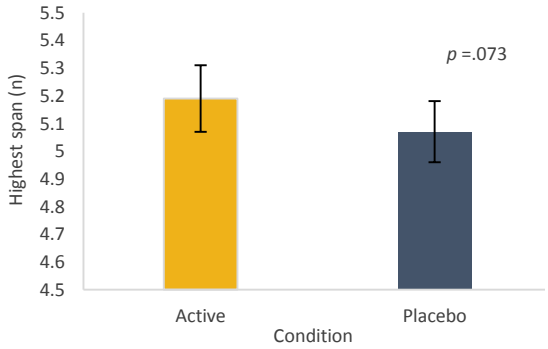


Figure 4.16 Highest span achieved on the Spatial Span (SSP). The x axis is condition and the y axis is the highest span achieved (n) over midpoint and endpoint. Columns denote the least squares means. Error bars denote the SE of the least squares means.

4.7.3 Effect of intervention on subjective evaluation of appetite, mood, motivation and mental alertness

Table 4.11 provides a summary of the means (± SE) for subjective evaluations completed prior to the cognitive measures at each test point for both conditions.

Table 4.11 Mean (± SE) for subjective evaluations undertaken pre-cognitive measures by condition and week

Outcome	Baseline *Week 0* Mean ± SE	Midpoint *Week 3* Mean ± SE	Endpoint *Week 6* Mean ± SE	Active vs. placebo
Subjective hunger (n)				
Active	6.26 ± 0.65	5.61 ± 0.64	5.06 ± .0.66	*F*(1,67) =
Placebo	6.23 ± .0.51	5.90 ± .0.56	5.61 ± 0.60[a]	0.36, *p* = .550
Subjective cheerfulness (n)				
Active	7.94 ± .0.38	8.52 ± .0.40	8.29 ± .0.36	*F*(1,66) =
Placebo	7.97 ± .0.43	9.05 ± .0.28	8.89 ± .0.38[a]	2.73, *p* = .103
Subjective ratings of bad temper (n)				
Active	1.71 ± 0.35	1.29 ± 0.18	1.87 ± 0.36	*F*(1,67) =
Placebo	1.46 ± .0.24	1.87 ± .0.34	1.26 ± .0.13[a]	0.51, *p* = .478
Subjective energy levels (n)				
Active	8.19 ± 0.45	7.90 ± 0.47	8.03 ± 0.48	*F*(1,67) =
Placebo	7.90 ± 0.46	8.41 ± 0.40	8.63 ± 0.35[a]	1.89, *p* = .174
Keenness to try hard (n)				
Active	8.61 ± 0.36	8.87 ± 0.32	8.90 ± 0.32	*F*(1,67) =
Placebo	8.85 ± 0.35	9.56 ± 0.20	9.37 ± 0.29[a]	0.16, *p* = .692
Subjective ease of distraction (n)				
Active	4.16 ± 0.63	3.23 ± 0.53	3.42 ± 0.54	*F*(1,66) =
Placebo	2.51 ± 0.44	2.21 ± 0.37	2.08 ± 0.35[a]	6.27, *p* = .015
Perceived ease of focussing (n)				
Active	7.19 ± 0.56	7.55 ± 0.47	8.35 ± 0.42	*F*(1,66) =
Placebo	8.46 ± 0.35	8.54 ± 0.37	7.87 ± 0.46[a]	0.72, *p* = .399
Wakefulness (n)				
Active	7.19 ± 0.56	7.55 ± 0.47	8.35 ± 0.42	*F*(1,67) =
Placebo	7.31 ± 0.53	7.51 ± 0.53	7.87 ± 0.46[a]	0.13, *p* = .722

Notes: Higher values represent a greater magnitude. [a]n=38.

4.7.3.1 Subjective hunger

No outlying observations were removed from the final model. Baseline subjective hunger was a significant covariate, $F(1,67) = 25.95$, $p < .001$, which showed a positive correlation with subjective hunger at subsequent test points. There was no significant main effect of condition,

$F(1,67) = 0.36$, $p = .550$, and no significant interaction between baseline*condition*week or baseline*condition, and these were removed from the final model.

4.7.3.2 Subjective cheerfulness

No outlying observations were removed from the final model. Baseline subjective cheerfulness was a significant covariate, $F(1,66) = 9.69$, $p = .003$; this being positively correlated with subjective ratings of cheerfulness at midpoint and endpoint. There was no significant main effect of condition, $F(1,66) = 2.73$, $p = .103$ and no significant baseline*condition interaction, $F(1,66) = 1.48$, $p = .228$. Baseline*condition*week was also nonsignificant and removed from the final model.

4.7.3.3 Subjective ratings of bad temper

In the final model, 4 outlying observations were removed to normalise residuals. Baseline subjective rating of bad temperedness was a significant covariate, $F(1,67) = 30.41$, $p < .001$, with this being positively correlated with perceptions of bad temperedness at midpoint and endpoint. There was no significant main effect of condition, $F(1,67) = 0.51$, $p = .478$, and no significant baseline*condition interaction and this was removed from the final model. The analysis also revealed a significant interaction between baseline*condition*week, $F(2,60) = 9.61$, $p < .001$, which is shown in Figure 4.17(A) and 14.17(B), and a trend for baseline*week, $F(1,60) = 3.01$, $p = .088$.

Inspection of post hoc comparisons in view of the baseline*condition*week interaction, revealed that at a log baseline subjective rating of 0.2 and greater (higher baseline self-perception of bad temperedness), those in the placebo condition rated themselves significantly higher for bad temperedness compared to the active condition at midpoint (0.21 ± 0.03 vs. 0.06 ± 0.03; $t(59) = 3.08$, $p = .016$). Moreover, at a log baseline subjective rating of 0.2 and greater, those in the the placebo condition rated themselves significantly higher for bad temperedness at midpoint (0.21 ± 0.03) relative to endpoint (0.09 ± 0.04; $t(59) = 3.30$, $p = .009$). There was also a trend towards those in the active condition rating themselves as lower for bad temperedness at midpoint compared to endpoint at a log baseline subjective rating of 0.5 and greater (0.14 ± 0.06 vs. 0.28 ± 0.06; $t(59) = -2.39$, $p = .090$).

Interaction between baseline*condition*week on subjective ratings of bad temper

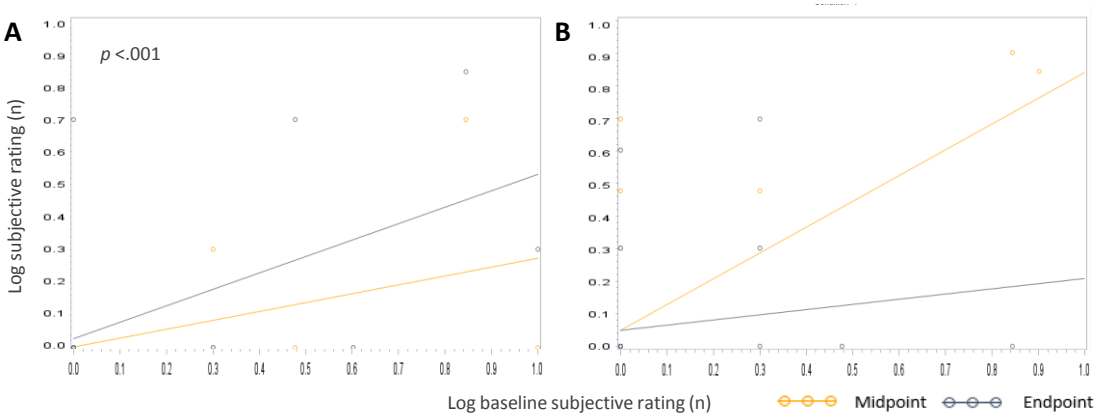


Figure 4.17 Subjective ratings of mood: Bad temper. The x axis represents log baseline subjective rating (n) and the y axis is the log self-rating (n) over subsequent test points Regression lines show relationship between x and y by week. (A) Is the active condition, (B) is the placebo condition.

4.7.3.4 Subjective energy levels

No outlying observations were removed from the final model. Subjective rating for energy levels at baseline was a significant covariate, $F(1,67) = 35.78$, $p < .001$, with subjective ratings at baseline being positively associated with subjective ratings of energy levels at later test points. There was no significant main effect of condition, $F(1,67) = 1.89$, $p = .174$, and no significant interaction between baseline*condition*week or baseline*condition, and these were removed from the final model.

4.7.3.5 Keenness to try hard

In the final model, one outlying observation was removed to normalise residuals. Baseline subjective rating was a significant covariate, $F(1,67) = 22.37$, $p < .001$, with subjective ratings at baseline being positively correlated with subjective ratings at midpoint and endpoint. There was no significant main effect of condition, $F(1,67) = 0.16$, $p = .692$ and no significant baseline*condition interaction, which was removed from the final model. The analysis revealed a significant baseline*condition*week interaction, $F(3,63) = 4.10$, $p = .010$.

Figure 4.18(A) (active) and 4.18(B) (placebo) present the relationship between baseline subjective ratings, condition and week. Inspection of post hoc comparisons revealed those in the placebo condition rated themselves significantly higher at midpoint (0.76 ± 0.09) compared to endpoint (0.43 ± 0.09; $t(64) = 3.57$, $p = .004$) at a log baseline subjective rating of 0.1 to 0.6,

inclusive. This was marginally significant at a log baseline subjective rating of 0.7 (0.92 ± 0.04 vs. 0.81 ± 0.04; $t(64) = 2.61$, $p = .054$).

Interaction between baseline*condition*week on subjective ratings of keenness to try hard

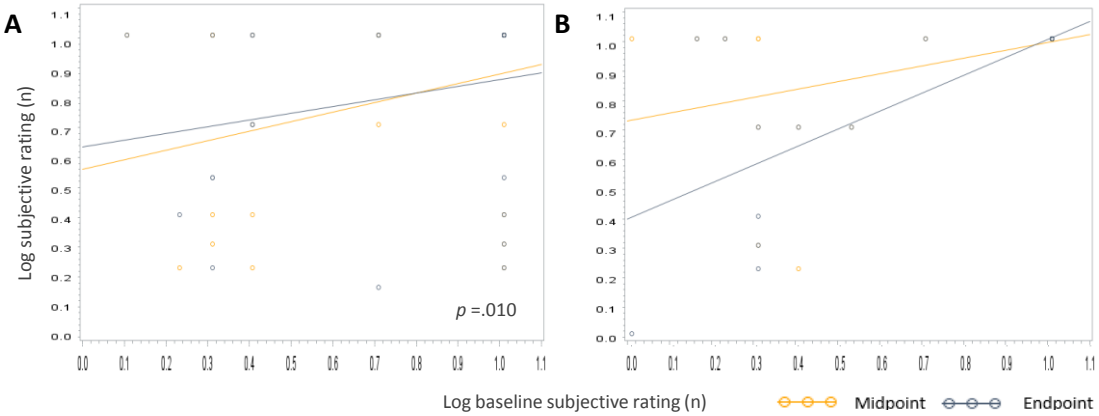


Figure 4.18 Subjective ratings of motivation: Keenness to try hard. The x axis represents log baseline subjective rating (n) and the y axis is the log self-rating (n) over subsequent test points. Regression lines show relationship between x and y by week. (A) Is the active condition, (B) is the placebo condition.

A main effect of week was marginally significant, $F(1,63) = 3.96$, $p = .051$, such that subjective ratings of keenness to try hard were higher at midpoint (0.88 ± 0.03) compared to endpoint (0.84 ± 0.03). A condition*week interaction was also significant, $F(1,63) = 8.99$, $p = .004$, such that those in the active condition were similarly keen on both test occasions (0.81 ± 0.04), while those in the placebo condition became less keen over time (midpoint:0.94 ± 0.04 vs. endpoint: 0.87 ± 0.04). However, post hoc comparisons did not reveal a significant difference.

4.7.3.6 Subjective ease of distraction

No outlying observations were removed from the final model. Baseline subjective rating for ease of distraction was a significant covariate, $F(1,66) = 19.43$, $p < .001$; this being positively associated with ease of distraction subjective ratings at midpoint and endpoint. Baseline*condition*week was not significant and was removed from the final model. A significant baseline*condition interaction was revealed, $F(1,66) = 4.99$, $p = .029$, shown in Figure 4.19.

Inspection of post hoc comparisons revealed those in the placebo condition (0.12 ± 0.04) rated themselves significantly less easily distracted compared to those in the active condition (0.29 ± 0.06; $t(66) = -2.32$, $p = .024$) at a log baseline subjective rating of 0.1. At a log baseline subjective

rating of 0.2, this same difference was marginally significant (0.18 ± 0.04 vs. 0.31 ± 0.05; $t(66) = -1.99$, $p = .050$).

Interaction between baseline*condition on subjective ratings of ease of distraction

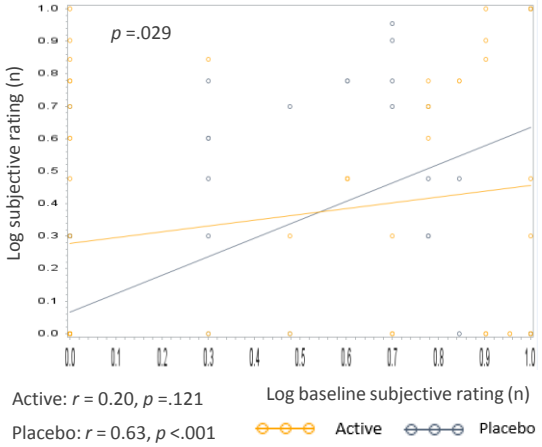


Figure 4.19 Subjective ratings of mental alertness: Ease of distraction. The x axis represents log baseline subjective rating (n) and the y axis is log self-rating (n) over subsequent test points. Regression lines show relationship between x and y by condition.

A significant main effect of condition, $F(1,66) = 6.27$, $p = .015$, was also found. Participants in the active condition reported being easily distracted to a greater extent (0.33 ± 0.05) compared to those in the placebo condition (0.24 ± 0.04). However, post hoc comparisons did not reveal a significant difference.

4.7.3.7 Perceived ease of focusing

No outlying observations were removed from the final model. Baseline perception of ease of focusing was a significant covariate, $F(1,66) = 17.13$, $p < .001$, with this being positively related to perceptions at midpoint and endpoint. There was no significant main effect of condition or baseline*condition interaction, $F(1,66) = 0.72$, $p = .399$ and $F(1,66) = 1.94$, $p = .168$, respectively. Baseline*condition*week was significant, $F(1,65) = 4.80$, $p = .032$, however, post hoc comparisons did not reveal a significant difference.

A significant interaction between condition*week was also found, $F(1,65) = 4.66$, $p = .035$. Participants in the placebo condition reported being able to focus more easily than their counterparts in the active condition both at midpoint (0.75 ± 0.05 vs. 0.71 ± 0.06) and endpoint (0.71 ± 0.05 vs. 0.63 ± 0.06). However, post hoc comparisons did not reveal a significant difference.

4.7.3.8 Wakefulness

No outlying observations were removed from the final model. Baseline perceived wakefulness was a significant covariate, $F(1,67) = 41.73$, $p < .001$, with this being positively related to perceived wakefulness at subsequent time points. There was no significant main effect of condition, $F(1,67) = 0.13$, $p = .722$, and no significant interaction between baseline*condition*week or baseline*condition, and these terms were removed from the final model. There was a significant main effect of week, $F(1,66) = 4.20$, $p = .045$, such that subjective ratings of wakefulness were lower (less awake) at midpoint (0.72 ± 0.04) compared to endpoint (0.79 ± 0.04).

4.7.4 Cognitive test evaluation ratings

Table 4.12 provides a summary of the means (± SE) for subjective evaluations completed post-cognitive measures at each test point for both conditions.

Table 4.12 Mean (± SE) for subjective evaluations undertaken post-cognitive measures by condition and week

Outcome	Baseline *Week 0* Mean ± SE	Midpoint *Week 3* Mean ± SE	Endpoint *Week 6* Mean ± SE	Active vs. placebo
Perceived test battery difficulty (n)				
Active	3.35 ± 0.50	3.00 ± 0.45	3.65 ± .0.55	$F(1,67) = 0.32$, $p = .574$
Placebo	3.15 ± 0.38	3.05 ± 0.37	3.62 ± 0.37	
Perceived concentration during test battery (n)				
Active	8.77 ± .0.35	8.65 ± .0.45	8.42 ± .0.46	$F(1,67) = 1.37$, $p = .247$
Placebo	8.85 ± 0.30	9.05 ± .0.29	8.90 ± .0.37	
Perceived performance (n)				
Active	7.65 ± 0.46	7.68 ± 0.47	7.77 ± 0.49	$F(1,67) = 1.73$, $p = .193$
Placebo	8.49 ± 0.33	7.64 ± .0.47	7.38 ± .0.43	
Frustration (n)				
Active	2.71 ± 0.44	3.19 ± 0.48	2.81 ± 0.47	$F(1,67) = 0.30$, $p = .585$
Placebo	2.85 ± 0.44	2.67 ± 0.47	3.31 ± 0.52	

Note: Higher values represent a greater magnitude.

4.7.4.1 Perceived test battery difficulty

No outlying observations were removed from the final model. Baseline subjective rating of test difficulty was a significant covariate, $F(1,67) = 25.61$, $p < .001$; this being positively correlated with perceived test difficulty at midpoint and endpoint. There was no significant main effect of condition, $F(1,67) = 0.32$, $p = .574$, and no significant interaction between baseline*condition*week or baseline*condition, and these were removed from the final model.

4.7.4.2 Perceived concentration during the test battery

In the final model, two outlying observations were removed to normalise residuals. Perceived concentration during the test battery at baseline was a significant covariate, $F(1,67) = 37.29$, $p < .001$, which was positively associated with perceptions of concentration during the test battery at later test points. There was no significant main effect of condition, $F(1,67) = 1.37$, $p = .247$, and no significant interaction between baseline*condition*week or baseline*condition, and these were removed from the final model.

4.7.4.3 Perceived performance

No outlying observations were removed from the final model. Baseline perceived performance was a significant covariate, $F(1,67) = 20.09$, $p < .001$, with this being positively related to cognitive test battery performance as perceived by participants at midpoint and endpoint. There was no significant main effect of condition, $F(1,67) = 1.73$, $p = .193$, and no significant interaction between baseline*condition*week or baseline*condition, and these were removed from the final model.

4.7.4.4 Frustration

No outlying observations were removed from the final model. Baseline subjective rating of frustration experienced during the cognitive test battery was a significant covariate, $F(1,67) = 17.72$, $p < .001$, which was positively associated with perceived frustration experienced during the measures at midpoint and endpoint. There was no significant main effect of condition, $F(1,67) = 0.30$, $p = .585$, and no significant interaction between baseline*condition*week or baseline*condition, and these were removed from the final model. There was a trend towards a condition*week interaction, $F(1,68) = 2.86$, $p = .096$. Participants in the placebo condition appeared to experience greater frustration over time (0.23 ± 0.06 at midpoint, 0.32 ± 0.06 at endpoint), whereas those in the active condition felt less frustrated across the test sessions (0.35 ± 0.06 at midpoint, 0.29 ± 0.06 at endpoint).

4.7.5 Summary of findings

4.7.5.1 Effects of a 6 week GPL with SM intervention on cognitive performance in children aged 6-8 years

A tabulated summary of cognitive performance outcomes following receiving Lacprodan® PL-20 or placebo for 6 weeks is shown in Table 4.13.

Table 4.13 Tabulated summary of cognitive performance outcomes following 6 weeks of supplementation

	Main effects		**Covariates**						
Cognitive outcome	**Condition**	**Week**	**Baseline**	**Age**	**IQ**	**Gender**	**Trial**	**Sum (total) of attempts**	**Highest span achieved**
Processing speed: Choice Reaction Time (CRT) & Simple Reaction Time (SRT)									
Movement time (correct trials; CRT)	NS	NS	S (p = <.001)	-	NS	MS (p = .050)	MS (p = .050)	/	/
Number of correct trials (CRT)	NS	NS	S (p = .006)	-	-	-	/	/	/
Reaction time (correct trials; CRT)	S (p = .009)	NS	S (p <.001)	-	NS	-	S (p <.001)	/	/
Number of correct trials (SRT)	NS	NS	S (p = .003)	-	NS	T (p = .084)	/	/	/
Reaction time (correct trials; SRT)	NS	S (p = .025)	S (p <.001)	-	NS	S (p = .031)	S (p = .002)	/	/
Movement time (correct trials; SRT)	NS	MS (p = .064)	S (p <.001)	MS (p = .058)	NS	-	-	/	/

	Main effects		Covariates						
Cognitive outcome	**Condition**	**Week**	**Baseline**	**Age**	**IQ**	**Gender**	**Trial**	**Sum (total) of attempts**	**Highest span achieved**
Verbal memory: Rivermead Behavioural Memory Test for Children (RBMT-C)									
Immediate recall	NS	NS	S (p < .001)	T (p = .073)	-	S (p = .047)	/	/	/
Delayed recall	MS (p = .055)	NS	-	MS (p = .065)	T (p = .077)	NS	/	/	/
Visuospatial recognition memory: Spatial Recognition Memory (SRM)									
Number of correct trials	NS	S (p = .047)	T (p = .074)	-	-	-	/	/	/
Reaction time (correct trials)	S (p = .047)	T (p = .071)	S (p <.001)	-	MS (p = .051)	-	S (p <.001)	/	/
Motor skills: Motor Screening Task (MOT)									
Reaction time	NS	NS	S (p = .012)	NS	NS	-	S (p <.001)	/	/
Distance	NS	S (p = .021)	S (p = .034)	-	-	S (p = .004)	T (p = .094)	/	/
Executive function (working memory): Spatial Span (SSP)									
Number of correct trials	T (p = .086)	NS	NS	S (p = .014)	S (p = .007)	S (p = .007)	/	S (p <.001)	S (p <.001)
Reaction time (correct trials)	NS	NS	S (p <.001)	-	NS	NS	S (p <.001)	S (p = .003)	/
Highest span achieved	T (p = .073)	NS	S (p <.001)	NS	-	-	/	S (p <.001)	/

Table 4.13 continued.

	Interaction terms							
Cognitive outcome	**Condition* week**	**Baseline* condition**	**Baseline*week**	**Baseline*condition* week**	**Trial*condition* week**	**Age*condition* week**	**IQ*condition* week**	**Gender*condition* week**
Processing speed: Choice Reaction Time (CRT) & Simple Reaction Time (SRT)								
Movement time (correct trials; CRT)	S (p = .003)	-	-	S (p = .039)	NS	NS	-	S (p <.001)
Number of correct trials (CRT)	NS	-	NS	-	/	-	-	S (p = .020)
Reaction time (correct trials; CRT)	S (p = .016)	S (p = .018)	-	-	-	-	S (p = .006)	S (p <.001)
Number of correct trials (SRT)	S (p = .019)	-	-	-	/	-	MS (p = .063)	S (p = .001)
Reaction time (correct trials; SRT)	NS	-	S (p = .021)	-	-	-	-	S (p = .002)
Movement time (correct trials; SRT)	S (p = .005)	S (p = .032)	-	-	S (p = .038)	S (p = .010)	S (p = .001)	S (p = .025)

	Interaction terms							
Cognitive outcome	**Condition* week**	**Baseline* condition**	**Baseline*week**	**Baseline*condition* week**	**Trial*condition* week**	**Age*condition* week**	**IQ*condition* week**	**Gender*condition* week**
Verbal memory: Rivermead Behavioural Memory Test for Children (RBMT-C)								
Immediate recall	MS (p = .065)	-	-	-	/	NS	-	NS
Delayed recall	NS	NS	NS	NS	/	-	-	NS
Visuospatial recognition memory: Spatial Recognition Memory (SRM)								
Number of correct trials	NS	-	-	NS	/	-	NS	-
Reaction time (correct trials)	NS	S (p = .045)	MS (p = .062)	S (p = .030)	-	NS	NS	NS
Motor skills: Motor Screening Task (MOT)								
Reaction time	S (p = .010)	-	S (p = .041)	S (p <.001)	NS	MS (p = .060)	S (p = .011)	-
Distance	NS	NS	NS	-	NS	NS	-	-
Executive function (working memory): Spatial Span (SSP)								
Number of correct trials	NS	-	-	-	/	-	NS	NS
Reaction time (correct trials)	S (p = .018)	-	-	S (p = .002)	-	S (p = .001)	S (p = .005)	S (p = .001)

	Interaction terms							
Cognitive outcome	**Condition* week**	**Baseline* condition**	**Baseline*week**	**Baseline*condition* week**	**Trial*condition* week**	**Age*condition* week**	**IQ*condition* week**	**Gender*condition* week**
Highest span achieved	NS	-	-	-	/	-	NS	-

Notes. MS: Marginally significant, NS: Nonsignificant, S: Significant. T; Trend; – indicates term removed from the final model for best fit, / indicates this was not entered into the model.

A summary of significant, marginally significant, and trends for covariates, main effects and interactions following the 6 week intervention is provided below:

Baseline performance as a covariate by cognitive measure outcome:

Cognitive performance at baseline was positively associated with subsequent cognitive performance on thirteen out of fifteen cognitive outcomes. These measures were of memory performance (verbal memory: RBMT-C immediate recall and visuospatial memory: SRM reaction time for correct trials and number of correct trials), motor skills (MOT reaction time and distance), working memory (SSP highest span achieved and reaction time for correct trials), and processing speed (SRT and CRT number of correct trials and reaction and movement time for correct trials respectively).

Age, IQ and gender of participants as covariates by cognitive measure outcome:

Age, as expected, was positively correlated with performance, such that on a measure of memory performance (RBMT-C immediate and delayed recall), older participants recalled more items (trend for immediate recall, marginally significant for delayed recall). On a measure of working memory (SSP number of correct trials), older participants were also more accurate. On this same outcome measure, those with a higher IQ were more accurate (more correct trials). In addition, on separate memory measures, specifically SRM reaction time for correct trials, participants with a higher IQ took longer to respond (marginally significant), whilst on the RBMT-C delayed recall, those with a higher IQ recalled more items (trend). Females recalled more items than boys on a measure of verbal memory (RMCT-C immediate recall) and were more accurate, responding closer to the stimulus, on a measure of motor skills (MOT distance). However, males performed better than females on a measure of working memory (SSP number of correct trials) and demonstrated faster reaction time and movement time on measures of processing speed (SRT reaction time for correct trials and CRT movement time (marginally significant)).

Other covariates based upon individual participant performance by cognitive measure outcome:

Trial, the total number of attempts at each span to the highest span achieved (by week) and highest span achieved (by week) were also added where appropriate as covariates to the model. Performance tended to fluctuate by trial, whereas performance was consistently positively associated with total number of attempts and highest span achieved on the SSP measure.

Main effect of condition by cognitive measure outcome:

Participants who received the placebo treatment recalled more items following a delay on a measure of verbal memory (RBMT-C, marginally significant) and demonstrated faster reaction

times on a measure of visuospatial memory (SRM reaction time for correct trials, trend). However, those in the active condition showed superior performance on a measure of working memory (SSP highest span achieved, trend). This was also observed (trend) on the same measure for the number of correct trials outcome, consistent with expectations given the number of correct trials was positively associated with highest span achieved.

Interaction between condition and week by cognitive measure outcome:

Those in the placebo condition also recalled more items compared to participants in the active condition on immediate verbal memory (RBMT-C, marginally significant), such that those in the active condition recalled significantly less items at midpoint relative to midpoint and endpoint performance of those in the placebo condition. On a measure of processing speed (SRT), participants in the placebo condition showed significantly faster movement times than those in the active condition at midpoint. However, participants in the active condition demonstrated significantly slower movement times at midpoint relative to their performance at endpoint. On a measure of motor skills (MOT reaction time), those in the placebo condition demonstrated faster reaction time at endpoint relative to those in the active condition (trend). Those in the placebo condition also got faster over time on a measure of working memory (SSP reaction time for correct trials). The active condition illustrated faster movement time over time on a measure of processing speed (CRT movement time) – both significantly slower at midpoint compared to endpoint.

Interaction between baseline performance and condition by cognitive measure outcome:

Performance by condition differed as a function of baseline performance for SRM reaction time for correct trials (visuospatial memory), SRT movement time and CRT reaction time (processing speed). Specifically, those in the placebo condition showed significantly faster reaction times on SRM for correct trials at poorer baseline performance. This was also observed for movement time on SRT measure when baseline movement times were slow. On CRT reaction time, those in the placebo condition were significantly slower compared to those in the active condition when baseline performance was faster, however, when the baseline performance was slower, those in the placebo condition showed quicker reaction times (significant and marginally significant) relative to participants in the active condition.

Interaction between baseline performance, condition and week by cognitive measure outcome:

Performance by condition and week also varied as a function of baseline performance. Participants in the placebo condition were significantly faster than those in the active condition at endpoint when baseline performance was poorer (and significantly slower at midpoint vs.

endpoint) on SRM reaction time for correct trials (visuospatial). On the MOT (motor skills), those with faster baseline reaction times showed significantly faster reaction time following the placebo than active treatment at endpoint. However, those in the active condition were significantly faster at midpoint compared to endpoint and those in the placebo condition responded more slowly at midpoint than at endpoint (marginally significant and a trend at different baseline performance). On the same measure at mid (marginally significant) to poorer (significant) baseline performance, those in the placebo condition were faster than those in the active condition at midpoint, however, at poorer baseline reaction times, participants in the active condition were significantly slower at midpoint relative to endpoint. Those in the placebo condition also demonstrated significantly faster reaction times compared to those in the active condition at endpoint at mid to poorer baseline performance (those in the placebo condition were also significantly slower at midpoint compared to endpoint) on the SSP measure (working memory).

Interaction between age of participants and condition and week by cognitive measure outcome:

Age*condition*week was a significant interaction on a measure of processing speed (SRT: movement time for correct trials) such that at 90 months of age, those in the placebo condition were significantly faster than those in the active condition at midpoint. However, those in the active condition were significantly slower at midpoint relative to endpoint at both 80 and 90 months of age. Lastly, at 100 months of age, participants in the placebo condition moved significantly faster at midpoint relative to endpoint. On a measure of executive function (SSP reaction time for correct trials), participants in the placebo condition reacted significantly slower at midpoint compared to endpoint at 80 and 90 months of age.

Interaction between IQ of participants and condition and week by cognitive measure outcome:

An IQ*condition*week interaction was found for the MOT (motor skills), such that those in the placebo condition demonstrated (significantly and marginally significant) faster reaction times compared to those in the active condition at endpoint at an IQ score of 100 and 110. Furthermore, at an IQ of 80, those in the active condition demonstrated significantly faster performance at midpoint relative to endpoint, whilst there was a trend towards those in the placebo condition responding more slowly at midpoint compared to endpoint on the SSP (executive function). On this same measure, at an IQ of 90 and 100, participants in the placebo condition showed significantly better performance at endpoint than at midpoint; this same pattern of performance was demonstrated by those in the active condition at an IQ of 100 and 110 (marginally significant/significant). IQ*condition*week interaction was also found for SRT

(processing speed), such that at an IQ score of 90, 100 and 110, those in the placebo condition demonstrated (significant and marginally significant) faster movement times compared to those in the active condition at midpoint, although the active condition was significantly slower at midpoint relative to endpoint. On the other measure of processing speed (CRT), those in the placebo condition showed marginally significantly faster response latencies at endpoint relative to midpoint at an IQ of 100 and 110 (marginally significant/significant).

Interaction between gender of participants and condition and week by cognitive measure outcome:

Gender*condition*week interactions were found for both measures of processing speed (movement time, reaction time and number of correct trials). On SRT, both males in the active condition (significant) and those in the placebo condition (marginally significant) demonstrated greater accuracy at midpoint than endpoint. Males in the placebo condition showed faster (marginally significant) movement times to females in the active condition at midpoint, although females in the active condition were slower (marginally significant) at midpoint compared to endpoint. On the same measure, females in the placebo condition responded significantly slower relative to males in the active condition at endpoint, although the reaction times of females in the placebo condition were significantly faster at midpoint compared to endpoint. On the CRT, males in the placebo condition completed less trials correctly than males in the active condition at endpoint (trend) and females in the active condition demonstrated significantly faster movement time over time, whereas females in the placebo condition showed significantly slower movement time over time. Females in the placebo condition showed slower reaction times (marginally significant) relative to males in the same condition at endpoint. Males in the placebo responded marginally significantly faster over time.

4.7.5.2 Effects of a 6 week GPL with SM intervention on subjective state in children aged 6-8 years

A summary of significant, marginally significant, and trends for covariates, main effects and interactions following the 6 week intervention is provided below:

Baseline self-ratings as a covariate on subjective state:

Baseline self-perceptions were positively associated with subsequent self-ratings (at midpoint and endpoint) on all subjective evaluations concerning appetite, mood, motivation and mental alertness.

Main effect of condition on subjective state:

Participants in the placebo condition rated themselves as more cheerful than those in the active condition (trend).

Interaction between baseline self-ratings and condition on subjective state:

Subjective ratings by condition varied as a function of baseline ratings for ease of distraction. For lower baseline ratings (less easily distracted), those in the placebo condition rated themselves (significantly and marginally significantly) less easily distracted compared to those in the active condition.

Interaction between baseline self-ratings, condition and week on subjective state:

Subjective ratings by condition and week also differed by baseline ratings. Participants in the placebo condition rated themselves higher for bad temperedness compared to those in the active condition at midpoint at lower-to-mid and higher baseline subjective ratings (significant). However, those in the placebo condition rated themselves higher for bad temperedness at midpoint relative to endpoint at the same baseline subjective responses (significant) and those in the active condition rated themselves as lower for bad temperedness at midpoint compared to endpoint (trend) at mid and higher baseline subjective ratings. Further, at lower-to-mid baseline subjective ratings, participants in the placebo condition reported greater keenness to try hard perceptions at midpoint relative to endpoint (significant and marginally significant).

4.7.5.3 Effects of a 6 week GPL with SM intervention on cognitive test evaluation ratings in children aged 6-8 years

A summary of significant, marginally significant, and trends for covariates, main effects and interactions following the 6 week intervention is provided below:

Baseline self-ratings as a covariate on cognitive test evaluations:

Baseline subjective ratings were positively associated with subsequent subjective ratings (at midpoint and endpoint) cognitive test evaluation ratings.

Interaction between condition and week on cognitive test evaluations:

Perceived frustration experienced during the cognitive measures seemed to change by condition and week (trend), such that those in placebo condition became more frustrated from midpoint to endpoint whereas those in the active condition became less frustrated over time.

4.8 Discussion

4.8.1 Overview of findings

The primary aim of the intervention study reported in this Chapter was to investigate whether supplementing usual diet with a GPL and SM bovine milk-based supplement, Lacprodan® PL-20, for 6 weeks would confer a cognitive performance advantage in children aged 6 – 8 years. A secondary aim was to assess whether supplementation with Lacprodan® PL-20 affected subjective evaluations of appetite, mood, motivation and mental alertness. It was hypothesised that Lacprodan® PL-20 supplementation would promote cognitive function, and that this would translate to better performance on cognitive measures assessing memory, motor skills, working memory and processing speed. The findings of this study provide limited support for a benefit of Lacprodan® PL-20 supplemented for 6 weeks on cognitive performance in a sample of school-aged children. Lacprodan® PL-20 was also not found to influence subjective evaluations. In respect of the primary outcome (CRT: Movement time), the lack of a significant (or marginally significant or trend) main effect of condition in favour of the active condition indicates that there was no advantage to those who received the active supplement. Baseline performance was positively related to performance on later test occasions, a common pattern seen across the measures. Interactions that remained significant following post hoc analysis indicated significant interactions between gender, condition and week, and condition and week. Females in the active condition were significantly slower at midpoint vs. endpoint, whilst females in the placebo condition were significantly faster at midpoint vs. endpoint. The former most likely underlies the condition and week interaction, such that those in the active condition performed significantly slower at midpoint vs. endpoint. Performance differences in favour of those in the active condition as shown on secondary outcomes include a trend towards superior performance (compared to the placebo condition) on the Spatial Span measure (working memory) for two outcomes including the number of correct trials and highest span achieved. Also on the CRT, there was a trend towards males in the active condition completing more trials correctly compared to males in the placebo condition at endpoint. Inspection of the findings for reaction time and movement time on the CRT indicates that there was no significant difference in either of these outcomes between males of each condition. This therefore suggests that there was no speed-accuracy trade-off by males in the placebo condition i.e. they got more correct but were not slower. On the same measure, at faster baseline reaction time, the active condition demonstrated significantly faster reaction time relative to the placebo condition.

In terms of mood, baseline subjective ratings of bad temperedness were related to subsequent ratings. Those in the placebo condition rated themselves significantly higher for bad temperedness compared to the active condition at midpoint. However, it was also found that at the same baseline subjective ratings, those in the placebo condition rated themselves significantly higher for bad temperedness at midpoint relative to endpoint. There was also a trend for the active condition rating themselves lower at midpoint than at endpoint. Therefore, taken together, it can be concluded that subjective ratings of bad temperedness are independent of treatment condition.

4.8.2 Possible explanations for the lack of a significant treatment effect on cognition and subjective evaluations following 6 week supplementation with Lacprodan® PL-20.

4.8.2.1 Study quality

The study design conformed to a randomised, double-blind, placebo-controlled study design, which is considered the gold-standard in intervention studies. A randomised sequence was used for participant allocation to treatment condition in an effort to distribute both known and unknown factors that could independently influence the outcome(s) of an intervention equally across conditions (Eccles, Grimshaw, Campbell, & Ramsay, 2003). However, there was a difference in the distribution of IQ, which was a significant covariate for some outcomes and may be a factor in the lack of effect/placebo advantage. Supplements were concealed in TetraBrik® cartons, with only the colour and fruit character appearing on the front of each carton (to identify flavour) and a condition code differing between cartons. All experimental drinks (supplement) were matched on taste and appearance and were consumed in a controlled manner under supervision, and the quantity consumed was documented on each occasion. All research staff, schoolteachers and children were blind as to which children were allocated to either of the two conditions (double-blind) and research staff were only unblinded following the completion of data analysis. All outcome data has been analysed and reported according to the *a priori* plan of measures utilised in the study. Taken together, the study demonstrates a low risk of bias and can be classed as being of good quality according to the Cochrane risk of bias tool for randomized clinical trials (Higgins et al., 2011).

The study also demonstrated good practice by familiarising participants with the cognitive measures, allowing sufficient practice prior to the study. This serves to reduce the extent of

practice effects, which are strongest between the first two study visits (Bell et al., 2018). Minimal improvements have been reported following a second test session in the context of multiple-testing using neuropsychological measures (Beglinger et al., 2005). In addition, parallel versions of the cognitive measures were used for practise, which is also recommended to minimise practice effects in the context of multiple testing (Beglinger et al., 2005; Bell et al., 2018).

Based on the number of participants included in the per protocol analysis, observed difference in LS-means and variability (standard deviation) on the primary outcome, an *a posteriori* calculation determined the power of the present study to detect a difference between the active and placebo conditions by week (baseline, midpoint and endpoint) was 0.051, 0.055, and 0.070, respectively. Furthermore, in order to achieve a power of 0.8, a sample size of 25,118, 6281 and 1571 would be required at baseline, midpoint and endpoint, respectively. It can therefore be concluded that the study was underpowered due to an insufficient number of participants.

4.8.2.2 Statistical approach

The statistical approach undertaken in the analysis of the data controlled for covariates that correlate with cognitive function i.e. age, IQ and gender (see section 4.6). Covariate adjustment can increase statistical power for continuous outcomes, as they may explain some of the variation in outcomes between participants, leading to smaller SEs for the treatment effect (Kahan, Jairath, Doré, & Morris, 2014). Importantly, the greater the correlation between a covariate and the outcome, the greater the increase in statistical power and reduction in SE (Pocock, Assmann, Enos, & Kasten, 2002). In eleven of the fifteen cognitive outcomes, demographic characteristics (age, IQ and gender) were either significant, marginally significant or showed a trend. Higher-order interactions included age on three outcomes; IQ featured in five outcomes and gender in six outcomes. Adjusting for IQ was especially important, as analysis of baseline demographic variables indicated that there was a marginally significant difference between the IQ of participants in the two conditions, such that IQ in the placebo condition was higher compared to those in the active condition. Further analysis showed that this difference was due to those in the placebo condition scoring significantly higher than participants in the active condition on the WASI vocabulary subcomponent. Figure 4.5 shows the distribution of scores on this measure and indicates that males and females in the placebo condition tended to demonstrate better performance than males and females in the active condition. This significant difference between the two conditions in WASI vocabulary scores may account for the condition*week interaction and main effect of condition on the immediate and delayed recall outcomes, respectively, of the Rivermead Behavioural Memory Test (a measure of verbal

memory) that are in favour of those in the placebo condition. Moreover, including baseline cognitive performance/subjective ratings as a covariate in the analysis adjusted for differences in performance on the cognitive measures/subjective ratings as assessed at baseline allowing an unbiased estimate of treatment effects (O' Connell et al., 2017). Baseline cognitive performance was a significant covariate in thirteen of fifteen outcomes underlining the importance for the analysis to be adjusted in this way. Similarly, baseline subjective ratings were significant covariates on all subjective evaluation outcomes. Therefore, it would be unwarranted to attribute the lack of a significant treatment effects to the statistical approach.

4.8.2.3 Duration of intervention

The half-life of GPLs in the human brain is currently unknown, making it difficult to estimate the optimal treatment length, assuming dietary consumption contributes to the incorporation of GPLs in the brain. A preliminary estimation of the half-life of DHA in the human brain (whole brain) is reported as 2.5 years, based on the global uptake of circulating unesterified DHA of 3.8 ± 1.7 mg per day and the reported 5 g of DHA in the human brain (Umhau et al., 2009). Consistent with this, another study has reported a daily whole brain incorporation rate of 3.8 ± 2.5 mg per day for APOE ε4 noncarriers, whilst for APOE ε4 carriers, the rate was 4.6 ± 3.3 mg per day (Yassine et al., 2017). Supplementation for 3 months equates to ~10% of this, therefore, the potential for even a slight increase in brain DHA levels following supplementation is likely to take at least a few months (Taha, Burnham, & Auvin, 2010). The plasma half-life of lysoPC (a product of PC hydrolysis by phospholipase A_2 (PLA_2)), ≈5–10 minutes, is comparably longer than the plasma half-life of unesterified DHA (≈ 30 seconds) (Lacombe et al., 2018). If the longer half-life of lysoPC relative to unesterified DHA in plasma is represented in the human brain, it is likely that GPL supplementation will be necessary over many months before there is an opportunity for GPL cell membrane replacement. However, caution should be taken, as data in rodent and human studies show that blood DHA concentration does not correlate with brain DHA integrity (Kuratko & Salem, 2009). This lack of relationship between plasma DHA content and brain DHA levels is also true of DHA esterified to PLs (Tu, Mühlhäusler, Yelland, & Gibson, 2013). This suggests the 6 week duration of the current study is insufficient to affect brain levels of GPLs.

4.8.2.4 Measurement of cognitive performance

Computerised cognitive assessments are ideal when measuring cognitive performance, as flexible test difficulty levels reduce the likelihood of floor and ceiling effects. Further, they afford standardisation of assessment, accurate recording of response times and automation of scoring (Wild, Howieson, Webbe, Seelye, & Kaye, 2008). Given the design of the study reported in this

Chapter, practical considerations included portability of assessment, quick and easy set-up and automated data capture. Another important consideration concerned the sensitivity of the cognitive assessment to detect subtle differences in cognitive performance following nutrient intervention. The impact of nutrition on cognitive performance is likely to be subtle given that it is one of multiple elements that impact the brain and cognitive function (Hughes & Bryan, 2003). To address these priorities and to enable the assessment of a range of cognitive domains, CANTAB was selected as the most suitable cognitive test battery for use in this study. This was for a variety of reasons. Firstly, it was compliant with Good Clinical Practice (GCP), having third party management of raw data. Secondly, it was suitable for use with young children (Isaacs & Oates, 2008). Thirdly, consistent with the practical aspects, CANTAB offers touchscreen response (ease of responding) and automated test presentation.

However, since the current study was conducted, a study assessing the internal consistency and 1-year stability of seven measures of the CANTAB in Finnish school-aged children reported a Cronbach's alpha reliability coefficient of .21 for the SRM measure. Moreover, many of the SRM patterns were found not to correlate and of those that did, correlations were modest. Overall it was concluded that the SRM was not a reliable measure (Syväoja et al., 2015). CANTAB has also been found to show weak-to-moderate correlations with principal components derived from traditional neuropsychological test measures, suggesting CANTAB measures may reflect general cognition and may not be able to measure discrete cognitive functions (Smith, Need, Cirulli, Chiba-Falek, & Attix, 2013). Consistent with this, confirmatory factor analysis revealed SSP highest span and CRT movement time did not consistently load onto the cognitive domain factors derived from traditional neuropsychological measures that assessed the same domains as the CANTAB measures were purported to assess. Although it was acknowledged that the lack of consistency may have been due to the CANTAB measures assessing a different component of the same cognitive domain or even multidimensional cognitive processes, for example, SSP showed shared variance, loading onto multiple factors (Lenehan, Summers, Saunders, Summers, & Vickers, 2016). Moreover, the construct validity of CANTAB has been largely founded on its sensitivity to discriminate clinical populations from healthy populations (Lenehan et al., 2016). The findings outlined above raise some reservations about the suitability (sensitivity and specificity) of CANTAB to assess the effects of nutritional interventions in children.

4.9 Conclusion

A randomised, double-blind, placebo-controlled, parallel group study was undertaken to examine the effect of supplementing a composite of GPLs and SM in school-aged children on cognitive performance and subjective state, specifically, appetite, mood, motivation and mental alertness. As GPLs are known to support cognitive function in multiple ways, it was hypothesised that a 6 week intervention would lead to an improvement in performance on cognitive measures from the CANTAB battery assessing memory, motor skills, working memory and processing speed. School children (n=132) aged 6 – 8 years were allocated to receive 6 weeks of the active (n=66) or placebo (n=66) bovine milk-based experimental drinks. Participants were seen in school 5 days per week and supplement drinks were consumed shortly before break-time in a controlled environment under supervision. The amount of supplement consumed on each occasion was assessed by weighing the supplement drinks prior to and post-consumption. Records of consumption per child were used to determine which participants should be included in a per protocol analysis (n=70). Test occasions took place every three weeks, at baseline (week 0), midpoint (week 3) and post-intervention at endpoint (week 6). The analysis of the study data adjusted for baseline cognitive performance/subjective rating (of subjective state), age, IQ and gender. The findings provided limited evidence for beneficial effects of the active supplement on cognitive performance. The active group were more accurate and achieved a higher span on a measure of working memory (executive function). However, for both outcomes, the performance differences by condition failed to reach statistical significance. There was also a trend towards males in the active condition demonstrating greater accuracy compared to males in the placebo condition at endpoint on a measure of processing speed. Subjective state was not influenced by the active supplement. Other possible explanations for the limited significant cognitive performance differences in favour of the active condition include the intervention period not being of a long enough duration and the possible inappropriate selection of a cognitive battery.

Chapter 5 A randomised placebo-controlled trial examining the effects of acute and chronic phospholipid intake on cognitive performance in adults aged 50 years and over with a subjective memory complaint (Study 2).

5.1 Cognitive decline in both healthy ageing and age-related disease

The strongest risk factor for cognitive decline is age (Troyer et al., 2014). Not all cognitive functions are equally affected by increasing age. Extant literature indicates that processing speed frequently declines with age (Deary et al., 2009b; Ebaid, Crewther, MacCalman, Brown, & Crewther, 2017; Eckert, Keren, Roberts, Calhoun, & Harris, 2010; Zaninotto, Batty, Allerhand, & Deary, 2018), with decline identified as being independent of motor control (Ebaid et al., 2017). Other functions commonly observed as declining with age include executive function (Fjell, Sneve, Grydeland, Storsve, & Walhovd, 2016; Isingrini et al., 2015; Kirova, Bays, & Lagalwar, 2015) and episodic memory (Harada, Love, & Triebel, 2013; Nyberg, 2017; Tromp, Dufour, Lithfous, Pebayle, & Després, 2015).

Processing speed, the capability of processing information rapidly (Ebaid et al., 2017), has been suggested to underlie age-related variation in cognitive functioning (Salthouse, 1996). Cognitive task performance on a range of tasks is vulnerable to, and restricted by, processing constraints and differences in the effectiveness of processes. When processing speed is slow, performance is impaired due to a lack of time to complete relevant operations and the output from earlier processing may not be available when the results of subsequent processing are derived (Salthouse, 1996). Processing speed has been assessed using a number of different variables, such as decision speed, perceptual speed and psychomotor speed (Salthouse, 2000). There is evidence to suggest that processing speed mediates other cognitive functions, for example, along with working memory and inhibitory control, processing speed has been acknowledged as contributing to age-related changes in episodic memory (Head, Rodrigue, Kennedy, & Raz, 2008).

Importantly, deficits in executive functioning may be a marker of likely progression to AD (Clark et al., 2012; Irwin, Sexton, Daniel, Lawlor, & Naci, 2018) and particular executive functions may demonstrate greater sensitivity to global cognitive decline than others. In a prospective longitudinal study (n=71, of which n=51 were cognitively normal and n=20 had MCI), performance differences on measures requiring inhibition and switching were shown to

differentiate between participants who demonstrated a significant decline on the Dementia Rating Scale (DRS) and those who did not over a 1 year period. Importantly, demographic characteristics and DRS score at baseline did not differ significantly between decliners and non-decliners (Clark et al., 2012). Working memory, a type of executive function (Diamond, 2013), facilitates online monitoring and temporarily holding auditory and visual information in mind (Baddeley, 1986; Baddeley, Eysenck, & Anderson, 2015). There is a great deal of empirical support for age-related decline in working memory function. Specifically, age-related decline in verbal and visuospatial working memory has been observed with increasing task complexity (Cansino et al., 2013; Klencklen, Banta Lavenex, Brandner, & Lavenex, 2017). In addition, increased susceptibility to intrusions (De Beni & Palladino, 2004), a decline (and absence in some) in the recency-effect in verbal working memory (Van den Noort, Haverkort, Bosch, Hugdahl, & Hugdahl, 2006), as well as an impairment in memory updating (De Beni & Palladino, 2004) have been documented with increased age.

Episodic memory is extremely vulnerable to cerebral ageing and neurodegeneration (Kinugawa et al., 2013), with deficits being more noticeable in tasks requiring recall rather than recognition (Nyberg et al., 2003). Episodic memory decline is a frequent occurrence in ageing and decline in episodic memory, along with executive function, is a characteristic of pathological ageing (Aretouli & Brandt, 2010; Baudic et al., 2006). Episodic memory performance tends to decline around 65 years of age (Kinugawa et al., 2013), although there is variation in onset and rate of decline (Gorbach et al., 2017). Indeed, the episodic memory trajectory in older adults has been related to ethnic background, years in education and presence of ε4 allele at APOE gene (Lee et al., 2018). Importantly, damage to the temporal lobe has been shown to impede re-experiencing a past event and imagining future events (Race, Keane, & Verfaellie, 2011). A critical part of the medial temporal lobe for episodic memory is the hippocampus and the involvement of the hippocampus in memory formation and maintenance is well established (Behrendt, 2013; Sadeh, Chen, Goshen-Gottstein, & Moscovitch, 2019; Wixted et al., 2018). In a longitudinal study, decline in episodic memory over 15 years in cognitively healthy individuals was significantly associated with reduced volume of the hippocampus over 4 years in those aged 65 - 80 years but not in those aged 55 - 60 years (Gorbach et al., 2017).

5.2 Age-related structural and functional brain changes and their association with cognitive function

Increasing age is not only associated with cognitive decline and neurodegeneration (Corrada, Brookmeyer, Paganini-Hill, Berlau, & Kawas, 2010), but also regional brain atrophy and disrupted

connectivity both in terms of structure and function (Hafkemeijer et al., 2014). The normal maturation of white matter has been suggested to follow an inverted U-trajectory with a maturation peak around midlife (Yeatman, Wandell, & Mezer, 2014). Indeed, middle adulthood has been suggested as the turning point from development to ageing in respect of white matter coherence (Giorgio et al., 2010). This is also reflected in findings from big data analytics, whilst grey matter continues to decrease from the second decade onwards (Coupé, Catheline, Lanuza, & Manjón, 2017). This pattern is reportedly also shown for volume trajectories across the lifespan of almost all subcortical structures except for the amygdala and hippocampus, with the amygdala presenting a more stable volume trajectory until around 80 years of age, whilst the hippocampus also demonstrates an inverted-U pattern although it has a longer maturation period (Coupé et al., 2017). There is also evidence that those areas that myelinate early show a more continuous trajectory of ageing relative to those that mature later (Sowell et al., 2003), while those that are the last to myelinate are also the earliest to deteriorate with age (Kochunov et al., 2007).

Loss of grey matter density (Sowell et al., 2003), white matter integrity (Bennett et al., 2017) and cortical thickness (Fjell et al., 2009), the presence of white matter signal abnormalities (Lindemer, Greve, Fischl, Augustinack, & Salat, 2017) and disruption of structural and functional brain systems (Andrews-Hanna et al., 2007) are all associated with ageing even in the absence of neurological disease. Importantly, age-related alterations are not homogenous but can display individual differences in respect of brain substrates and cognitive functions (Persson et al., 2016; Raz et al., 2005). Indeed, onset and rate of decline vary across individuals (Raz et al., 2005). Notably, individual differences in respect of cognitive dysfunction with age and discrepancies between the extent of cerebral atrophy and clinical outcomes has been discussed previously in the context of cognitive reserve, brain reserve and brain maintenance (Nyberg, Lövdén, Riklund, Lindenberger, & Bäckman, 2012a). For example, education as a proxy of cognitive reserve has been observed as attenuating age-related alterations in cognition (Farina, Paloski, de Oliveira, de Lima Argimon, & Irigaray, 2018; Roldán-Tapia, Cánovas, León, & García-Garcia, 2017). However, any resilience afforded by education may be limited in respect of protective effects (Lavrencic et al., 2018) and the stage in life a person receives further education may be an important factor (Thow et al., 2018). That is, the likelihood of cognitive reserve facilitated by education is increased by recent educational experiences rather than remote (i.e. schooling), which is limited in its contribution, that is, towards premorbid cognitive function only (Wilson et al., 2019).

5.2.1 Changes in white matter integrity with ageing

Diffusion Tensor Imaging (DTI) relies upon MRI signal detection, with the MRI signal intensity reflecting distance and direction of water diffusion (Alger, 2012). Whereas fluid filled areas in the brain, and to a lesser extent grey matter diffusion, is non-directional (isotropic) and unrestricted, diffusion within white matter is anisotropic (exhibiting variations in physical properties when measured in different directions); tissue integrity is inferred based on rate and directionality of molecular diffusion (Bennett & Madden, 2014). In a review of cross-sectional and longitudinal studies using DTI to explore white matter integrity in ageing, a common finding includes decreased fractional anisotropy (degree of anisotropic diffusion) and increased mean diffusivity (average diffusion rate) with increasing age. Although results showed individual differences and overlap across age ranges, the findings suggest deterioration in both the integrity and composition of white matter in ageing (Bennett & Madden, 2014). Further, another common finding is that radial diffusivity (diffusion perpendicular to axonal bundles) tends to be increased in old age (Bennett & Madden, 2014; Bennett et al., 2010), this being a surrogate marker of myelin damage (Winklewski et al., 2018; Zhong et al., 2012). Notably, in one study on age-related changes in grey and white matter across adulthood, reduction in fractional anisotropy was found to be driven by an increase in radial diffusivity, possibly reflecting alterations in myelin sheaths or loss of axons in fibre tracts (Giorgio et al., 2010). Previously, it was suggested that frontal brain regions were disproportionally affected by increasing age (Madden, Bennett, & Song, 2009; Sullivan et al., 2001; Sullivan & Pfefferbaum, 2003), however, others have identified large age-related declines in posterior regions (Bennett et al., 2010; Giorgio et al., 2010; Salat et al., 2005).

Numerous DTI studies have associated white matter integrity with cognitive function, where increased integrity is associated with superior cognitive performance. For example, deterioration in white matter connections have been related to poorer episodic memory (Lockhart et al., 2012; Metzler-Baddeley, Jones, Belaroussi, Aggleton, & O'Sullivan, 2011; Sexton et al., 2010; Yassa, Muftuler, & Stark, 2010), a lack of adoption of the fast speed-accuracy trade-off (Forstmann et al., 2011), and poorer executive function (Jacobs et al., 2013; Ystad et al., 2011), reasoning and cognitive flexibility (Borghesani et al., 2013), grammar learning (Antonenko, Meinzer, Lindenberg, Witte, & Flöel, 2012), interindividual reaction time performance (Fjell, Westlye, Amlien, & Walhovd, 2011) and balance performance (Van Impe, Coxon, Goble, Doumas, & Swinnen, 2012) in older adults. Notably, where effect sizes have been reported in studies that have observed these relationships, the largest were found for executive function and processing speed (Bennett & Madden, 2014). There is also recent evidence

showing white matter integrity is compromised in those with subjective cognitive decline, and this is associated with poorer executive function performance (Ohlhauser, Parker, Smart, & Gawryluk, 2019). Although in another study, white matter integrity was relatively preserved in those with subjective cognitive decline whilst being disrupted in those with amnestic MCI (aMCI) (Wang et al., 2016). Alternatively, no difference was found in white matter integrity of normal and cognitively impaired-not demented (CIND) oldest old (90 - 103 years) (Bennett et al., 2017). Therefore, overall, the findings are inconclusive.

5.2.2 The presence of white matter hyperintensities with age

Increasing age is also associated with macrostructural changes in the brain. That is, increasing age is a risk factor for white matter hyperintensities (WMHs; Zhuang, Chen, He, & Cai, 2018), which along with lacunas and microbleeds, are indicative of cerebral small vessel disease (Frey et al., 2019; Gouw et al., 2011). WMHs are known to increase an individual's risk of stroke and dementia (Prins & Scheltens, 2015; Wardlaw, Valdés Hernández, & Muñoz-Maniega, 2015). Specifically, increased deep WMHs have been found to promote the likelihood of vascular dementia (Smith et al., 2016). Interestingly, in a meta-analysis, the association between WMH and incident dementia was found for population-based studies but not in studies that examined high-risk populations (typically with mild cognitive impairment or a history of stroke). However, it was suggested that this finding be considered with caution owing to small samples and methodological flaws (Debette & Markus, 2010).

Crucially, WMHs have been associated with decline in global cognition, episodic, semantic and working memory and perceptual speed, and been found to contribute to an increased risk of developing MCI in a 6 year longitudinal study (adjusted for age, gender, total grey matter volume, vascular risk factors, and vascular diseases) in older adults assessed as cognitively normal at baseline (Boyle et al., 2016). Moreover, increasing severity of periventricular hyperintensities identified in healthy older adults have been associated with greater decline in processing speed and executive function following adjustment for vascular risk and depressed mood (Prins et al., 2005), and development of persistent cognitive impairment after adjusting for age, APOE ε4 status, incident hypertension, intercranial volume, entry MMSE score, baseline hippocampus and ventricular cerebrospinal fluid volume and rate of ventricular cerebrospinal fluid volume change (Silbert, Howieson, Dodge, & Kaye, 2009). Similarly, increases in white matter signal hyperintensity have been related to a decline in executive function over a ~2 year period in healthy adults aged 50 years plus (Kramer et al., 2007), whereas progression of periventricular hyperintensities has been associated with declines in processing speed over 3

years in healthy adults aged 60-90 years (van Dijk et al., 2008). Additionally, progression of subcortical white matter hyperintensity volume has been related to decline in episodic memory in a longitudinal study (maximum follow-up period of 18 years; $\bar{x}$ 9.1 years) that recruited healthy adults aged ≥ 65 years (Silbert, Nelson, Howieson, Moore, & Kaye, 2008). Notably, in a review of studies considering the relationship between the location of WMHs and cognitive function in older adults, the association between periventricular hyperintensities and impaired memory, executive function and processing speed was identified to a greater extent relative to the association with subcortical hyperintensities. Importantly, the review concluded that it was not possible to determine whether WMHs in different brain regions have differential effects on cognition. However, caution must be exercised when considering these findings given that the review included studies that had recruited not only healthy older adults but also those with MCI and certain dementias (Bolandzadeh, Davis, Tam, Handy, & Liu-Ambrose, 2012).

It has been proposed that WMHs as identified in conventional MRI scans represent the final stage of disease progression (Jokinen et al., 2015). In contrast, DTI is able to detect subtle declines in white matter tract integrity even before WMHs are identifiable (Prins & Scheltens, 2015). The relationship between WMHs and cognition is not straightforward. Indeed, WMHs may highlight a variety of symptoms, not necessarily related to cognitive dysfunction, or those with WMHs may even remain asymptomatic (Tomimoto, 2015). Further, other types of brain damage, for example, grey matter atrophy, may confound any relationship between WMHs and cognitive dysfunction (Tomimoto, 2015; Xiong & Mok, 2011).

5.2.3 Grey matter volume reductions with age

A longitudinal study across 6 years that recruited healthy adults aged 21 – 80 years examined age-related alterations in topological organisation of structural brain networks. Using measurements of regional grey matter volume, more localised organisation was identified in middle-to-old aged individuals (> approximately 50 years of age). This pattern of activity was the opposite of that seen in those identified as young to middle-aged, who showed greater global efficiency (< approximately 50 years of age; Wu et al., 2013). Consistent with this, examination of structural covariance networks to study the pattern of covariance in grey matter volumes across different regions of the cortex revealed age-related topological changes. For younger individuals (18-23 years), topological organisation tended to be more distributed whereas middle-aged individuals (30-58 years) had more localised topology, which was sustained in those aged 61-89 years (Li et al., 2013). Age-related grey matter volumetric reductions have been observed in frontal, temporal and parietal lobes, whilst the occipital lobe seems to be relatively

unaffected (Ramanoël et al., 2018). Similarly, in a 4-year longitudinal study, the highest rates of regional grey matter loss in non-demented 65-82 year olds (n=1,172) were in the frontal and parietal cortices, temporal cortex as well as in the hippocampi and the middle and superior occipital gyri. Moreover, the annual rate of global grey matter atrophy was significantly larger for women, chiefly in the frontal and parieto-occipital cortices, whereas the bilateral hippocampi showed age-related accelerated atrophy, which was similar across gender. Interestingly, educational attainment was found not to attenuate grey matter loss, which may reflect education promoting cognitive reserve (e.g. connectivity efficiency) rather than the number of neurons (Crivello, Tzourio-Mazoyer, Tzourio, & Mazoyer, 2014). Grey matter atrophy, WMHs and white matter integrity (fractional anisotropy) have been found to be independently associated with cognitive function but of these, grey matter volume was more closely associated with cognitive performance in a sample, aged 60 years and over, of cognitively healthy and impaired adults. This finding is likely due to grey matter atrophy representing damage to neuronal soma, dendrites and synapses, thereby causing impairment in nodes in the neural networks, whereas WMHs and deterioration in white matter integrity may only impede tract transmission between regions that may be connected by multiple fibre pathways (He et al., 2012).

Global grey matter volume corrected for brain atrophy has been suggested as predicting executive function in cognitively healthy older adults, with those with larger grey matter volumes showing better performance on the Trail Making Test B (Laubach et al., 2018). Importantly, grey matter network disintegration was found to partially mediate the relationship between age and cognitive function. Specifically, grey matter integrity of the fronto-occipital, temporal, limbic, cuneal and secondary somatosensory networks has been associated with memory performance. Integrity of the cerebellar, fronto-parietal, temporal, secondary somatosensory, limbic and sensorimotor networks was associated with executive function in healthy older adults (Koini et al., 2018).

5.2.4 Alterations in functional connectivity with age

The measurement of functional connectivity (FC) aligns with the notion that coordinated activity between distinct, segregated brain regions supports cognition and where connectivity decreases imply compromised information transfer between brain regions (Hermundstad et al., 2013; Sala-Llonch, Bartrés-Faz, & Junqué, 2015). Resting-state FC is measured by taking fMRI scans sequentially during a period of undirected wakefulness (resting-state fMRI) to identify low frequency BOLD fluctuations that are correlated across distant brain areas (Sala-Llonch et al.,

2015). Resting-state FC of healthy adults (59-74 years) has shown decreased connectivity relative to younger adults within and between functional networks except for somatomotor and visual networks, which demonstrated an increase (Geerligs, Renken, Saliasi, Maurits, & Lorist, 2015). Moreover, age-related differences in intrinsic connectivity patterns have also been seen in a sample of healthy young and middle-aged adults (18-65 years). Specifically, with increasing age, increases in FC were seen in paralimbic cortical and subcortical regions, whilst decreases were found in visual cortex and several nodes of the DMN, a resting-state functional network, including the medial prefrontal, posterior cingulate (although this disappeared when head motion was controlled for), precuneus, lateral parietal and middle frontal gyrus. The increase in connectivity shown was proposed to reflect age-related changes in emotion processing and regulation capacity (Hampson et al., 2012).

The reduction in FC in the DMN is consistent with previous findings using resting-state fMRI in healthy adults (Andrews-Hanna et al., 2007; Betzel et al., 2014; Onoda, Ishihara, & Yamaguchi, 2012; Song et al., 2014; Tomasi & Volkow, 2012; Wang et al., 2010). Notably, the DMN has received most attention compared to other resting-state functional networks (Sala-Llonch et al., 2015; Tao et al., 2015) and reduced FC has been proposed to be a potential biomarker for AD (Balthazar, de Campos, Franco, Damasceno, & Cendes, 2014; Mohan et al., 2016), in which FC reduction is particularly acute (Dennis & Thompson, 2014; Klaassens et al., 2017).

Integrity of the network associations with the default mode regions in healthy adults has been implicated in cognitive function. Previous research reports reduced FC is associated with poorer working memory performance (Hampson, Driesen, Skudlarski, Gore, & Constable, 2006; Sambataro et al., 2010), associative memory (Wang et al., 2010), episodic memory, processing speed and executive function composites (Andrews-Hanna et al., 2007), verbal and visual memory (Sala-Llonch et al., 2014) and general intelligence (van den Heuvel, Stam, Kahn, & Hulshoff Pol, 2009). Also, within-DMN FC has been specifically associated with episodic memory (Greicius, Srivastava, Reiss, & Menon, 2004; Staffaroni et al., 2018).

5.2.5 Interim summary: Age-related cognitive decline and structural and functional brain changes, and their association with cognitive function

Ageing is the strongest risk factor for cognitive decline. Cognitive domains including processing speed, executive function and memory are found to be predominantly compromised with age. Anatomical and functional brain alterations are associated with healthy ageing and cognitive impairment. A decline in grey matter volume is seen post the first 10 years of life, whilst white matter coherence starts to diminish in mid-life. Volume trajectories of most subcortical

structures follow the same pattern as grey matter volume decline except for the amygdala, the trajectory of which is more stable, and the hippocampus, which peaks during midlife. The stable development of the amygdala until 80 years of age may explain emotional stability observed in ageing (Scheibe & Carstensen, 2010).

With increasing age, grey matter has been observed as showing more localised organisation. In healthy older adults, the highest rates of regional grey matter loss have been reported in frontal and parietal cortices, temporal cortex, hippocampi and the middle and superior occipital gyri. Global grey matter volume has been related to cognition, with greater volume being more favourable for executive function. DTI has revealed that white matter integrity and composition deteriorates with age, which has been found to relate to poorer cognitive performance in multiple domains such as episodic memory, executive function, processing speed, reasoning as well as balance. Further, myelin damage is also associated with ageing, possiby reflecting axonal loss, which is commonly observed in normal ageing and in age-related diseases (Salvadores, Sanhueza, Manque, & Court, 2017). Similarly, poorer global cognition as well as reduced memory, executive function and processing speed performance has also been related to the presence of WMHs, the likelihood of which is increased with age. Moreover, WMHs have also been found to increase the risk of stroke and cognitive impairment (both MCI and DM). However, WMH lesions are not consistently associated with cognitive dysfunction and in some cases, there may be no symptoms at all. Grey matter volume was found to associate more closely with cognitive performance in cognitively healthy and impaired older adults relative to WMHs and white matter integrity, which are all independently related to cognitive function. Resting-state FC measures show coordinated activity of spatially-distinct brain regions when at rest (undirected wakefullness) and also show age-related differences. Reduced FC in the DMN, a resting-state functional network, is important for memory, executive function, processing speed and general intelligence, and has been proposed as a potential biomarker for AD.

5.3 Cell membranes in ageing

5.3.1 Decline in cell membrane lipids in healthy ageing

Exploration of the membrane lipid composition in the frontal and temporal cortices in healthy adults (no pathological changes; 20 – 100 years of age) has revealed that PLs diminish linearly from 20 years of age (Svennerholm, Boström, Jungbjer, & Olsson, 1994). It has been reported that the rate of decline in PL quantity is slow between the ages of 20 and 80, after which, it becomes fairly marked (Svennerholm, Boström, Helander, & Jungbjer, 1991; Svennerholm et al.,

1994). Reductions in myelin lipids have also been identified as occurring from 20 years of age also (Svennerholm et al., 1994). Ageing is also associated with a decline in cerebral omega-3 PUFA levels owing to reduced absorption, decreased capacity to cross the blood-brain barrier (BBB) and reduced conversion of shorter-chained FAs to longer FAs (Yehuda, 2012). Furthermore, as discussed in Chapter 2 (section 2.6), the supply of omega-3 PUFA from a Western diet is suboptimal for the aged brain (Woo, 2011).

However, as discussed in Chapter 2, impairment of mitochondrial bioenergetic function and increased oxidative stress promote lipid peroxidation, which disturbs the physicochemical properties of membrane lipids and their normal behaviour. For a full discussion concerning mitochondrial dysfunction, oxidative stress and inflammation, and the impact of these on GPLs as well as the potential for GPL supplementation to restore and ameliorate both respectively, see Chapter 2 (sections 2.7.5 and 2.7.6).

5.3.2 Cell membrane alterations in neurodegeneration

Degradation of cellular membranes is a hallmark of neuronal degeneration. Pronounced alterations in neural membrane GPL composition have been observed in neurological disorders (Shamim, Mahmood, Ahsan, Kumar, & Bagga, 2018). Neurological disorders and neurodegenerative disease both involve uncontrolled lipid metabolism (Shamim et al., 2018). Changes in the molecular species of GPLs have also been identified such that PC containing DHA (PC-DHA) have been found to be selectively depleted in the grey matter of patients with AD; and in brain regions with pronounced Aβ deposition, the extent of PC-DHA reduction was significantly related to disease duration (Yuki et al., 2014). Post-mortem brain sample studies highlighted abnormal metabolism of PLs in AD during the 1980s and 1990s. Crucially, a previous study reported that increased membrane PL degradation, including reductions in PC and PE, was specific to AD and no other forms of neurodegeneration, such as Parkinson's disease (Nitsch et al., 1992). Findings from experimental studies suggest PL deterioration intensifies the formation of amyloid, which has a reciprocal effect, and the viability of cholinergic neurons may be vulnerable to the degradation of choline containing compounds including PC (Klein, 2000). The study of autopsy brain samples from AD patients has further revealed that concentrations of PI and PE are significantly reduced, and the availability of PA has also been found to be lower in the superior temporal gyrus in AD brains. These findings were not affected by APOE genotype (Pettegrew, Panchalingam, Hamilton, & McClure, 2001). More recently, other findings from AD post-mortem brain tissues report that pooled data from the frontal cortex, temporal cortex, and cerebellum showed significant reductions in the levels of PC, phosphatidylcholine plasmalogen,

and lysoPC (Grimm et al., 2011). Further, AD has been observed as being associated with aberrant lipid profiles in lipid rafts taken from the frontal cortex (Martín et al., 2010). Similarly, alterations in the lipid rafts from frontal and entorhinal cortices in AD brains have been identified in the initial stages of the disease. Alterations in the lipid matrix impacted lipid classes and FAs in cortical lipid rafts and these alterations were paralleled by a rise in microviscosity, membrane order and amyloid precursor protein accumulation within rafts (Diaz, Fabelo, Ferrer, Marin, & Martin, 2017).

AD is accompanied by increased lipid peroxidation (Shamim et al., 2018), leading to the formation of α, β-unsaturated aldehydes, which in turn mediate inflammation (Lee & Park, 2013). As discussed in Chapter 2 (section 2.7.6) inflammation is associated with neurodegenerative diseases (Amor, Puentes, Baker, & van der Valk, 2010; Chitnis & Weiner, 2017; Stephenson, Nutma, van der Valk, & Amor, 2018). Oxidising conditions brought on by ROS cause protein cross-linking and Aβ aggregation (Shamim et al., 2018). Indeed, Aβ plaques identified in AD have been associated with oxidative damage and PET studies have shown reduced brain metabolism prior to the development of neuropsychological impairment and anatomical alterations (Sullivan & Brown, 2005). Accumulation of neurotoxic proteins in AD typically co-occurs with mutations in mtDNA, impairment of mitochondrial integrity and oxidative phosphorylation, depletion of ATP and greater oxidative stress and subsequent necrosis (Aufschnaiter et al., 2017). Importantly, mitochondrial generation of ATP is directly linked to the regulation of synthesis, trafficking and degradation of GPLs (Paradies, Paradies, Ruggiero, & Petrosillo, 2013), therefore, depletion of ATP will likely have consequences for these processes. Mitochondrial derived ROS are frequently seen in neurodegenerative disease and can result in lipid peroxidation and changes in membrane lipid composition (Aufschnaiter et al., 2017), as discussed in Chapter 2 (section 2.7.5).

Changes in mitochondrial associated membranes (MAMs) are claimed to play a role in several neurodegenerative diseases (Vance, 2014). Mitochondrial dysfunction as seen in AD, amyotrophic lateral sclerosis, Huntington's disease and Parkinson's disease (Lezi & Swerdlow, 2012) is known to happen early on in these disease states, suggesting this may be at the root of these neurodegenerative diseases, with protein oligomerization following as a result (Lane, Hilsabeck, & Rea, 2015). Consistent with this, mitochondrial abnormality has been identified as a universal prelude to neurodegeneration (Kidd, 2005). Indeed, brain samples from patients with MCI show elevated levels of aldehydes, suggesting a possible role of lipid peroxidation in the early stages of neurodegeneration (Butterfield et al., 2006; Reed et al., 2008; Reed, Pierce, Markesbery, & Butterfield, 2009).

5.4 Previous empirical findings reporting positive effects of GPLs on cognitive function

In respect of the aims of the present study (see section 5.5), the systematic research review in Chapter 3 identified two studies of particular interest. The first reported a treatment advantage (300 mg of bovine cortex PS per day) on immediate and delayed visual and verbal associative (Name-face association) acquisition and delayed recall at 3 and 6 weeks following the start of the intervention in a sample of middle-aged adults meeting the criteria for AAMI (Crook et al., 1991). The second found a benefit of PS-DHA/EPA (300 mg PS, 79 mg DHA+EPA) after 15 weeks of treatment in a sample of older adults with subjective memory complaints. Specifically, beneficial treatment effects were reported for immediate verbal recall (trial 1 of Rey Auditory Verbal Learning Test (RAVLT), while sub-group analysis including participants that demonstrated better performance at baseline found a benefit for immediate verbal recall, verbal total learning (sum of scores of trial 1-5) and delayed verbal recall (Vakhapova et al., 2010). Also of interest are the three studies that reported a treatment advantage following the administration Lacprodan® PL-20 for either 3 (Hellhammer et al., 2010) or 6 weeks (Boyle et al., 2019; Schubert et al., 2011). Positive treatment effects reported included a significant reduction in reaction time on an attention switch measure (Boyle et al., 2019), significantly better visual-spatial memory performance, although this was found only after post-hoc analysis split by age (Schubert et al., 2011), and marginally significantly faster reaction time on a measure of verbal recognition memory after controlling for inter-individual variation in cortisol concentration (Hellhammer et al., 2010). However, unlike the participants recruited in the present study, the samples in these studies included young adults and the experimental procedure aimed to induce acute stress to enable exploration of the potential protective effects of PLs on cognitive function.

Other empirical work undertaken to explore treatment effects of GPLs in cognitively healthy older adults with subjective memory complaints not meeting the criteria for inclusion in the systematic research review (Chapter 3) have also reported a benefit to cognitive function. Moré et al. (2014) recruited adults who reported some memory problems in daily life in a single-centre randomised, double-blind, placebo-controlled, parallel group study. Participants consumed capsules containing either 100 mg PS + 80 mg PA mixed with lecithin or a placebo (500 mg lecithin) three times per day for 3 months. The PS + PA condition demonstrated significantly better performance on the information, visual memory and memorising numbers components of the Wechsler Memory Scale compared to those in the placebo condition. In a pilot study (open-label), eight older adults with subjective memory complaints took PS-omega-3 capsules that provided 300 mg PS and 37.5 mg EPA + DHA per day for 6 weeks (Richter, Herzog, Cohen,

& Steinhart, 2010). Significant improvements were reported for delayed verbal recall (word list) and reaction time of detections in the vigilance task. However, although these findings are promising, this pilot study used a small sample, did not use a control group and did not correct for multiple testing despite using the Cognitive Drug Research battery to assess participants. Therefore, the findings should be considered with caution.

5.4.1 Interim summary: Age-related changes to cell membranes and GPL supplementation studies in adults with a subjective memory complaint.

PL reduction in cell membranes has been identified in frontal and temporal cortices from twenty years of age in healthy adults. Ageing is also accompanied by reduced cerebral levels of omega-3 PUFA due to changes in absorption, poorer capacity to reach the brain and reduced FA conversion of shorter-chained FAs. Moreover, comsuming a Westernised diet contributes to suboptimal provision of omega-3 PUFA for the aged brain.

Cellular membrane degradation is a typical characteristic of neuronal degeneration (e.g. dementia). Alterations in brain membrane GPL concentrations and composition including their constituent FAs, and aberrant lipid metabolism are recognised in neurological disorders, although the presentation of such may vary by disease state. Similarly, alterations in lipid rafts have been associated with AD with a concomitant reduction in lipid fluidity, increase in membrane order and amyloid precursor protein accumulation within rafts. Mitochondrial dysfunction and oxidative stress leading to lipid peroxidation and inflammation are observed in normal ageing and in neurodegeneration. In AD, inflammation is related to Aβ aggregation seen prior to the development of neuropsychological impairment and anatomical alterations. This accumulation may have a knock-on effect on the regulation of synthesis, trafficking and degradation of GPLs, due to depletion of ATP. Importantly, mitochondrial dysfunction may have a causal effect in numerous neurodegenerative diseases.

Previous studies that have found a benefit of GPL supplementation on cognitive function that are of relevance to the present study have recruited middle-aged and older adults with subjective memory complaints and reported treatment-related improvements in memory. In addition, three studies supplementing Lacprodan® PL-20 to explore whether the composite supplement protects against stress-induced cognitive performance impairments also reported a benefit to memory and vigilance in young adults.

5.5 Aims of Study 2 and related hypotheses

The primary aim of the study presented in this Chapter was to assess whether acute (90 minutes) and/or chronic (12-weeks) supplementation of Lacprodan® PL-20 enhances cognitive performance on measures assessing memory and executive function in adults aged 50 years or over with a subjective memory complaint (SMC). Adults with a SMC were recruited to the study, as SMCs have been claimed to potentially provide an early clinical marker of deterioration prior to the onset of MCI due to AD (Burmester, Leathem, & Merrick, 2016; Jessen et al., 2010; Slot et al., 2019; Studart & Nitrini, 2016). Hence this sample enabled the opportunity to explore whether a GPL supplement (also containing SM) could foster cognitive performance improvements in an at-risk group (i.e. those at risk of further cognitive decline). Indeed, diet and nutrition are reported to be important modifiable risk factors that may provide the potential to delay or even prevent the onset of cognitive decline (Caracciolo, Xu, Collins, & Fratiglioni, 2014; van de Rest, Berendsen, Haveman-Nies, & de Groot, 2015). In view of the potential for GPLs to protect and facilitate cognitive function (Chapter 2, section 2.7), their involvement in supporting cellular function, and the reported benefit of GPL supplementation in a similar sample (Crook et al., 1991; Moré et al., 2014; Vakhapova et al., 2010), it was hypothesised that supplementation with Lacprodan® PL-20 would promote better cognitive performance relative to the control group on the cognitive measures employed in the study. In line with empirical work indicating age-related decrements in cognitive function (see sections 5.1 and 5.2), it was hypothesised that cognitive performance would be inversely correlated with age. Further, given that individuals with higher IQ scores typically perform better on measures assessing mental abilities (Checa & Fernández-Berrocal, 2015; Haier, 2014), it was also hypothesised that those with a higher IQ score would show superior performance relative to those with a lower IQ score. Females were expected to show a performance advantage on measures examining verbal memory (Asperholm, Nagar, Dekhytar & Herlitz, 2019; Herlitz & Yonker, 2002; McCarrey, An, Kitner-Triolo, Ferrucci & Resnicj, 2016) and face-name associative memory (see Table 5.3) (Herlitz & Yonker, 2002). There is some evidence to suggest a male advantage for pattern separation performance (reviewed in Yagi & Galea, 2019). It was therefore anticipated that males may show greater performance than females on a measure assessing this (see Table 5.3). No further hypotheses could be proposed regarding gender performance differences, either because such differences have not been identified in the previous literature, they do not exist (e.g. N-back; Schmidt et al., 2009) or there are inconsistent findings. A secondary aim was to explore whether Lacprodan® PL-20 supplementation had any effect on self-perceptions of cognitive failures as measured by the Cognitive Failures Questionnaire (Broadbent, Cooper, FitzGerald, & Parkes,

1982). Consistent with the expectation of Lacprodan® PL-20 being favourable for cognitive function, it was hypothesised that those allocated to receive the supplement would report experiencing less cognitive failures over the intervention period. Again, based upon the inverse relationship between age and cognitive function (see sections 5.1 and 5.2), it was further anticipated that the frequency of cognitive failures would be greater for older participants.

5.6 Methods

5.6.1 Participants

Fifty adults aged 50 years and over were recruited to take part in the study from across Yorkshire. All participants were cognitively healthy but felt that their memory had deteriorated with age and therefore presented with a SMC. Of the fifty recruited to the study, two participants withdrew following baseline (week 0) assessment and one other participant withdrew post-midpoint (week 6) assessment making the final sample 47 participants. A CONSORT figure showing the flow of participants through each phase of the trial is shown by Figure 5.1.

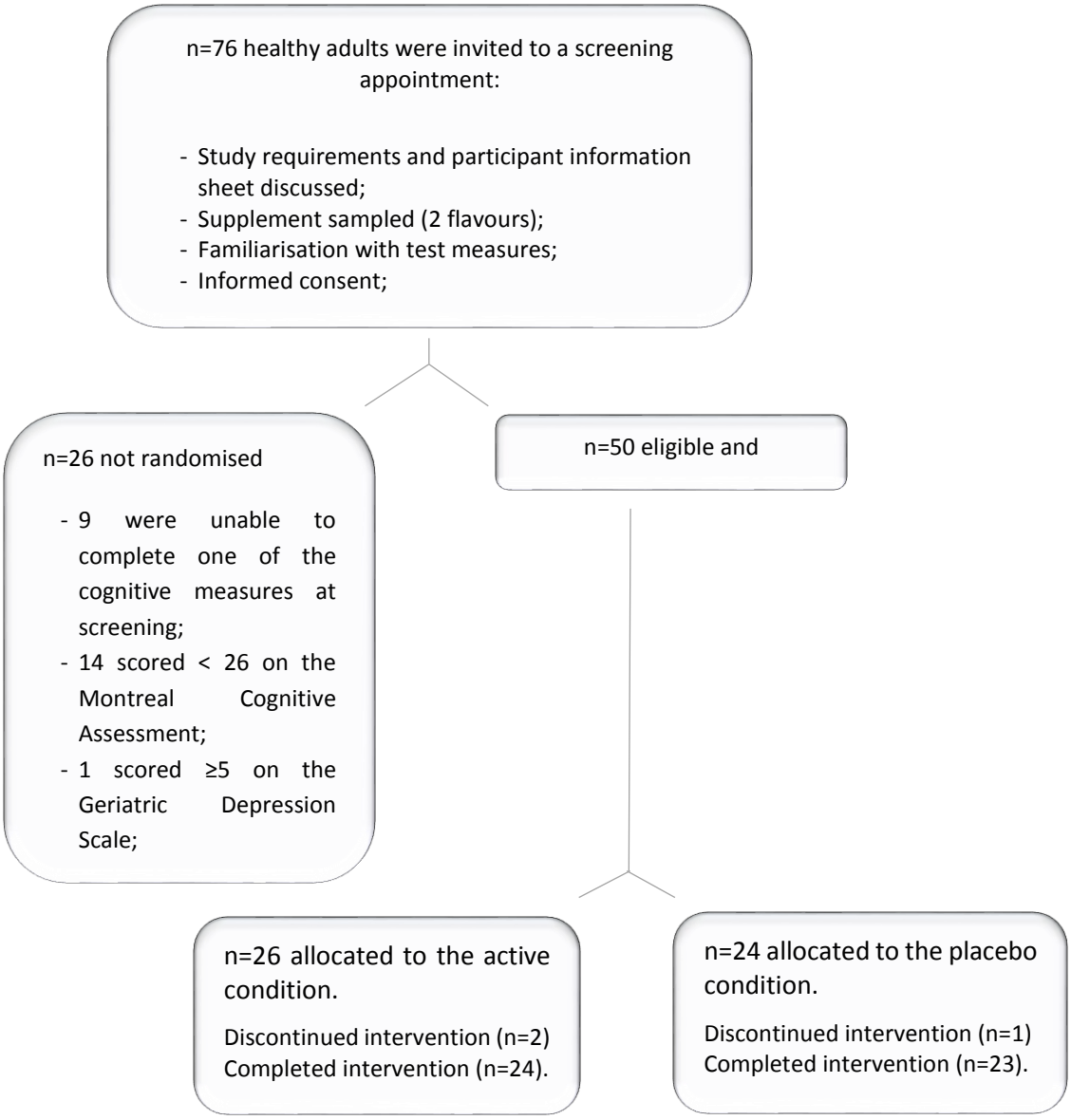


Figure 5.1 CONSORT figure showing the flow of participants through each phase of the trial (screening, randomisation and intervention).

5.6.1.1 Eligibility criteria

The inclusion and exclusion criteria used to assess volunteer eligibility is presented in Table 5.1. During initial contact from an individual expressing an interest in the study, the researcher confirmed they were 50 years of age or over and had a SMC. The latter was ascertained by asking the individual 'Do you feel that your memory is worse than it used to be?'. Any individual not 50 years of age or over and/or who did not present with a SMC were informed that they were not eligible for the study. Medication and supplement use were also queried. All reported medications were checked for side effects. In cases where medication use increased the risk of experiencing cognitive or mood disturbances, individuals were asked whether they had experienced such side effects. Providing no side effects of either nature had been experienced

and the medication had been taken continually for a minimum of 6 months, the individual was invited to be screened. The remaining eligibility criteria were checked during the screening appointment during which the researcher went through each criterion with individuals in person. All measures used in assessing eligibility are covered in section 1.6.3.1.

Table 5.1 Inclusion and exclusion criteria for study sample

Inclusion criteria	Exclusion criteria
- Aged ≥ 50 years;	- Any self-reported psychiatric or medical disorder that could interfere with cognitive function;
- Presents with a subjective memory complaint;	- Use of any drugs directly active on CNS, including prescription, herbal and diet supplements;
- Has adequate intellectual function;	- Evidence of delirium, confusion or other disturbance of consciousness;
- Has capacity to consent to participation and is willing to consume supplement and complete consumption diary daily for 12 weeks;	- Self-reported neurological disorders including dementia, Parkinson's disease, stroke, focal brain lesions, multiple sclerosis, and epilepsy;
- Willing and able to participate in screening and testing measures on four occasions (screening x 1, testing x 4);	- Current diagnosis or history of alcoholism or drug dependence;
- Able to follow verbal and simple written instructions in English;	- History of any of infective or inflammatory brain disease;
- Has normal vision and hearing, with appropriate corrective aids if required;	- History of head injury;
- Able to understand cognitive testing instructions and responding requirements;	- Has colour vision deficiency and/or dyslexia;
- Comfortable with a researcher conducting screening and testing measures.	- Are lactose intolerant and/or suspects or know they have an allergy to any ingredient in the active and placebo supplements.[a]

Note. [a]A list of ingredients was shown at screening.

5.6.1.2 Recruitment

A study advert (Appendix 29) was placed locally around the University campus, in Community and Leisure Centres, and in local Libraries. A recruitment advertisement with details of the study was emailed to members of the University mailing lists and to individuals who had previously expressed a wish to be contacted concerning future research studies. The advertisement was

also emailed to Marks and Spencer stores across West Yorkshire with a request that it be placed on staff noticeboards. The advertisement was also posted on a closed Facebook group. Details of the study were registered with the NIHR Join Dementia Research initiative, which has both cognitively impaired and cognitively healthy adult volunteers. The study was also advertised in local magazines and the Yorkshire Evening Post, in which it featured as part of a wider focus on how to promote healthy cognition. Individuals who met the initial eligibility criteria concerning age, having a SMC and not experiencing any medication side-effects (where medication was taken) were sent a participant information sheet (Appendix 30). The researcher then made contact a week later to follow-up and schedule a screening appointment where appropriate.

5.6.2 Experimental design

This study conformed to a randomised, double-blind, placebo-controlled, parallel groups design comprising two treatment conditions. A breakdown of the allocation by condition is provided in the CONSORT diagram (Figure 5.1). Allocation to condition using a pre-prepared randomisation list took place prior to screening. This avoided the risk of unblinding an individual during their screening in the case of any minor differences between the active and placebo supplements (e.g. colour) when they sampled the intervention products. Block randomisation (quasi-randomisation) was used to allocate individuals to a condition. This promotes equality in the demographic characteristics across experimental conditions (Efird, 2011). Experimental blocks were created based on gender, age and level of education. Participants were seen on four occasions including one screening session and four test sessions (time 1 – 4) as shown in Figure 5.2.

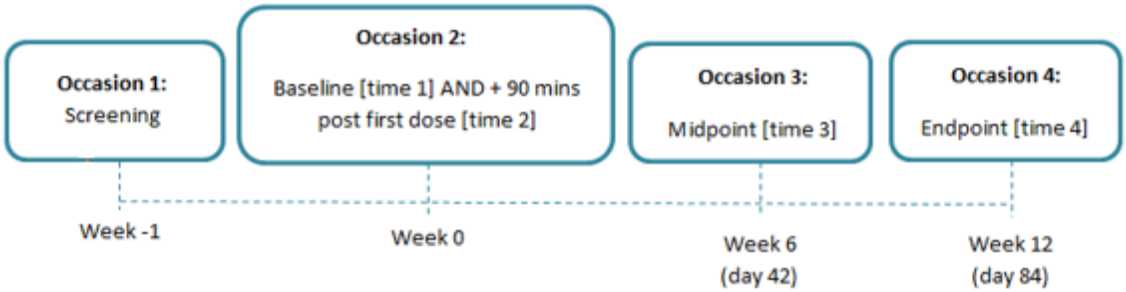


Figure 5.2 Study appointment schedule.

5.6.2.1 Sample size calculation

An *a priori* power calculation was conducted based upon the Attention Switching Task (switch cost reaction time outcome) measure administered as part of a 6 week randomised, double-blind, placebo-controlled, parallel groups trial that supplemented the diet of males (n=54) over

the age of 18 with Lacprodan® PL-20 (Boyle et al., 2019). To detect a difference in log hazard of -0.1758 between placebo and treatment group with a SE of 0.0392, a sample size of 53 was determined i.e. 27 per arm.

5.6.2.2 Ethical approval

This study received ethical approval from the Institute of Psychological Science Research Ethics Committee (ref no:PSC-129; date approved: 14/11/2017). An amendment was subsequently submitted on 14th February 2018 to allow screening appointments to be undertaken in the homes of those interested in participating in the study (ref no:PSC-289). This amendment was approved on 14th February 2018. A fieldwork risk assessment had previously been completed and submitted with the initial application for ethical approval (PSC-129).

During the screening appointment, participants were given written and verbal information about the purpose of the study and all procedures. Participants were made aware they could withdraw at any point without having to give a reason. Permission was sought from all participants that withdrew from the study for the use of their data collected up until the point they withdrew. Participants were also asked to contact the researcher in the first instance in case of any adverse events. Ongoing oral assent was obtained from the participants throughout the trial.

5.6.3 Intervention

5.6.3.1 Supplements

Either an active or placebo isovolumetric supplement drink was consumed by participants over 12 weeks:

a) Active: Containing Lacprodan® PL-20 (10.67 g PL-20 with 120ml water);
b) Placebo: Matched for taste and appearance against the active supplement.

The ingredients within the active and placebo supplements (%) are provided in Table 5.2 by flavour.

Table 5.2 Composition of active and placebo supplements by flavour (%).

Ingredient	Chocolate active	Strawberry Active	Chocolate placebo	Strawberry placebo
Lacprodan® PL-20	54.00	54.86	0.00	0.00
Sugar, sucrose, (white)	0.00	18.87	0.00	16.32
Maltodextrin	0.00	0.00	60.13	60.96
Nesquik, Cocoa powder	19.43	0.00	16.84	0.00
Strawberry Flavour	0.00	0.51	0.00	0.44

Ingredient	Chocolate active	Strawberry Active	Chocolate placebo	Strawberry placebo
Skim Milk Powder	25.30	25.71	21.93	22.23
Cocoa	1.27	0.00	1.10	0.00
Red colouring	0.00	0.05	0.00	0.04

The composition of Lacprodan® PL-20, specifically, the quantities of GPLs and SM have been described previously in Chapter 4 (section 4.5.3.1). Participants were required to take one supplement per day at a time convenient for them. However, the researcher encouraged participants to take the supplement at a regular time to enable the consumption of the supplement to become a routine and therefore increase the likelihood of remembering to take it each day. Supplements were in powdered form and were added to 120ml water prior to oral consumption. Two flavours were available, strawberry and chocolate. Single supplement portions were contained in individual sachets that required dry storage. Participants were expected to consume 84 sachets over the 12-week intervention period. All supplement packs were similar in appearance and identified with only a 3-digit code. Supplement shelf-life was extended to March 2019 following microbiological assessment by Arla Food Ingredients P/S, Denmark. This enabled the study to recruit participants over a longer period.

5.6.3.2 Known drug reactions and interaction with other therapies

An expert panel approved Lacprodan® PL-20 as part of nutrition bars and milk-based nutritional beverages as safe, suitable and GRAS in July 2014 (see Appendix 5). As both supplements contain ingredients available from / used in regular food products only, it was not anticipated that the supplements will interact with other therapies or lead to a drug reaction. All adverse events were recorded (see section 5.6.3.5).

5.6.3.3 Study restrictions

Participants were asked to maintain their usual diet throughout the 12-week study period.

5.6.3.4 Assessment of compliance

A consumption diary (excerpt: Appendix 31) was supplied to participants, who were asked to record consumption of the supplements daily. In addition, participants were required to store all part-empty and fully empty sachets, which were collected by the researcher at midpoint and endpoint appointments (up to 42 each time). This afforded an additional measure of consumption. On collection, a record was made as to the number of days the supplement had been taken for as indicated on the consumption diary as well as the total number of empty sachets returned. In the case of part-empty sachets, it was recommended by Arla Food

Ingredients P/S, Denmark, that the amount of remaining supplement be weighed and deducted from the total weight of the supplement contained in each sachet. However, all returned sachets from participants were fully empty. In the case of an inconsistency in respect of the number of empty sachets returned and the number of days the participant had indicated they had taken the supplement for in the consumption diary, clarification was sought from the participant. Where a participant could not be sure or where clarification could not be gained, the lesser of the two (count vs. sachets returned) was recorded as the number of days the supplement had been taken for.

The inclusion of a participant in the PP analyses was determined by their consumption of the supplement across the intervention period. It was recommended by Arla Food Ingredients P/S, Denmark, that participants who had consumed the supplement drinks on at least 33 days between test sessions (33 of 42 days from baseline to midpoint and 33 of 42 days from midpoint to endpoint) were eligible to be included in the PP analysis (n=50).

5.6.3.5 Adverse events

Participants were advised during the screening appointment in cases of an adverse event taking place whilst enrolled on the study to contact the researcher as soon as possible following the onset of the symptom(s). Adverse events were recorded using an adverse event form (see Appendix 32). Each case was followed until the matter was resolved. The researcher liaised with Arla Food Ingredients Group P/S throughout. Participants were able to leave the study at any point. Adverse events which occurred during the study are described in section 5.12.

5.6.3.6 Blinding

Both participants and researchers were blind to the treatment condition (active/placebo) throughout the study and data analysis. Both treatments were matched for colour and taste as far as possible.

5.6.4 Screening

The screening appointment lasted between 1 hour 30 minutes to 2 hours. Appointments took place in the Human Appetite Research Unit (HARU), School of Psychology, or if requested, in the home of an individual interested in the study. At the start of the appointment, the researcher fully briefed individuals by going through the participant information sheet, ensuring they fully understood the expectations of study participants. All questionnaires to be completed throughout the study and the supplement consumption diary were talked through. A sample of either the active or placebo supplement (pre-determined by condition allocation) in both

flavours were prepared prior to the appointment, enabling individuals to try these after checking that they were not allergic or had an intolerance to any of the ingredients. Once the individual had confirmed they enjoyed the supplement and felt comfortable with the expectation that they would consume the supplement daily over the 12-week intervention period, individuals completed a practice version of the cognitive measures to be used during each test session. Individuals were informed the data collected as part of this activity would not be used for any purpose, and that the activity simply allowed the individual to become familiar with the measures and provide the researcher an opportunity to ensure the individual fully comprehended the test requirements. It also served to minimise practise effects likely to affect later cognitive performance where an individual entered the study. Before being asked to sign the consent form (Appendix 33), individuals were given the opportunity to ask questions. Following this form being signed, screening measures and questionnaires were then administered (see sections 5.6.4.1 and 5.6.4.2), after which, the waist:hip ratio of willing individuals was measured as per standard operating procedures (Appendix 34).

If during the screening appointment, an individual met the criteria for cognitive impairment and/or depression, the individual was informed of this by the researcher who then went through the assessment measure with them and how their score had led to this conclusion. In addition, it was made clear that the assessment used to screen for cognition/mood (whichever applied) is a quick measurement that is convenient for use in the study and as such, it would be advisable for them to see their GP in the interests of a fuller, more comprehensive examination being undertaken. Also, in respect of the Montreal Cognitive Assessment (MoCA; Nasreddine et al., 2005) used to assess cognition, it was explained that the test specificity is 50%, which can lead to false positives. Fifteen individuals met the criteria for cognitive impairment or depression (see Figure 5.1). Contact details of support services such as counselling available in Leeds and Bradford were made available to these fifteen individuals.

5.6.4.1 Screening measures

SMCs have consistently been found to be associated with depression (Balash et al., 2013; Brigola et al., 2015; Buckley et al., 2013; Lehrner et al., 2014; Montejo Carrasco et al., 2017; Sousa, Pereira, & Costa, 2015). Poorer cognitive function, physical function and state of health are also related to depression in older adults (Gale et al., 2011) and increasing age raises the likelihood of each of these (Bishop, Lu, & Yankner, 2010; Harada et al., 2013; Metti, Best, Shaaban, Ganguli, & Rosano, 2018; Murman, 2015; Payette et al., 2011; Sibbritt, Byles, & Regan, 2007). It has been proposed that cognitive impairment represents a core feature of depression, with deficits

identified in attention, memory and executive function domains, as demonstrated by depressed patients (Rock, Roiser, Riedel, & Blackwell, 2014). Consistent with this, increased severity of depression has been found to be significantly related to reduced performance in processing speed, episodic memory and executive function (McDermott & Ebmeier, 2009). In order to control for the risk of cognitive dysfunction associated with depression, the Geriatric Depression Scale 15-item questionnaire (GDS-15; Yesavage & Sheikh, 1986) was administered at the screening appointment to screen for depression. One individual met the criteria for depression and therefore was not recruited to the study.

Additionally, the MoCA (Nasreddine et al., 2005) was undertaken during the screening appointment to ensure participants did not have cognitive impairment either due to having MCI or dementia. There are two types of MCI, namely aMCI and non-amnestic-MCI (naMCI), both of which have different presentations in respect of structural alterations in the brain and patterns of performance on cognitive measures (Csukly et al., 2016). aMCI is distinguished from naMCI by the presence of memory impairment, with both subtypes affecting either single or multiple domains (Dugger et al., 2015; Petersen et al., 2014). SMCs have been identified as a risk factor for incident aMCI (Zammit, Lipton, Katz, Derby, & Hall, 2017). With this in mind, the MoCA was chosen to screen for cases of cognitive impairment, as this has been reviewed favourably as a brief screening measure in detecting aMCI (Ozer, Young, Champ, & Burke, 2016) but also MCI more generally and dementia (Wojtowicz & Larner, 2017). Fourteen individuals met the criteria for cognitive impairment and therefore was not recruited to the study.

5.6.4.1.1 Geriatric Depression Scale 15-item questionnaire (GDS-15)

To screen for cases of clinical depression, a short form of the full Geriatric Depression Scale (Yesavage et al., 1982), the GDS-15 (Yesavage & Sheikh, 1986), was employed. This measure consists of 15 questions, which respondents complete individually by selecting either 'yes' or 'no' (Appendix 35). Responses that are indicative of possible depression are scored a 1 otherwise a score of 0 is given for each item. A composite score is calculated from the 15 items, with a total score of ≥5 suggesting depression. Minimum and maximum total scores are between 0 – 15, with a total score between 5-10 indicating mild depression, and a total score of ≥11 suggesting severe depression. Reliability coefficient (Cronbach's alpha; r_α) has been reported as r_α = .80, with an overall sensitivity and specificity of 97% and 95% respectively (Nyunt, Fones, Niti, & Ng, 2009).

5.6.4.1.2 Montreal Cognitive Assessment (MoCA)

The MoCA (Nasreddine et al., 2005) was developed to identify MCI earlier relative to other clinical tools such as the Mini Mental State Examination (Trzepacz, Hochstetler, Wang, Walker, & Saykin, 2015) and scores can range from 0 - 30, with higher scores suggesting better performance (Appendix 36). Items concern orientation, drawing figures, verbal fluency naming objects, memory, recall, attention, vigilance, processing speed, repetition and abstraction. Administration takes ~10 minutes and the administration and scoring instructions for the MoCA suggests a score of ≥ 26 is classed as non-demented (Nasreddine et al., 2005; Smith, Gildeh, & Holmes, 2007). One point is added to the final score when an individual has 12 years or less of formal education (Nasreddine et al., 2005). The MoCA has been shown to demonstrate a high sensitivity using this cut off score for MCI and DM identification, 83% and 94%, respectively, however, specificity is fairly poor - 50% for both (Smith et al., 2007). Confirmatory factor analysis has led to the proposal of a two-factor model factorial structure; the first factor being memory (memory, language and orientation subtests) and the second factor being Attention/Executive Functions (attention, executive functions and visuospatial abilities subtests) (Duro, Simões, Ponciano, & Santana, 2010).

5.6.4.1.3 Wechsler Abbreviated Scale of Intelligence (WASI)

To assess of intelligence, the WASI (Wechsler, 1999) was employed. This measure was administered as previously described in Chapter 4 (section 4.5.4.1.1).

5.6.4.1.4 Ishihara colour blindness test

Colour deficiency was tested for due to two of the cognitive measures used on test days requiring participants to pay close attention to coloured pictorial presentations. This measure was administered as previously described in Chapter 4 (section 4.5.4.1.2). No participants were identified as having colour vision deficiencies.

5.6.4.2 Self-report forms completed during screening

5.6.4.2.1 Sociodemographic form

Sociodemographic details (Appendix 37) were recorded including age, gender, level of education ('A' level or equivalent or higher, 'O' level or equivalent, trade (commercial or clerical), none), language spoken at home and in country of birth, employment status, and marital status.

5.6.4.2.2 Socioeconomic indicator

A measure of level of deprivation, previously used in a survey of household resources and standards of living (Townsend, 1979), comprising eight questions was utilised (Appendix 38). The total score reflects the number of items an individual does not have due to not being able to afford them rather than not wanting them but otherwise being able to afford them. Income has been acknowledged as influencing cognitive performance in adults aged 50 years and above in a study that used cross-sectional data from the World Health Organization Study on Global Ageing and Adult Health (SAGE) Wave 1 (2007–2010) (Basu, 2013). Moreover, an indicator of cognition (processing speed) was found to be significantly associated with poverty index scores after controlling for ethnicity, age, health status, gender and childhood socioeconomic status (Zhang et al., 2015).

5.6.5 Testing procedure

Ethical approval was obtained to conduct test appointments either in the Human Appetite Research Unit (HARU) or in participants' homes, depending on what was most convenient for each participant. The date and time of the first test appointment (baseline) determined the date and time of subsequent appointments (assuming the availability of the participant and researcher). Appointments took place during the morning, afternoon and evenings to accommodate participant availability; however, preference was given towards morning appointments, as this time of the day is more favourable when assessing cognitive performance in older adults (Anderson, Campbell, Amer, Grady, & Hasher, 2014; Knight & Mather, 2013; West, Murphy, Armilio, Craik, & Stuss, 2002).

5.6.5.1 Test session

At the start of each test appointment, participants completed three questionnaires measuring subjective state: State Anxiety Scale (S-anxiety; Spielberger, Gorsuch, Lushene, Vagg, & Jacobs, 1983), Stress Arousal Checklist (SACL; Mackay, Cox, Burrows, & Lazzerini, 1978) and the Profile of Mood States – Short form (POMS; Shacham, 1983). Hence, participants' anxiety, stress, arousal and mood could be accounted for at the time of cognitive measure completion. Importantly, these subjective states have been identified as affecting cognitive performance. For example, acute cognitive performance anxiety has been shown to impair performance on an N-back measure (Angelidis, Solis, Lautenbach, van der Does, & Putman, 2019), while acute stress has been observed as affecting performance on a stroop-like task (Kohn, Hermans, & Fernández, 2017). Further, acute mood (induced positive and negative moods) is known to effect task-switch performance (Hsieh & Lin, 2019). In addition, participants also completed the

Cognitive Failures Questionnaire (CFQ) (Broadbent et al., 1982) to document cognitive slip occurrence over the 6 weeks prior to the appointment. This was followed by presentation of the cognitive measures in a set order (parallel versions were used for each appointment). The researcher remained with the participant whilst they completed the questionnaires and cognitive tests in case the participant had any questions or experienced any difficulty. This also allowed the researcher to audio record responses to two of the measures. At the end of the baseline and midpoint appointments, participants were provided with 45 sachets of supplement and a supplement consumption diary. At the end of midpoint and the final appointment (endpoint), empty/part-empty supplement sachets and completed consumption diaries were collected by the researcher. Each test appointment took approximately 1 hour to complete, and participants received £10.00 to cover any travel expenses and compensate for time lost.

The baseline appointment took place 7 days after the screening appointment subject to participant availability and was split over two parts. To test whether there is an acute benefit of consuming Lacprodan® PL-20 on cognitive performance, following completing the cognitive measures for the first time since screening, participants were given their first dose of the supplement prior to a 90-minute break, after which time the questionnaires and cognitive measures were administered for a second time. Only dose 1 was taken in the company of the researcher, with all subsequent doses being taken by participants in their own homes. Figure 5.3 presents the test appointment schedule and details of appointment agendas.

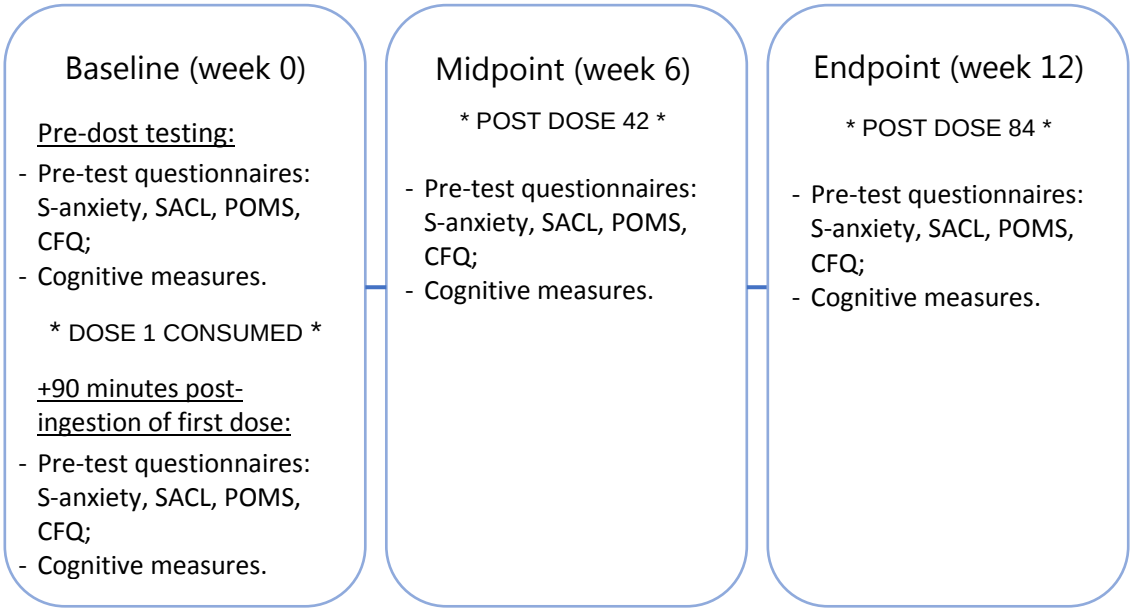


Figure 5.3 Schedule of study appointments.

5.6.5.1.1 Self-report measures completed during test sessions

5.6.5.1.1.1 State-Trait Anxiety Inventory (STAI)

The STAI (Spielberger et al., 1983) is comprised of 2 subscales, each consisting of 20 questions. The Trait Anxiety Scale (T-Anxiety) assesses the relatively stable propensity to be anxious, whereas The State Anxiety Scale (S-Anxiety – Appendix 39) evaluates how anxious the respondent feels at the time of completing the scale. The former taps into states of calmness, security and confidence, whereas the latter focuses on tension, nervousness, apprehension, worry and arousal of the autonomic nervous system. Respondents can select a single response as per the frequency of feelings 'in general' on the T-Anxiety (Almost Never, Sometimes, Often, Almost always). For the S-Anxiety, responses are in the context of their current feelings 'at this moment' (Not at all, Somewhat, Moderately so, Very much so). Responses indicating a lower frequency (T-Anxiety) and a lesser prevalence (S-Anxiety) produce a lower score, e.g. 'Almost Never' / 'Not at all' correspond to a score of 1, whereas, 'Almost always' / 'Very much so' correspond to a score of 4. Nineteen of the 40 items are anxiety-absent items. Scores on these items are reversed. Overall, a higher score represents greater anxiety. Reliability coefficients of the STAI range between r_{α} **=** .86 - .95 and test-retest reliability coefficients range between r = .65 - .75 over a 2-month period (Spielberger et al., 1983).

5.6.5.1.1.2 The Stress Arousal Checklist (SACL)

The SACL (Mackay et al., 1978) is a mood adjective checklist of 30 adjectives (Appendix 40) that distinguish between stress (18 items, e.g. 'comfortable', 'nervous', 'tense') and arousal (12 items, e.g. 'activated', 'drowsy', 'idle'). Respondents are asked to indicate how much each adjective represents how they are feeling at the time of completing the checklist using a 4-point Likert scale (long scoring method: Definitely feel = 4, Feel more or less = 3, Do not understand the adjective / cannot decide = 2, Definitely not feel = 1). Scores are reversed on negatively weighted items. The possible minimum and maximum scores are 18 – 72 and 12 – 48 on the stress and arousal sections of the checklist respectively. Reliability coefficients for the stress section are between r_{α} = .86 – .89 and for the arousal section, r_{α} = .74 – .84 (King, Burrows, & Stanley, 1983; Mackay et al., 1978; Mccormick, Walkey, & Taylor, 1985).

5.6.5.1.1.3 The Profile of Mood States – Short form (POMS-SF)

The POMS-SF (Shacham, 1983) is a mood adjective checklist of 37 items (Appendix 41) that measures 6 dimensions of distinct, transient affective states (Tension-Anxiety, Depression – Dejection, Anger – Hostility, Fatigue – Inertia, Vigour – Activity and Confusion – Bewilderment).

Respondents are asked to indicate how much each adjective represents how they are feeling at the time of completing the form. A Total Mood Disturbance (TMD) and individual dimension scores can be calculated based upon responses according to a 5-point Likert scale (Not at all = 0, A little = 1, Moderately = 2, Quite a bit = 3, Extremely = 4). For this study, only the TMD score was analysed and this was calculated in line with a previous method (Curran, Andrykowski, & Studts, 1995). Reliability coefficients for the POMS-SF subscale scores range between r_α = .76 - .95, and for the TMD, r_α = .87 - .92. Confirmatory factor analysis supports the 6-factor interpretation (Depression, Vigour, Anger, Tension, Confusion and Fatigue) (F. Baker, Denniston, Zabora, Polland, & Dudley, 2002; Curran et al., 1995; Shacham, 1983).

5.6.5.1.1.4 Cognitive Failures Questionnaire (CFQ)

The CFQ (Broadbent et al., 1982) measures everyday cognitive failures reflecting errors and slips in functioning (Wallace, Kass, & Stanny, 2002). In a review of cognitive failures in daily life in healthy populations, cognitive failures were suggested to reflect fluctuations in cognitive capacity (Carrigan & Barkus, 2016). Functionally, cognitive failures are minor slips that result in the usual smooth flow of either a physical or mental intended action being disrupted (Broadbent et al., 1982). The CFQ is a 25-item self-report measure in which respondents are asked to indicate how often they have experienced each failure over the previous 6 months (Appendix 42). For the purpose of this study, the reference period was altered to 6 weeks. This enabled measurement of cognitive failures between each chronic test session. Respondents select 1 of 5 possible responses available from a 5-point Likert scale (Very Often = 4, Quite Often = 3, Occasionally = 2, Very rarely = 1, Never = 0). A total score is calculated by summing all item responses, with a higher score representing a greater number of cognitive failures. Reliability coefficients range between r_α = .85 - .90. Studies examining the structure of the CFQ using Factor analysis have reported 3, 4 and 5 factors, however, all have identified memory and action slips as core factors (Broadbent et al., 1982; Pollina, Greene, Tunick, & Puckett, 1992; J. C. Wallace et al., 2002).

5.6.5.1.2 Assessment of cognitive performance

The order of the cognitive measures that were administered along with respective completion times, the cognitive domains assessed, and outcome variables generated are listed in Table 5.3.

Table 5.3 Order and nature of cognitive measure presentation during test sessions

Cognitive measure	Cognitive domain(s) assessed	Outcome(s)	Measure duration
Visual Verbal Learning Test (VVLT)	Assesses episodic verbal immediate and delayed memory of two-word lists, A and B.	Number of words correctly recalled in any order: 1. Rate of learning (word list A). A higher score is better; 2. New learning (word list B). A higher score is better; 3. Retroactive interference. A lower score is better; 4. Proactive interference. A lower score is better; 5. Delayed recall. A higher score is better.	10 - 15 minutes in total
N-back Task	Dependent on age: 41-60-year olds:- attentional and executive processes; > 60-year olds:- attentional and executive processes (including updating) and verbal memory.	1. **Target accuracy**: total of correctly identified targets as a % of the total number of targets (number of targets identified/66*100). A higher score is better; 2. **Total accuracy**: total of correctly identified targets – number of false alarms as a % of the total number of targets (number of targets identified - false alarms/66*100). A higher score is better; 3. Reaction time (RT) for identification of targets. A faster time is better; 4. RT for identification of non-targets. A faster time is better.	5 minutes
Face-Name Associative Memory Exam	Assesses associative memory between pictorial (visual working memory) and verbal (verbal memory) presentations.	1. The total of fore- and surnames correctly recalled immediately. A higher score is better; 2. The total of fore- and surnames correctly recalled after a delay. A higher score is better.	5 minutes in total
Attention Switching Task	Assesses executive function (cognitive flexibility)	1. **Target accuracy**: total of correctly identified targets by trial type (switch, nested, pre-switch). A higher score is better; 2. **Target RT**: RT of correctly identified targets by trial type (switch, nested, pre-switch). A faster time is better;	3 minutes

Cognitive measure	Cognitive domain(s) assessed	Outcome(s)	Measure duration
		3. Switch cost (within each presentation colour i.e. blue / red): - accuracy (as a % of the total number of targets (number of targets identified/72*100) for switch trials (trial 1 (switch) – trial 2 (nested)) and repeat trials (trial 3 (pre-switch) – trial 2 (nested)). A higher score is better; - RT for switch trials (trial 1 (switch) – trial 2 (nested)) and repeat trials (trial 3 (pre-switch) – trial 2 (nested)). A faster time is better.	
Pattern Separation Task	Assesses episodic memory	1. **Pattern separation score**: pattern separation rate – similar bias rate. A higher score is better; 2. **Recognition score:** hit rate – false alarm rate. A higher score is better.	4 minutes 30 seconds
Rapid Visual Information Processing Task (RVIP)	Assesses working memory and visual sustained attention	1. Total hits (targets). A higher score is better; 2. Total false alarms. A lower score is better; 3. RT for hits. A faster time is better.	6 minutes

5.6.5.1.2.1 Cognitive measures

As discussed in section 5.1, age-related cognitive decline is associated with both healthy and unhealthy ageing. In both cases, processing speed, memory and executive functioning can become compromised (Harada et al., 2013; Hedden & Gabrieli, 2004). Notably, declines in episodic and working memory are more likely to accompany advancing age than other types of memory, such as procedural (Nyberg, Lövdén, Riklund, Lindenberger, & Bäckman, 2012b). As indicated in Table 5.3, all cognitive measures used in the present study target these domains.

The selection of cognitive measures employed in the current study was informed by previous research (see section 5.4) that recruited adults with subjective memory complaints and found significant treatment effects on memory performance: a Name face association measure (acquisition and delayed recall; Crook et al., 1991) and verbal memory on the RAVLT (Vakhapova et al., 2010). In addition, other studies were considered that have reported a benefit of Lacprodan® PL-20 in spite of these recruiting younger adults and assessing participants under conditions of acute stress, particularly Boyle et al. (2019), who utilised the Attention Switching Task. The Pattern Separation Task was utilised to assess the potential for the derivative of PI, PI(4,5)P_2, to support LTD induction in the CA1 subregion of the hippocampus, which is thought to be involved in pattern separation (see section 2.7.2). Based on the empirical studies reviewed in Chapter 3, it was concluded that there is a paucity of literature exploring the effects of GPL supplementation in the areas of working memory performance and sustained attention. The present study therefore seeks to address this.

Cognitive measures were presented on a Hewlett-Packard laptop using PsychoPy software (Peirce et al., 2019) and included practise trials (except for VVLT and the Face-Name Associative Memory Exam, as this would increase memory load and therefore most likely interfere with test performance). Where stimuli were re-presented during a single test session, re-presentations were randomised to avoid the serial position effect i.e. words on the VVLT measure and faces on the Face-Name Associative Memory Exam.

5.6.5.1.2.1.1 Visual Verbal Learning Test (VVLT)

Similar to other verbal memory measures including the Californian Verbal Learning Test (CVLT; Delis, Kramer, Kaplan, & Ober, 1987), the Hopkins Verbal Learning Test (HVLT; Brandt, 1991) and the Rey Auditory Learning Test (RAVLT; Rey, 1941), the VVLT measures episodic verbal learning of a list of unrelated words. Essentially, the measure is a visual analogue of the RAVLT and examines immediate memory span, new learning, susceptibility to interference (retroactive and

proactive) and long-term memory (+30-minute delay post-word list presentation). Two-word lists (List A and B) of 16 words each were visually presented in a random order, one word at a time, for 1 second with a 2 second inter-stimulus interval. Participants recalled the words verbally in any order over a one-minute interval. Recall of the word lists was audio recorded.

List A was presented repeatedly on 3 occasions before List B was shown (trials A1-A3). List B is intended to interfere with the memory for words in List A. After the recall of List B (trial B1), participants were instructed to recall List A (trial A4) once again without list A being re-presented. Delayed memory for List A (trial A5) was assessed by asking participants to recall this list within 1 minute after a 30-minute interval, again in the absence of List A being re-presented. Participants completed other cognitive measures during the 30-minute interval between the immediate and delayed recall stages. Different word lists were used for each test session (parallel versions) and versions were counterbalanced across test sessions. Word lists were selected from the MRC linguistic database and matched on concreteness, imageability, familiarity, age of acquisition and word length.

The outcome variable for each trial was the number of correctly recalled words. For rate of learning, the analysis included trials A1-A3, where trial was added to the model to explore how performance varied across trials A1-A3 (inclusive). New learning explored performance for trial B1. Retroactive interference is characterised by the learning of new material interfering with the recall of old material. In this way, the number of words forgotten was calculated as the difference between the number of words correctly recalled on Trial A3 and Trial A4. Alternatively, proactive interference is the interference of old material on the recall of new material. This was calculated as the difference between correct recall of words from Trial A1 and Trial B1. The number of correct words recalled for Trial A5 determined the delayed memory score.

5.6.5.1.2.1.2 Pattern Separation Task

Pattern separation is the storage of similar or partially overlapping representations as distinct memories thus avoiding interference between memories (Deng, Aimone, & Gage, 2010; Yassa & Stark, 2011). Pattern separation is a crucial aspect of episodic memory (Leal & Yassa, 2018) and is vulnerable to age-related deficits (Stark & Stark, 2017). Numerous studies using both animals and humans, as well as computational models of hippocampal learning, indicate functional roles of the dentate gyrus and the CA1 (Hanert et al., 2019) and CA3 subregions of the hippocampus (Bakker, Kirwan, Miller, & Stark, 2008; Lacy, Yassa, Stark, Muftuler, & Stark,

2011; Leutgeb, Leutgeb, Moser, & Moser, 2007; Leutgeb & Leutgeb, 2007) in pattern separation. From middle-age, the volume of the hippocampus has been shown to reduce in healthy adults (Driscoll et al., 2009), which has been associated with a decline in episodic memory and dentate gyrus (cortical region within the hippocampal formation) function (Kramer et al., 2007; Small, Tsai, DeLaPaz, Mayeux, & Stern, 2002).

This is a continuous recognition task adapted from a visual short-term memory task (Smith, McKeown, & Bunce, 2017) in which door stimuli (pictures of doors obtained from Baddeley, Hitch, Quinlan, Bowes, & Stone, 2016) were presented for 2 seconds with a 2.5 second inter-stimulus interval sequentially over 60 trials. On the presentation of each door, participants were instructed to identify whether the door had not been seen previously (classed as new), was similar to a door that has been seen previously (classed as similar) or had been presented previously (classed as old). Reponses were made by pressing number keys 1, 2 and 3 on the keyboard, respectively, within the 2 second item presentation time. Pattern separation and recognition scores were calculated based on the following matrix (Table 5.4):

		Response type made		
		New	***Old***	***Similar***
Door type presented	***New***	Correct rejection rate	False alarm rate	Similar bias rate
	Old	Miss rate	Hit rate	Incorrect
	Similar	Incorrect	Pattern completion rate	Pattern separation rate

Table 5.4 Pattern Separation Task score matrix

Door pairs were matched on perceptual and categorical similarity. Specifically, all door pairs were independently rated as being perceptually similar (Smith et al., 2017). Four intervening items (doors) were presented between each old pair and similar pair. Each trial block of 60 trials comprises 20 single 'new' doors, 10 'similar' door pairs and 10 'old' door pairs. Different door stimuli were used for each test session (parallel versions) and the presentation of each of the 60 trial blocks were counterbalanced across test sessions.

5.6.5.1.2.1.3 Face-Name Associative Memory Exam

The lack of relatedness between a person's face and their name makes associating one with the other a particularly difficult task (Werheid & Clare, 2007). An adaption of the Face-Name

Associative Memory Exam (Rentz et al., 2011; Sperling et al., 2001) was administered in which participants were shown (familiarisation stage) 10 colour photographs of unfamiliar male or female faces (from shoulders up on a plain background) with blank expressions, one at a time (5 male, 5 female in each test session) for 2 seconds. Following this, the same photographs were presented individually for 5 seconds along with name details (a forename and surname situated centrally underneath each photograph). Participants were asked to associate the name details with the corresponding photograph. Subsequently, participants were asked to recall (immediate recall) the full name (forename and surname) verbally on the re-presentation of each of the 10 photographs (presented successively for 5 seconds), which was audio recorded. Following a 30-minute interval, participants were shown the same photographs individually and asked to recall (delayed recall) the corresponding name details verbally for the second time, which was also audio recorded (5 seconds were allowed per photograph). Participants completed other cognitive measures during the 30-minute interval between the immediate and delayed recall stages. The order of presentation of each photograph across the measure was randomised and the presentation of each was followed by an inter-stimulus interval of 250 milliseconds (ms).

Older adults regularly struggle with remembering proper names (Leirer, Morrow, Sheikh, & Pariante, 1990). The lack of context hinders the formation of an association between a name and a novel face, which is complex (Werheid & Clare, 2007) and places more of a demand on cognition (Rentz et al., 2011). Previous work that assessed recognition memory for face-name pairs indicated that greater activation in the anterior hippocampal formation bilaterally and left inferior prefrontal cortex accompanied successful recognition (Sperling et al., 2003). Performance on the face-name task has been found to be inversely associated with Aβ deposition in frontal and precuneus, posterior cingulate, and lateral parietal cortices in cognitively healthy older adults, irrespective of cognitive reserve (Rentz et al., 2011). Moreover, evidence suggests that the face-name task is sensitive to memory impairments as seen in prodromal and early stages of AD (Werheid & Clare, 2007).

Males and females featured in each photograph used in this study were aged 50 years or over at the time of the photograph being taken (database: Minear & Park, 2004). It was thought that this was most appropriate given the age range of the study sample. Selection of the photographs was determined following a validation study (ref no: 17-0138; date approved: 16-May-2017) that explored which photographs from a possible 280 were similar for attractiveness and distinctiveness. The attractiveness and distinctiveness of facial stimuli has been shown to affect

memory performance (Wiese, Altmann, & Schweinberger, 2014). Adults aged 50 years or over (n=13) were recruited for the purpose of the validation study. Participants viewed photographs (n=280) of unfamiliar male or female faces with blank expressions on a laptop (from shoulders up on a plain background) and rated each for attractiveness and distinctiveness using a 7-point Likert scale (1-7 inclusive) featured below each photograph. Specifically, all participants saw each photograph during a familiarisation stage. Following this, each participant was shown each photograph for a second and third time (immediately after the familiarisation stage). On the second presentation, participants were asked to rate each person for attractiveness, whereas on the third presentation, participants rated each for distinctiveness. There was no time limit on each presentation and participants were able to take a comfort break when needed. The Likert scale anchor points represented extreme ratings (not at all attractive/distinctive – very attractive/distinctive). Participants were instructed to think of a distinctive face as one that could be classed as unusual, that would stand out in a crowd of more typical faces.

Forenames associated with each photograph were taken from the Top Names of the 1960s as published by the Social Security Administration (Federal Agency) (https://www.ssa.gov/oact/babynames/decades/names1960s.html). Forenames were selected randomly from the list for each gender. Attention was paid towards the nationality of each to ensure the use of those selected were not specific to a particular country. Forenames were taken from this period (as opposed to more recent years), as the youngest participants were born within this decade and it was therefore expected that such names would be more representative. Surnames associated with the photographs were derived from The Oxford Dictionary of Family Names in Britain and Ireland (https://global.oup.com/academic/product/the-oxford-dictionary-of-family-names-in-britain-and-ireland-9780199677764?cc=gb&lang=en&) and were randomly selected. All forenames and surnames were of two syllables and 6 letters in length. Photograph and name pairings, and the allocation of the 20 face-name pairing trials to each block (1 block per test session) was randomised. The presentation of each block was counterbalanced across test sessions and different face-name pairs were employed on each session (parallel versions). No photograph or name was used more than once.

5.6.5.1.2.1.4 Attention Switching Task

This measure required participants to switch their visual attention (perceptual switching) depending on a visual cue (colour of stimuli) presented in each trial based upon the rule for that trial set (rule switch). Whilst performing the measure, multiple goals operate simultaneously,

and the behaviour exhibited by participants is dependent upon the conditions of each trial and the specific rule in operation. During rule switching, working memory is updated so that the correct behaviour is performed (Ravizza & Carter, 2008). Poorer performance is typically seen immediately following task switch (first trial of new rule implementation) indicated by greater error and longer response latencies. This is referred to as the switch cost (Monsell, 2003). Importantly, the left inferior frontal junction has been reported to play a crucial role in task-switching and stronger connectivity between this and the bilateral posterior parietal cortex, the right middle frontal gyrus, and the bilateral middle occipital gyrus, in which the left inferior frontal junction acts as a hub to bring together task switch-related information, is related to better performance in task-switching i.e. reduced switch cost (Yin, Wang, Pan, Liu, & Chen, 2015). These findings complement other reports that the inferior frontal junction coordinates thoughts and actions as per internal goals (Brass, Derrfuss, Forstmann, & von Cramon, 2005; Derrfuss, Brass, Neumann, & von Cramon, 2005).

The task-switch measure is based upon a measure that combines a task-switch paradigm with a Go/No-go task (Wylie, Javitt, & Foxe, 2003) and administration of the measure followed a previous study considering the benefits of Lacprodan® PL-20 (Boyle et al., 2019). Letter-number pairs e.g. 'M + 9' or '7 + R', were presented in a quasi-random sequence in succession for 1 second with an inter-stimulus interval of 120 ms. The stimuli set included 8 letters (vowels: A, E, I, and U; consonants: G, K, M, and R) and 8 numbers (even: 2, 4, 6, and 8; odd: 3, 5, 7, and 9). Every three trials, letter-number pairs were presented in alternating colours (e.g. red or blue). The colour determined the rule for that trial set and therefore, the required response. For example, participants were asked to respond by pressing the space bar when the letter in the pair was a vowel but not respond when it was a consonant when the pair was presented in red. Alternatively, participants were asked to respond (space bar press) when the number in the pair was even but not when it was odd when the pair was presented in blue. Target trials (vowel letters and even numbers) were balanced across trials within each colour (24 for trial 1, 24 for trial 2 and 24 for trial 3 by colour) with a 50% probability of being presented amongst the 144 trials. Neighbouring trials featured different letters and numbers. Half of the trials (n=72) included congruent pairs (e.g. 8 + U) whereas the remaining 72 trials included incongruent pairs (e.g. K + 2). Parallel versions were used across test sessions in which only the colours differed i.e. the letter-number pairs remained consistent across test sessions. The presentation of each test version was counterbalanced across test sessions.

Two of the outcomes for this measure, switch cost accuracy and reaction time, are calculated by comparing switch and pre-switch trials to the nested trial by subtracting. Here, nested trials are the comparator trial (control) with a level of performance least affected by the switch. Therefore, performance can be compared to this trial to indicate the level at which a cost in performance is evident.

5.6.5.1.2.1.5 Rapid Visual Information Processing Task (RVIP)

This measure is a serial discrimination task (Neale, Johnston, Hughes, & Scholey, 2015) devised by Bakan (Bakan, 1959) and is designed to assess working memory and visual sustained attention (Coull, Frith, Frackowiak, & Grasby, 1996; Neale et al., 2015). With respect to task completion, it has been suggested that a right frontoparietal network supports sustained attention, whereas a left frontoparietal network facilitates the phonological loop component of working memory (Coull et al., 1996). However, more recently, functional neuroimaging research using block analysis has shown increased activation in frontal, parietal, occipital and cerebellar regions (Neale et al., 2015). Event related potential analysis has shown similar activation when considering correct responses only, particularly in the activation of the medial and inferior frontal and precuneus and cerebellum areas (Neale et al., 2015).

Single digits (1-9) were presented in a quasi-random sequence one at a time in the centre of the screen, each being presented for 600 ms (100 per minute). Digits were presented with no inter-stimulus interval. Participants were asked to respond when they identified three consecutive odd or even numbers (targets) by pressing the space bar. Eight targets were randomly presented per minute, 48 in total. Parallel versions were used for each test session, with version presentation being counterbalanced across test sessions.

5.6.5.1.2.1.6 N-back Task

This measure is a continuous performance task and has been frequently used to measure working memory (Gajewski, Hanisch, Falkenstein, Thönes, & Wascher, 2018; Yaple, Stevens, & Arsalidou, 2019). N-back can be presented in various forms including visual, auditory, and olfactory, placing demands on separate processing systems (Owen, McMillan, Laird, & Bullmore, 2005). Working memory load is manipulated by altering the distance between trials that are compared (1, 2 or 3 trials back), with 3 back being the most difficult. Gajewski and colleagues (2018) have reported age-related differences in performance, with reaction times increasing and accuracy decreasing progressively with increasing age. Findings from a meta-analysis of fMRI studies assessing N-back performance across the healthy adult lifespan indicates the parietal

and cingulate cortices as well as the insula, claustrum and cerebellum are engaged during the task for all ages. However, prefrontal cortex engagement illustrates a gradual linear decline with age, which is absent in older adults (Yaple et al., 2019). Correlating performance on the N-back with other measures of cognitive function has also revealed age differences. Specifically, younger adults have been found to employ mainly executive functions, whilst performance by middle-aged adults has been found to involve attentional and executive functions. Moreover, older adults have been found to typically utilise attentional, updating and verbal memory functions and executive functions to a lesser extent (Gajewski et al., 2018).

This measure employed a working memory load of 2 (2-back) in which a series of digits (0-9) were presented one at a time in a quasi-random sequence in blocks of 50 trials. Administration of the measure followed a previous study considering the benefits of Lacprodan® PL-20 (Boyle et al., 2019). Digits were presented for 500 ms (single trial) with an inter-stimulus interval of 1 second, and 4 blocks of trials were presented at each test session. Participants were asked to respond on all trials, for both non-targets and targets by pressing the number keys 1 and 2 on the keyboard, respectively. A target is classified as the presentation of the same digit being shown in both the current trial and two trials back (3, 0, 3), whereas a non-target is identified when there is a lack of correspondence between the digits (2, 0, 3). Target stimuli were presented randomly with a probability of 33% (over the 200 trials) and the first three trials were always non-targets. Parallel versions were used for each test session, with version presentation being counterbalanced across test sessions.

5.7 Data analysis

The analysis of the data collected in the present study was carried out in two separate stages. The first stage involved analysing cognitive measure data collected prior to and following acute supplementation i.e. time 1 (baseline, pre-first dose) and time 2 (+90 minutes post-first dose). The second stage involved analysing cognitive and subjective data (Cognitive Failures Questionnaire) collected prior to and following chronic supplement administration i.e. baseline (pre-first dose), midpoint (+6 weeks baseline, after 42 supplement doses) and endpoint (+12 weeks baseline, after 84 supplement doses).

As with Study 1 (Chapter 4), baseline performance, age, IQ and gender were controlled for in the analysis of the cognitive measure data and subjective data from the Cognitive Failures Questionnaire. In respect of the first data analysis stage, controlling for baseline performance

(time 1) resulted in data being available for one test point only, time 2 (+90 minutes post-first dose). Due to this, the factor time was removed from the model following independent statistical advice (Quadt Consultancy BV, personal communication), which meant the main effect of time and interactions with time could not be requested. As for the second data analysis stage, this was similar to that carried out on the data from Study 1 (see section 4.6).

In the present study, the Attention Switching Task (switch cost reaction time) was the primary outcome variable, whilst other cognitive and subjective measures were secondary outcome variables. Cognitive measure data were extracted from PsychoPy (Peirce et al., 2019) and entered in Excel and checked for accuracy. Participant responses from the Visual Verbal Learning Test (VVLT) and the Face-Name Associative Memory Exam were audio recorded and scored according to the marking scheme. Subjective data was also scored. Scores were tallied by participant and week, entered and checked for accuracy in Excel.

Trial was included in a model as a nominal variable for the Attention Switching Task outcomes and the rate of learning outcome of the VVLT where this led to an improvement in the model fit (Quadt Consultancy BV, personal communication). The total number of correct trials obtained (total correct) at each test point was also entered in a model as a covariate for all reaction time outcome measures of the N-back task and the Rapid Visual Information Processing Task, again, where this led to an improvement in model fit. This also applied to target accuracy and switch cost accuracy in the analysis of target reaction time and switch cost reaction time, respectively, from the Attention Switching Task measure. Responses to subjective state questionnaires (POMS, SACL and STAI) were analysed prior to cognitive measure data analysis to determine whether anxiety and/or mood were statistically different between conditions prior to cognitive test administration. No statistical differences in the subjective responses of participants were found and therefore these variables were not included as covariates in the analysis of cognitive measure data.

5.8 Results

Fifty participants enrolled on to the study and completed the acute assessment. Two participants withdrew following this, prior to midpoint (data collected at midpoint corresponds to forty-eight participants). One further participant withdrew for health reasons between midpoint and endpoint (data collected at endpoint corresponds to forty-seven participants). Forty-seven participants complete the full trial (see Figure 5.1).

5.8.1 Adverse events

Five participants reported adverse events following supplementing their usual diet with the study supplement. One participant (active condition) withdrew from the study after having an allergic reaction to the supplement within days of commencing the intervention. At their final appointment, a second participant (placebo condition) revealed they had been experiencing a variety of symptoms including pains in their stomach, belching and diarrhoea for 9 weeks over the course of the 12-week intervention period. However, they suspected these symptoms were due to stress. A third participant (active condition) reported high potassium levels and a fourth (active condition) reported increased systolic blood pressure whilst on the study but it is thought that these conditions are unrelated to supplementation with the study products and both completed the study and were included in the analysis. A fifth participant (active condition) experienced diarrhoea following consuming the supplement for one week and withdrew from the study. Thus, 3 of the participants who reported adverse events were included in the analysis.

5.8.2 Participant characteristics

The characteristics of the 50 middle-aged and older adults with a subjective memory complaint that met the experimental drink consumption threshold (per protocol analysis) across the 12 weeks are presented in Table 5.5.

Table 5.5 Participant characteristics of those included in the analyses from the two conditions

	Active (n=26) Mean ± SE /Median (Range)	**Placebo (n=24) Mean ± SE /Median (Range)**	**Active vs. placebo condition**
Age (months)	**742.88** ± 14.79	**749.42** ± 16.30	$t(48) = -.30$, $p = .767$[a]
IQ	118.0 *(98, 130)*	118.5 *(87, 129)*	$U = 350.5$, $p = .453$
Gender			
M	7 (50%)	7 (50%)	$X^2(1, N = 50) = .03$, $p = .860$
F	19 (53%)	17 (47%)	

	Active (n=26) Mean ± SE /Median (Range)	Placebo (n=24) Mean ± SE /Median (Range)	Active vs. placebo condition
Language spoken at home			X^2(1, N = 50) =.94, p = 1.00[b]
English	25	24	
Arabic	1	0	
Employed			X^2(1, N = 50) =.79, p = .407
Yes	13	15	
No	13	9	
SES	3 (11%)	0 (0%)	U = 288.0, p = .170
Waist:hip ratio[c]	0.86 (0.69, 1.07)	0.82 (0.72, 1.06)	U = 248.5, p = .217

[a]Equal variances assumed. [b]Exact test. [c]Waist/hip measurements. SES: Socioeconomic status

5.8.3 Acute effect of intervention on cognitive performance in middle-aged and older adults with a subjective memory complaint

5.8.3.1 Measures of executive function performance

Table 5.6 provides a summary of the means (± SE) across measures of executive function performance for active and placebo conditions.

Table 5.6 Mean (± SE) on measures of executive function performance (Attention Switching Task, Rapid Visual Information Processing and N-back Task) by condition and test occasion

Outcome	Time 1 (*pre-first dose*)[a] Mean ± SE	Time 2 *(+90 minutes post time 1 & first dose)* Mean ± SE	Active vs. placebo
Attention Switching Task: Switch cost reaction time (s)			
Active (switch trials)	0.27 ± 0.56	-0.24 ± 0.59	F(1,91) = 7.46, p = .008
Placebo (switch trials)	-0.44 ± 0.65	-0.75 ± 0.58	
Active (repeat trials)	1.09 ± 0.43	0.64 ± 0.46	
Placebo (repeat trials)	-0.03 ± 0.62	-0.12 ± 0.56	
Attention Switching Task: Switch cost accuracy (%)			
Active (switch trials)	-1.21 ± 1.10	-2.56 ± 1.16	F(1,92) = 0.02, p = .898
Placebo (switch trials)	-1.34 ± 1.31	-2.10 ± 1.22	
Active (repeat trials)	1.15 ± 0.75	0.99 ± 0.96	
Placebo (repeat trials)	0.70 ± 0.99	0.65 ± 1.01	

Outcome	Time 1 (*pre-first dose*)[a] Mean ± SE	Time 2 *(+90 minutes post time 1 & first dose)* Mean ± SE	Active vs. placebo
Attention Switching Task: Target accuracy (n)			
Active (switch trials)	12.82 ± 0.82	13.85 ± 0.95	
Placebo (switch trials)	13.12 ± 0.69	13.65 ± 0.86	
Active (nested trials)	13.69 ± 0.89	15.69 ± 0.96	$F(1,142) = 0.96$, $p = .328$
Placebo (nested trials)	14.08 ± 1.14	15.17 ± 1.07	
Active (pre-switch trials)	14.52 ± 0.99	16.41 ± 1.11	
Placebo (pre-switch trials)	14.59 ± 1.01	15.64 ± 1.05	
Attention Switching Task: Target reaction time (s)			
Active (switch trials)	9.08 ± 0.63	9.97 ± 0.70	
Placebo (switch trials)	9.36 ± 0.72	9.72 ± 0.77	
Active (nested trials)	8.81 ± 0.70	10.21 ± 0.62	$F(1,141) = 4.24$, $p = .041$
Placebo (nested trials)	9.80 ± 0.84	10.47 ± 0.74	
Active (pre-switch trials)	9.90 ± 0.72	10.85 ± 0.73	
Placebo (pre-switch trials)	9.76 ± 0.79	10.35 ± 0.72	
Rapid Visual Information Processing (RVIP): Hits (n)			
Active	18.00 ± 1.94	19.92 ± 2.00	$F(1,46) = 6.80$, $p = .012$
Placebo	16.88 ± 1.68	15.17 ± 1.87	
Rapid Visual Information Processing (RVIP): False alarms (n)			
Active	16.85 ± 2.65	14.04 ± 1.73	$F(1,42) = 0.44$, $p = .508$
Placebo	22.17 ± 3.36	19.05 ± 3.48	
Rapid Visual Information Processing (RVIP): Reaction time for hits (s)			
Active	0.46 ± 0.00	0.46 ± 0.00	$F(1,864) = 8.41$, $p = .004$
Placebo	0.46 ± 0.01	0.47 ± 0.00	

Outcome		Time 1 (*pre-first dose*)[a] Mean ± SE	Time 2 *(+90 minutes post time 1 & first dose)* Mean ± SE	Active vs. placebo
N-back Task: Target accuracy (%)				
	Active	57.46 ± 4.55	59.03 ± 4.70	$F(1,44) = 7.55$, $p = .009$
	Placebo	50.44 ± 4.93	55.87 ± 4.07	
N-back Task: Total accuracy (%)				
	Active	49.13 ± 4.21	52.97 ± 4.31	$F(1,44) = 3.57$, $p = .066$
	Placebo	44.95 ± 4.81	51.39 ± 3.88	
N-back Task: Reaction time for targets (s)				
	Active	0.53 ± 0.00	0.52 ± 0.00	$F(1,1891) = 1.98$, $p = .160$
	Placebo	0.56 ± 0.00	0.55 ± 0.00	
N-back Task: Reaction time for nontargets (s)				
	Active	0.54 ± 0.00	0.53 ± 0.00	$F(1,4971) = 147.23$, $p < .001$
	Placebo	0.54 ± 0.00	0.53 ± 0.00	

Notes. [a]Time 1 represents baseline performance; n: the number of trials; s: seconds. For more information on cognitive outcomes, please refer to Table 5.3.

5.8.3.1.1 Attention Switching Task

5.8.3.1.1.1 Switch cost reaction time

No outlying observations were removed from the final model. Accuracy was a significant covariate, $F(1,91) = 324.43$, $p < .001$, this being positively related to switch cost reaction time. There was a trend towards gender being a significant covariate, $F(1,91) = 2.89$, $p = .093$, with females (-0.21 ± 0.15) responding more quickly compared to males (0.28 ± 0.24). Baseline performance and age were not significant covariates, $F(1,91) = 0.03$, $p = .863$ and $F(1,91) = 1.27$, $p = .262$, respectively. IQ was also not a significant covariate and was removed from the final model. There was a marginally significant interaction between baseline*condition, $F(1,91) = 3.86$, $p = .052$.

There was a significant main effect of condition, $F(1,91) = 7.46$, $p = .008$ (Figure 5.4(A)), such that those in the active condition (0.36 ± 0.19) responded significantly slower than those in the placebo condition (-0.29 ± 0.19).

There was also a significant main effect of trial (switch costs vs. repeat costs), $F(1,91) = 6.38$, $p = .013$, shown in Figure 5.4(B). Just as with accuracy, reaction time switch costs (0.36 ± 0.19) were significantly greater compared to repeat costs (-0.30 ± 0.19) across both conditions. There was no significant trial*condition interaction, $F(1,91) = 0.00$, $p = .989$.

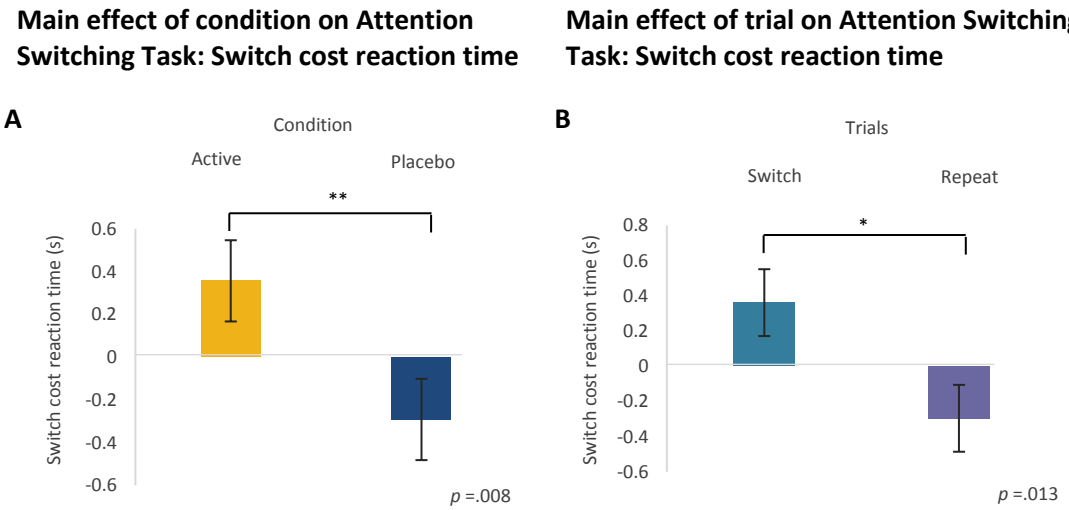


Figure 5.4 Switch cost reaction time on the Attention Switching Task. (A) The x axis is condition and the y axis is switch cost reaction time (s) over midpoint and endpoint. Columns denote the least squares means. Error bars denote the SE of the least squares means. (B) The x axis represents trials and the y axis is the switch cost reaction time (s) over midpoint and endpoint. Columns denote the least squares means. Error bars denote the SE of the least squares means.* p <.05, ** p <.01.

5.8.3.1.1.2 Switch cost accuracy

No outlying observations were removed from the final model. Baseline was a significant covariate, $F(1,92) = 11.63$, $p = .001$, with this being positively related to performance at time 2. Age was a significant covariate, $F(1,92) = 4.44$, $p = .038$, such that better performance was shown with increasing age. IQ and gender were not significant covariates, $F(1,92) = 0.58$, $p = .447$ and $F(1,92) = 1.62$, $p = .206$, respectively. There was a significant main effect of trial (switch cost vs. repeat cost), $F(1,92) = 5.55$, $p = .021$ (Figure 5.5). Accuracy switch costs (-2.27 ± 0.76) were significantly higher (less accurate) compared to repeat costs (0.12 ± 0.74) across both conditions. There was no significant main effect of condition, $F(1,92) = 0.02$, $p = .898$. Trial*condition was

also nonsignificant, $F(1,92) = 0.12$, $p = .727$. There was also no significant baseline*condition interaction, and this term was removed from the final model.

Main effect of trial on Attention Switching Task: Switch cost accuracy

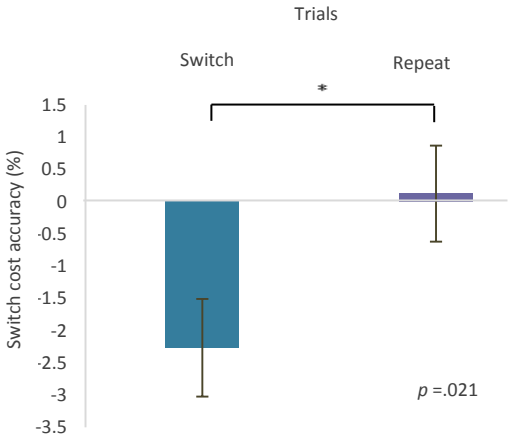


Figure 5.5 Switch cost accuracy on the Attention Switching Task. The x axis represents trials and the y axis is the switch cost accuracy (%) over midpoint and endpoint. Columns denote the least squares means. Error bars denote the SE of the least squares means. * p <.05

5.8.3.1.1.3 Target accuracy

One outlying observation was removed from the final model. Baseline target accuracy was a significant covariate, $F(1,142) = 168.68$, p <.001, which was positively correlated with performance at time 2. Age, IQ and gender were not significant covariates and were removed from the final model. There was no significant main effect of condition or trial, $F(1,142) = 0.96$, $p = .328$ and $F(2,142) = 1.11$, $p = .334$, respectively. Further, trial*condition was not significant, $F(2,142) = 0.06$, $p = .939$. There was also no significant baseline*condition interaction, and this term removed from the final model.

5.8.3.1.1.4 Target reaction time

No outlying observations were removed from the final model. Accuracy, as expected, was a significant covariate, $F(1,141) = 605.26$, $p < .001$, such that the greater the number of targets identified, the longer the reaction time. Baseline target reaction time was not a significant covariate, $F(1,141) = 0.92$, $p = .339$. Age, IQ and gender were also not significant covariates and were removed from the final model. There was a significant baseline*condition interaction, $F(1,141) = 6.20$, $p = .014$, such that there was consistency in performance across test sessions; those who were slower at baseline remained slower at time 2, and this was also the case for those who were faster at baseline. In respect of condition, those in the active condition were marginally slower compared to those in the placebo condition before and after treatment.

Inspection of post hoc comparisons revealed that participants in the active condition (10.44 ± 0.41) were significantly slower than those in the placebo condition (9.49 ± 0.39; $t(141) = 2.00$, $p = .048$) if their baseline reaction time for targets was 1. The participants in the active condition (10.41 ± 0.36) were marginally significantly slower than those in the placebo condition (9.59 ± 0.35; $t(141) = 1.91$, $p = .058$) if their baseline reaction time for targets was 2. There was a trend towards participants in the active condition (10.38 ± 0.32) responding more slowly than their counterparts in the placebo condition (9.69 ± 0.32; $t(141) = 1.81$, $p = .073$) if their baseline reaction time for targets was 3 or 4. There was also a trend towards those in the active condition (10.14 ± 0.15) demonstrating faster reaction time relative to those in the placebo condition (10.49 ± 0.14; $t(141) = -1.71$, $p = .090$) if their baseline reaction time for targets was 11. Finally, those in the active condition (10.11 ± 0.19) were significantly faster than those in the placebo condition (10.59 ± 0.16; $t(141) = -2.07$, $p = .040$) if their baseline reaction time for targets was 12 or greater (slower reaction time). No other post hoc comparisons were significant.

There was a significant main effect of condition, $F(1,141) = 4.24$, $p = .041$, such that participants in the active condition (10.18 ± 0.13) demonstrated faster reaction time for targets compared to their counterparts in the placebo condition (10.33 ± 0.13), however, post hoc tests did not reveal a significant difference.

There was also a significant main effect of trial, $F(2,141) = 5.90$, $p = .004$, shown in Figure 5.6. As expected, irrespective of condition, participants demonstrated slowest target reaction times on switch trials i.e. on occasions when a new rule had to be applied to make a correct response. Following this, target reaction time decreased, such that the fastest reaction time for targets was demonstrated for the pre-switch trial. Inspection of post hoc comparisons revealed there was a significant difference in reaction time for switch trials vs. nested trials and for switch trials vs. pre-switch trials. Specifically, reaction time was significantly slower on switch trials (10.71 ± 0.16) compared to nested trials (10.08 ± 0.16; $t(141) = 2.73$, $p = .019$) and pre-switch trials (9.97 ± 0.16; $t(141) = 3.20$, $p = .005$) across both conditions. There was no significant trial*condition interaction, $F(2,141) = 1.41$, $p = .248$.

Main effect of trial on Attention Switching Task: Target reaction time

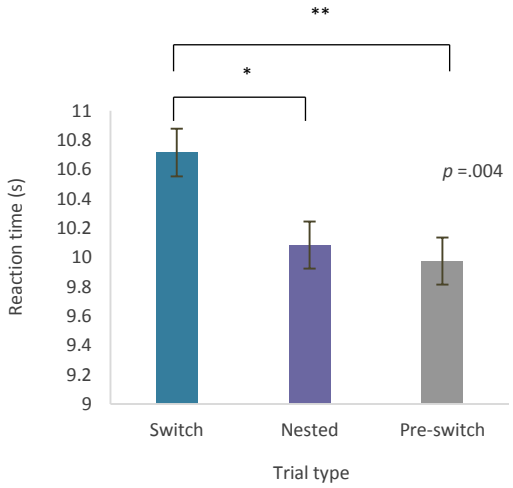


Figure 5.6 Reaction time for targets on the Attention Switching Task. The x axis represents trial type and the y axis is the reaction time (s) for targets over midpoint and endpoint. Columns denote the least squares means. Error bars denote the SE of the least squares means. * p <.05, ** p <.01.

5.8.3.1.2 Rapid Visual Information Processing Task (RVIP)

5.8.3.1.2.1 Hits

One outlying observation was removed from the final model. Baseline performance was a significant covariate, $F(1,46) = 158.97$, p <.001, and was positively related to performance at time 2. Age, IQ and gender were not significant covariates and were removed from the final model. There was a significant main effect of condition, $F(1,46) = 6.80$, p = .012 (Figure 5.7). Figure 5.7 presents that those in the active condition (0.35 ± 0.03) were significantly more accurate (more correct trials) than participants in the placebo condition (0.25 ± 0.03). There was no significant baseline*condition interaction, and this term was removed from the final model.

Main effect of condition on Rapid Visual Information Processing Task (RVIP): Hits

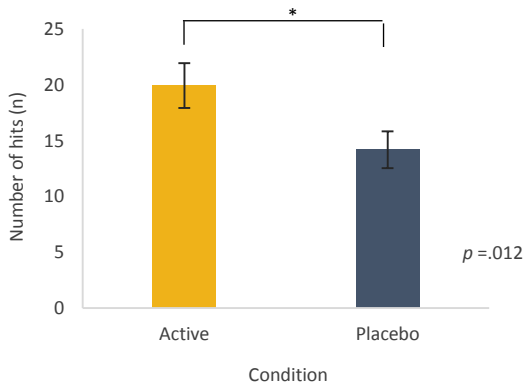


Figure 5.7 Hits achieved on the Rapid Visual Information Processing Task (RVIP). The x axis is condition and the y axis is the number of hits (n) over midpoint and endpoint. Columns denote the mean. Error bars denote the SE of the mean.* p <.05

5.8.3.1.2.2 False alarms

Two outlying observations were removed from the final model. Baseline performance was a significant covariate, $F(1,42) = 91.37$, p <.001, such that this showed a positive relationship with the incidence of false alarms at time 2. IQ was a significant covariate, $F(1,42) = 10.44$, $p = .002$, and was negatively correlated with performance i.e. the prevalence of false alarms decreased with increasing IQ. Gender was not a significant covariate, $F(1,42) = 0.55$, $p = .461$. Age was also not a significant covariate and was removed from the final model. There was no significant main effect of condition, $F(1,42) = 0.44$, $p = .508$. There was also no significant baseline*condition interaction, $F(1,42) = 1.19$, $p = .281$.

5.8.3.1.2.3 Reaction time for hits

Eleven outlying observations were removed from the final model. Baseline reaction time was a significant covariate, $F(1,864) = 83.16$, p <.001, with baseline performance being positively correlated with reaction time at time 2. Age was a significant covariate, $F(1,864) = 12.54$, p <.001, such that with increasing age, reaction time became slower. Gender was also a significant covariate, $F(1,864) = 8.02$, $p = .005$, with females reacting significantly more quickly (0.72 ± 0.01) relative to males (0.78 ± 0.02). As expected, the total number of correct trials (hits) was also a significant covariate, $F(1,864) = 25.49$, p <.001, such that reaction time was negatively correlated with the number of correct trials. IQ and trial were not significant covariates and were removed

from the final model. There was a significant interaction between baseline*condition, $F(1,864) = 10.86$, $p = .001$, shown in Figure 5.8.

Interaction between baseline*condition on Rapid Visual Information Processing Task (RVIP): Reaction time for hits

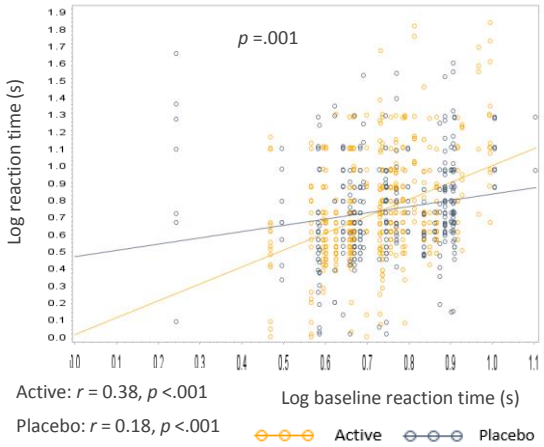


Figure 5.8 Reaction time for hits on the Rapid Visual Information Processing Task (RVIP). The x axis represents log baseline reaction time (s) for hits and the y axis is the log reaction time (s) for hits over subsequent test points. Regression lines show relationship between x and y by condition.

Figure 5.8 shows that at faster baseline reaction time, those in the active condition demonstrated faster performance compared to participants in the placebo condition. However, the opposite was shown at slower baseline reaction latencies. Inspection of post hoc comparisons revealed those in the active condition (0.45 ± 0.04) were significantly faster than those in the placebo condition (0.58 ± 0.04; $t(864) = -2.37$, $p = .018$) if their log of baseline reaction time was 0.4 or lower (faster baseline reaction time). Those in the active condition (0.55 ± 0.03) were also marginally significantly faster than the placebo condition (0.63 ± 0.03; $t(864) = -1.96$, $p = .050$) at a log of baseline reaction time of 0.5. Conversely, participants in the active condition (0.83 ± 0.02) were significantly slower than those in the placebo condition (0.76 ± 0.02; $t(864) = 3.16$, $p = .002$) if their log of baseline reaction time was 0.8 or above (slower reaction time). No other post hoc comparisons were significant.

A significant main effect of condition was also found, $F(1,864) = 8.41$, $p = .004$. Inspection of post hoc comparisons revealed a trend towards those in the active condition (0.77 ± 0.01) demonstrating slower reaction latencies compared to those in the placebo condition (0.73 ± 0.02; $t(864) = 1.80$, $p = .072$).

5.8.3.1.3 N-back Task

5.8.3.1.3.1 Target accuracy

No outlying observations were removed from the final model. Baseline performance was a significant covariate, $F(1,44) = 84.60$, $p < .001$, such that baseline target accuracy was positively associated with subsequent performance at time 2. IQ was a significant covariate, $F(1,44) = 7.30$, $p = .010$, being positively related to greater target accuracy, such that those with a higher IQ achieved better target accuracy. There was a trend towards gender being a significant covariate, $F(1,44) = 2.89$, $p = .096$, with females (7.48 ± 0.17) achieving greater target accuracy relative to males (6.91 ± 0.28). Age was not a significant covariate and was removed from the final model. There was a significant baseline*condition interaction, $F(1,44) = 6.90$, $p = .012$ (Figure 5.9). Inspection of post hoc comparisons revealed that those in the active condition (5.25 ± 0.29) showed significantly poorer target accuracy compared to those in the placebo condition (6.27 ± 0.29; $t(44) = -2.47$, $p = .018$) if their square root baseline target accuracy was 5 or lower (poorer performance). Moreover, performance by those in the active condition (6.13 ± 0.23) was marginally significantly less accurate to that of those in the placebo condition (6.76 ± 0.23; $t(44) = -1.96$, $p = .056$) if their square root baseline target accuracy was 6. No other post hoc comparisons were significant.

Interaction between baseline*condition on N-back Task: Target accuracy

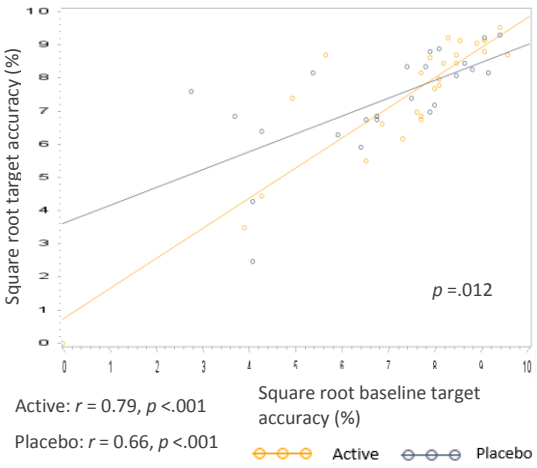


Figure 5.9 Target accuracy on the N-back Task. The x axis represents square root baseline target accuracy (%) and the y axis is the square root target accuracy (%) over subsequent test points. Regression lines show relationship between x and y by condition.

There was also a significant main effect of condition, $F(1,44) = 7.55$, $p = .009$, such that those in the active condition performed less well (7.09 ± 0.21) relative to those in the placebo condition (7.30 ± 0.22), however, post hoc tests did not reveal a significant difference.

5.8.3.1.3.2 Total accuracy

No outlying observations were removed from the final model. Baseline total accuracy was a significant covariate, $F(1,44) = 65.56$, $p < .001$, with this being positively correlated with performance at time 2. IQ was a significant covariate, $F(1,44) = 7.21$, $p = .010$, such that total accuracy increased with increasing IQ. Gender was not a significant covariate, $F(1,44) = 2.12$, $p = .152$. Age was also not a significant covariate and was removed from the final model. There was a marginally significant baseline*condition interaction, $F(1,44) = 3.82$, $p = .057$ and condition was also marginally significant, $F(1,44) = 3.57$, $p = .066$. Performance between the two conditions differed such that participants in the placebo condition (51.08 ± 2.65) achieved greater total accuracy compared to those in the active condition (50.16 ± 2.56).

5.8.3.1.3.3 Reaction time for targets

No outlying observations were removed from the final model. Baseline was a significant covariate, $F(1,1891) = 252.76$, $p < .001$, with this being positively related to reaction time for targets at time 2. Trial was a significant covariate, $F(1,1891) = 5.25$, $p = .022$, with performance fluctuating across the trials. As expected, the total number of correct trials achieved was a significant covariate, $F(1,1891) = 6.90$, $p = .009$, and reaction time decreased as a function of a greater number of correct trials obtained. There was a trend towards gender being a significant covariate, $F(1,1891) = 2.79$, $p = .095$, with females (-0.28 ± 0.00) reacting to targets faster than males (-0.29 ± 0.00). IQ was not a significant covariate, $F(1,1891) = 1.30$, $p = .255$. Age was not a significant covariate and was removed from the final model. There was no significant main effect of condition, $F(1,1891) = 1.98$, $p = .160$, and no significant baseline*condition interaction, $F(1,1891) = 1.56$, $p = .211$.

5.8.3.1.3.4 Reaction time for nontargets

Seventeen outlying observations were removed from the final model. Baseline was a significant covariate, $F(1,4971) = 228.61$, $p < .001$, and was positively associated with reaction time for nontargets at time 2. Age was a significant covariate, $F(1,4971) = 3.87$, $p = .049$, with reaction time increasing with age. IQ was also a significant covariate, $F(1,4971) = 172.29$, $p < .001$, such that those with a higher IQ responded more slowly compared to those with a lower IQ. Gender

was a significant covariate, $F(1,4971) = 19.18$, $p < .001$, with males (0.52 ± 0.00) responding significantly more quickly compared to females (0.53 ± 0.00). Trial and the total number of correct trials achieved were also significant covariates, $F(1,4971) = 4.92$, $p = .027$ and $F(1,4971) = 335.01$, $p < .001$, respectively. Reaction time for nontargets by trial varied and decreased with increasing number of correct trials achieved. There was a significant baseline*condition interaction, $F(1,4971) = 138.37$, $p < .001$, shown in Figure 5.10(A).

Figure 5.10(A) indicates that at faster baseline reaction time for nontargets, those in the active condition showed faster performance compared to those in the placebo condition, however, the opposite was found at slower baseline reaction time, such that those in the active condition demonstrated slower performance relative to those in the placebo condition. Inspection of post hoc comparisons revealed that those in the active condition (0.52 ± 0.00) responded significantly faster than those in the placebo condition (0.53 ± 0.00; $t(4971) = -2.97$, $p = .003$) if their baseline reaction time for nontargets was 0.55 or less (faster performance). Conversely, participants in the active condition were significantly slower at responding (0.56 ± 0.00) compared to those in the placebo condition (0.54 ± 0.00; $t(4971) = 5.08$, $p < .001$) if their baseline reaction time for nontargets was 0.60 or greater (slower performance).

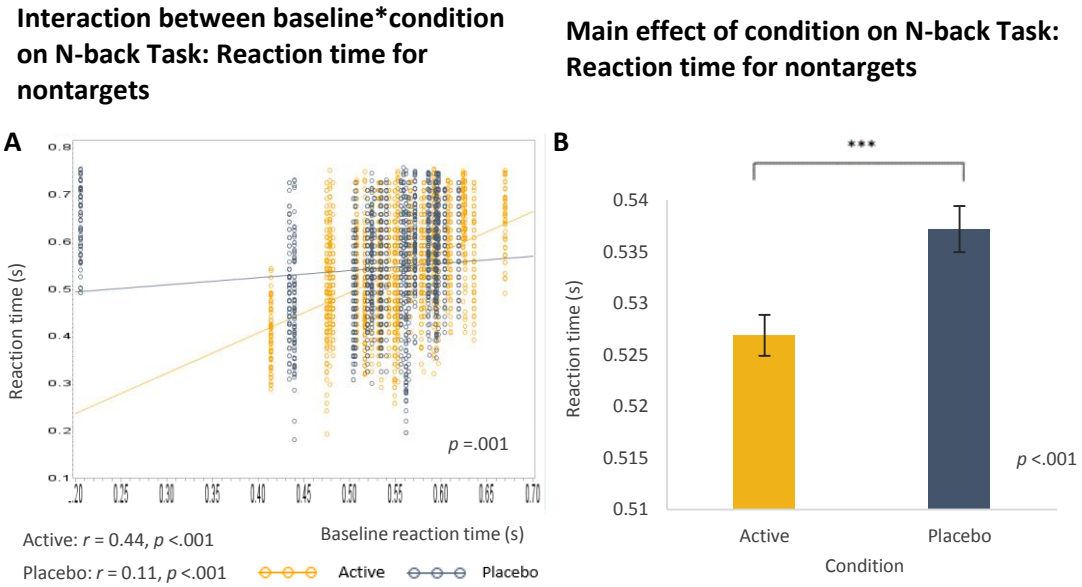


Figure 5.10 Reaction time for nontargets on the N-back Task. (A) The x axis represents baseline reaction time (s) for nontargets and the y axis is reaction time (s) for nontargets over subsequent test points. Regression lines show relationship between x and y by condition. (B) The x axis is condition and the y axis is reaction time (s) for nontargets over midpoint and endpoint. Columns denote the mean. Error bars denote the SE of the mean.*** $p < .001$.

There was also a significant main effect of condition, $F(1,4971) = 147.23$, $p < .001$ (Figure 5.10(B)). Inspection of post hoc comparisons revealed those in the active condition (0.52 ± 0.00) demonstrated significantly faster performance relative to the placebo condition (0.53 ± 0.00; $t(4971) = -4.64$ $p < .001$).

5.8.3.2 Measures of memory performance

Table 5.7 provides a summary of the means (± SE) across measures of memory performance for active and placebo conditions.

Table 5.7 Mean (± SE) on measures of memory performance (Pattern Separation Task, Face-Name Associative Memory and Visual Verbal Learning Test) by condition and test occasion

Outcome	Time 1 (*pre-first dose*)[a] Mean ± SE	Time 2 *(+90 minutes post time 1 and first dose)* Mean ± SE	Active vs. placebo
Pattern Separation Task: Pattern separation score (n)			
Active	-4.08 ± 1.07	-3.92 ± 0.93	$F(1,44) = 1.04$, $p = .312$
Placebo	-3.96 ± 0.90	-2.75 ± 0.83	
Pattern Separation Task: Recognition score (n)			
Active	2.58 ± 0.48	1.96 ± 0.72	$F(1,45) = 4.60$, $p = .038$
Placebo	2.54 ± 0.47	1.67 ± 0.59	
Face-Name Associative Memory Exam: Immediate recall (n)			
Active	5.5 ± 0.52	4.12 ± 0.48	$F(1,46) = 0.00$, $p = .978$
Placebo	4.38 ± 0.62	3.71 ± 0.46	
Face-Name Associative Memory Exam: Delayed recall (n)			
Active	4.85 ± 0.58	2.69 ± 0.44	$F(1,46) = 3.60$, $p = .064$
Placebo	3.5 ± 0.57	2.83 ± 0.50	
Visual Verbal Learning Test (VVLT): Rate of learning (n)			
Active (trial A1)	7.85 ± 0.56	7.04 ± 0.55	$F(1,140) = 0.11$, $p = .737$
Placebo (trial A1)	7.75 ± 0.40	6.54 ± 0.47	
Active (trial A2)	10.27 ± 0.56	9.62 ± 0.44	
Placebo (trial A2)	10.38 ± 0.49	9.50 ± 0.50	
Active (trial A3)	11.38 ± 0.48	10.96 ± 0.48	

Outcome	Time 1 (*pre-first dose*)[a] Mean ± SE	Time 2 *(+90 minutes post time 1 and first dose)* Mean ± SE	Active vs. placebo
Placebo (trial A3)	11.21 ± 0.51	10.96 ± 0.53	
Visual Verbal Learning Test (VVLT): New learning (n)			
Active	7.04 ± 0.53	6.69 ± 0.52	$F(1,44) = 0.16$, $p = .689$
Placebo	6.08 ± 0.54	6.21 ± 0.58	
Visual Verbal Learning Test (VVLT): Retroactive interference (n)			
Active	2.96 ± 0.46	5.23 ± 0.58	$F(1,45) = 1.46$, $p = .233$
Placebo	2.79 ± 0.32	4.08 ± 0.43	
Visual Verbal Learning Test (VVLT): Proactive interference (n)			
Active	0.81 ± 0.49	0.35 ± 0.48	$F(1,45) = 0.09$, $p = .770$
Placebo	1.67 ± 0.48	0.33 ± 0.51	
Visual Verbal Learning Test (VVLT): Delayed recall (n)			
Active	8.23 ± 0.60	5.5 ± 0.78	$F(1,44) = 3.94$, $p = .053$
Placebo	7.83 ± 0.75	6.42 ± 0.71	

Notes. [a]Time 1 represents baseline performance; n: the number of trials; s: seconds. For more information on cognitive outcomes, please refer to Table 5.3.

5.8.3.2.1 Pattern Separation Task

5.8.3.2.1.1 Pattern separation score

Two outlying observations were removed from the final model (see section 4.6). Baseline performance was a significant covariate, $F(1,44) = 30.71$, $p < .001$, such that this showed a positive relationship with behavioural pattern separation performance at time two. There was a trend towards gender being a significant covariate, $F(1,44) = 3.05$, $p = .088$, with males (0.58 ± 0.04) obtaining a higher score than females (0.49 ± 0.03). Age and IQ were not significant covariates and were removed from the final model. There was no significant main effect of condition, $F(1,44) = 1.04$, $p = .312$. There was also no significant baseline*condition interaction, and this term was removed from the final model.

5.8.3.2.1.2 Recognition score

No outlying observations were removed from the final model. Baseline performance was a significant covariate, $F(1,45) = 36.05$, $p < .001$, being positively correlated with recognition score at the subsequent test point. Gender was not a significant covariate, $F(1,45) = 0.37$, $p = .547$. Age and IQ were not significant covariates also and were removed from the final model. There was a significant baseline*condition interaction, $F(1,45) = 8.25$, $p = .006$ (Figure 5.11). Inspection of post hoc comparisons revealed those in the active condition (0.41 ± 0.04) obtained significantly higher recognition scores compared to those in the placebo condition (0.31 ± 0.04; $t(45) = 2.03$, $p = .048$) if their log baseline recognition score was 0.4 or greater (improved performance).

There was also a significant main effect of condition, $F(1,45) = 4.60$, $p = .038$, such that participants in the active condition obtained a higher recognition score (0.33 ± 0.03) compared to those in the placebo condition (0.28 ± 0.03). However, post hoc comparisons did not reveal a significant difference.

Interaction between baseline*condition on Pattern Separation Task: Recognition score

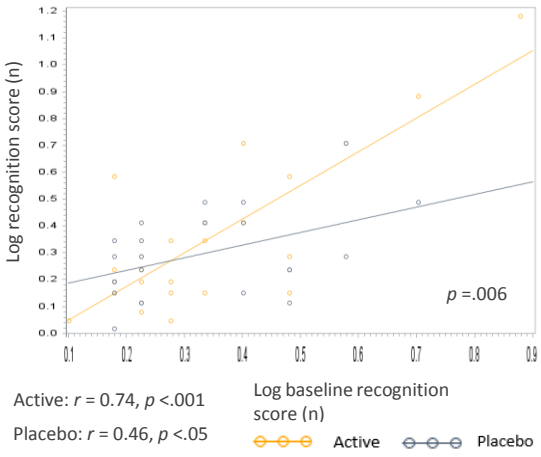


Figure 5.11 Recognition score on the Pattern Separation Task. The x axis represents log baseline recognition score (n) and the y axis is the log recognition score (n) over subsequent test points. Regression lines show relationship between x and y by condition.

5.8.3.2.2 Face-Name Associative Memory Exam

5.8.3.2.2.1 Immediate recall

No outlying observations were removed from the final model. Baseline immediate recall performance was a significant covariate, $F(1,46) = 10.60$, $p = .002$, and was positively associated

with performance at time 2. Gender was not a significant covariate, $F(1,46) = 1.97$, $p = .167$. Age and IQ were not significant covariates also and were removed from the final model. There was no significant main effect of condition, $F(1,46) = 0.00$, $p = .978$. There was also no significant baseline*condition interaction, and this term was removed from the final model.

5.8.3.2.2.2 Delayed recall

No outlying observations were removed from the final model. Baseline performance was a significant covariate, $F(1,46) = 45.09$, $p < .001$, and was positively related to delayed recall at time 2. IQ was not a significant covariate, $F(1,46) = 2.03$, $p = .161$. Age and gender were not significant covariates also and were removed from the final model. Condition was marginally significant, $F(1,46) = 3.60$, $p = .064$. Performance between the two conditions differed such that those in the placebo condition (3.25 ± 0.35) recalled more items correctly compared to those in the active condition (2.31 ± 0.34). There was no significant baseline*condition interaction, and this term was removed from the final model.

5.8.3.2.3 Visual Verbal Learning Test (VVLT)

5.8.3.2.3.1 Rate of learning (word list A)

One outlying observation was removed from the final model. Baseline performance was a significant covariate, $F(1,140) = 25.36$, $p < .001$, such that performance at baseline was positively related to performance at time 2. Age and IQ were also significant covariates, $F(1,140) = 7.21$, $p = .008$ and $F(1,140) = 17.22$, $p < .001$, respectively. Rate of learning reduced with age, whereas rate of learning was augmented by increased IQ. Gender was not a significant covariate and was subsequently removed from the final model. There was a significant main effect of trial, $F(2,140) = 20.29$, $p < .001$, such that rate of learning improved over successive trials (Figure 5.12).

Inspection of post hoc comparisons revealed there was a significant difference in the number of items correctly recalled, with significantly less items being correctly recalled for trial 1 (7.41 ± 0.32) compared to trials 2 (9.38 ± 0.29; $t(140) = -4.44$, $p < .001$) and 3 (10.43 ± 0.30; $t(140) = -6.32$, $p < .001$). Moreover, significantly less items were correctly recalled on trial 2 (9.38 ± 0.29) relative to trial 3 (10.43 ± 0.30; $t(140) = -2.55$, $p = .031$). There was no significant main effect of condition, $F(1,140) = 0.11$, $p = .737$. There was also no significant trial*condition interaction, $F(2,140) = 0.06$, $p = .941$, nor a significant baseline*condition interaction, and this term was removed from the final model.

Main effect of trial on Visual Verbal Learning Test (VVLT): Rate of learning

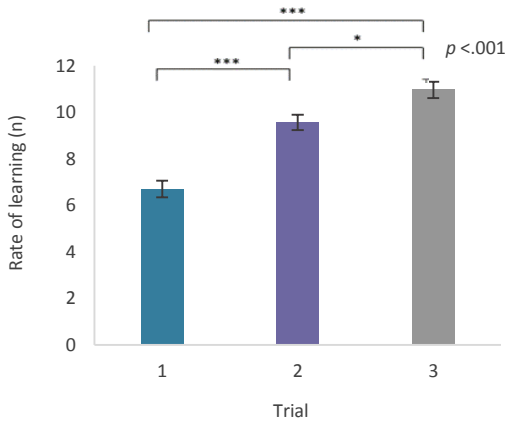


Figure 5.12 Rate of learning on the Visual Verbal Learning Test (VVLT). The x axis represents trial and the y axis is the rate of learning (n) over midpoint and endpoint. Columns denote the mean. Error bars denote the SE of the mean. * p <.05, *** p <.001.

5.8.3.2.3.2 New learning (word list B)

No outlying observations were removed from the final model. Baseline was a significant covariate, $F(1,44) = 15.69$, p <.001, with the number of items recalled from list B at baseline being positively related to performance at time 2. Age was also a significant covariate, $F(1,44) = 4.30$, $p = .044$, with performance worsening with age. Gender was not a significant covariate, $F(1,44) = 0.92$, $p = .343$. IQ was also nonsignificant and removed from the final model. There was no significant main effect of condition, $F(1,44) = 0.16$, $p = .689$, and no significant baseline*condition interaction, $F(1,44) = 0.16$, $p = .690$.

5.8.3.2.3.3 Retroactive interference (Trial A3-Trial A4)

No outlying observations were removed from the final model. Baseline and gender were not significant covariates, $F(1,45) = 2.43$, $p = .126$ and $F(1,45) = 0.54$, $p = .468$, respectively. Age and IQ were also not significant covariates and were removed from the final model. There was no significant main effect of condition, $F(1,45) = 1.46$, $p = .233$, and no significant baseline*condition interaction, $F(1,45) = 0.26$, $p = .609$.

5.8.3.2.3.4 Proactive interference (Trial A1-Trial B1)

No outlying observations were removed from the final model. Age was a significant covariate, $F(1,45) = 4.46$, $p = .040$, such that better performance was demonstrated by younger participants. Baseline and gender were not significant covariates, $F(1,45) = 1.38$, $p = .247$ and

F(1,45) = 0.79, *p* = .379, respectively. IQ was also not a significant covariate and was removed from the final model. There was no significant main effect of condition, *F*(1,45) = 0.09, *p* = .770. There was also no significant baseline*condition interaction, and this term was removed from the final model.

5.8.3.2.3.5 Delayed recall

No outlying observations were removed from the final model. Baseline was a significant covariate, *F*(1,44) = 36.21, *p* <.001, and was positively related to performance at time 2. Age was also a significant covariate, *F*(1,44) = 6.05, *p* = .018, such that performance showed age-related decline. Gender was not a significant covariate, *F*(1,44) = 0.13, *p* = .722. IQ was also nonsignificant and removed from the final model. The main effect of condition just failed to reach significance, *F*(1,44) = 3.94, *p* = .053. Performance between the two conditions differed such that those in the active condition (5.23 ± 0.53) recalled less items correctly compared to those in the placebo condition (6.51 ± 0.54). There was no significant baseline*condition interaction, *F*(1,44) = 2.01, *p* = .164.

5.8.4 Chronic effect of intervention on cognitive performance in middle-aged and older adults with a subjective memory complaint

5.8.4.1 Measures of executive function performance

Table 5.8 provides a summary of the means (± SE) across measures of executive function performance for active and placebo conditions.

Table 5.8 Mean (± SE) on measures of executive function performance (Attention Switching Task, Rapid Visual Information Processing and N-back Task) by condition and week

Outcome	Baseline (*week 0*)[a] Mean ± SE	Midpoint (*week 6*)[a] Mean ± SE	Endpoint (*week 12*)[b] Mean ± SE	Active vs. placebo
Attention Switching Task: Switch cost reaction time (s)				
Active (switch trials)	0.29 ± 0.56	-1.08 ± 0.52	-1.09 ± 49	
Placebo (switch trials)	-0.44 ± 0.65	-0.32 ± 0.60	-0.87 ± 0.73	*F*(1,45) = 0.05, *p* = .833
Active (repeat trials)	0.88 ± 0.36	-0.19 ± 0.36	0.10 ± 0.30	
Placebo (repeat trials)	-0.03 ± 0.62	0.35 ± 0.55	0.08 ± 0.39	

Outcome	Baseline (*week 0*)[a] Mean ± SE	Midpoint (*week 6*)[a] Mean ± SE	Endpoint (*week 12*)[b] Mean ± SE	Active vs. placebo
Attention Switching Task: Switch cost accuracy (%)				
Active (switch trials)	-0.91 ± 1.12	-3.59 ± 0.97	-3.27 ± 0.80	
Placebo (switch trials)	-1.34 ± 1.31	-1.89 ± 1.26	-2.82 ± 1.39	$F(1,45) = 2.85, p = .098$
Active (repeat trials)	0.89 ± 0.69	0.33 ± 0.74	0.14 ± 0.59	
Placebo (repeat trials)	0.70 ± 0.99	0.72 ± 1.01	0.24 ± 0.81	
Attention Switching Task: Target accuracy (n)				
Active (switch trials)	12.88 ± 0.88	14.04 ± 0.91	14.77 ± 0.83	
Placebo (switch trials)	13.12 ± 0.69	12.85 ± 0.85	13.40 ± 0.92	
Active (nested trials)	13.54 ± 0.94	16.63 ± 0.96	17.13 ± 0.67	
Placebo (nested trials)	14.08 ± 1.14	14.21 ± 1.13	15.43 ± 1.03	$F(1,45) = 0.65, p = .426$
Active (pre-switch trials)	14.18 ± 1.04	16.86 ± 0.84	17.23 ± 0.76	
Placebo (pre-switch trials)	14.59 ± 1.01	14.73 ± 1.03	15.60 ± 0.93	
Attention Switching Task: Target reaction time (s)				
Active (switch trials)	9.02 ± 0.68	10.31 ± 0.65	10.88 ± 0.64	
Placebo (switch trials)	9.36 ± 0.72	9.28 ± 0.67	9.53 ± 0.71	
Active (nested trials)	8.74 ± 0.73	11.39 ± 0.60	11.97 ± 0.48	
Placebo (nested trials)	9.80 ± 0.84	9.60 ± 0.80	10.40 ± 0.75	$F(1,46) = 0.65, p = .424$
Active (pre-switch trials)	9.62 ± 0.75	11.20 ± 0.54	12.07 ± 0.48	
Placebo (pre-switch trials)	9.76 ± 0.79	9.95 ± 0.75	10.49 ± 0.65	
Rapid Visual Information Processing (RVIP): Hits (n)				
Active	18.25 ± 2.08	20.00 ± 2.14	18.75 ± 2.36	$F(1,43) = 0.46, p = .502$
Placebo	16.88 ± 1.68	15.17 ± 1.92	17.35 ± 1.93	
Rapid Visual Information Processing (RVIP): False alarms (n)				
Active	17.46 ± 2.83	17.46 ± 1.88	17.92 ± 1.96	

Outcome	Baseline (*week 0*)[a] Mean ± SE	Midpoint (*week 6*)[a] Mean ± SE	Endpoint (*week 12*)[b] Mean ± SE	Active vs. placebo
Placebo	22.17 ± 3.36	17.96 ± 3.40	21.65 ± 4.87	$F(1,41) = 7.22$, $p = .010$
Rapid Visual Information Processing (RVIP): Reaction time for hits (s)				
Active	0.45 ± 0.00	0.45 ± 0.00	0.46 ± 0.00	$F(1,42) = 1.88$, $p = .178$
Placebo	0.46 ± 0.01	0.47 ± 0.01	0.46 ± 0.01	
N-back Task: Target accuracy (%)				
Active	56.94 ± 4.92	61.24 ± 5.18	61.05 ± 4.24	$F(1,45) = 3.36$, $p = .073$
Placebo	50.44 ± 4.93	54.67 ± 4.00	58.17 ± 4.33	
N-back Task: Total accuracy (%)				
Active	48.99 ± 4.55	54.29 ± 5.80	54.17 ± 4.90	$F(1,45) = 4.66$, $p = .036$
Placebo	44.95 ± 4.81	49.12 ± 4.19	53.89 ± 4.37	
N-back Task: Reaction time for targets (s)				
Active	0.53 ± 0.00	0.52 ± 0.00	0.53 ± 0.00	$F(1,43) = 3.60$, $p = .064$
Placebo	0.56 ± 0.00	0.55 ± 0.00	0.54 ± 0.00	
N-back Task: Reaction time for nontargets (s)				
Active	0.54 ± 0.00	0.53 ± 0.00	0.52 ± 0.00	$F(1,42) = 0.02$, $p = .878$
Placebo	0.54 ± 0.00	0.53 ± 0.00	0.53 ± 0.00	

Notes. [a]n=48; [b]n=47; n: the number of trials; s: seconds. For more information on cognitive outcomes, please refer to Table 5.3.

5.8.4.1.1 Attention Switching Task

5.8.4.1.1.1 Switch cost reaction time

No outlying observations were removed from the final model. Accuracy was a significant covariate, $F(1,127) = 487.83$, $p < .001$, this being positively related to switch cost reaction time. Baseline and gender were not significant covariates, $F(1,127) = 1.25$, $p = .266$ and $F(1,45) = 1.11$, $p = .297$, respectively. Age and IQ were also nonsignificant covariates and were removed from the final model. There was no significant main effect of condition, $F(1,45) = 0.05$, $p = .833$ and no significant baseline*condition interaction, and this term was removed from the final model.

No higher order interactions were significant. There was a significant baseline*week interaction, $F(1,127) = 4.07$, $p = .046$, and a significant interaction between condition and week, $F(1,42) = 5.36$, $p = .026$, however, post hoc comparisons did not reveal any significant differences.

There was a main effect of trial (switch costs vs. repeat costs), $F(1,47) = 11.66$, $p = .001$, shown in Figure 5.13. This shows that reaction time switch costs were significantly greater for switch trials (-0.03 ± 0.15) than repeat trials (-0.59 ± 0.14). Trial*condition and trial*condition*week interactions were not significant and were removed from the final model.

Main effect of trial on Attention Switching Task: Switch cost reaction time

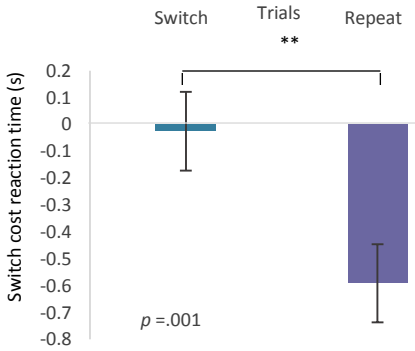


Figure 5.13 Switch cost reaction time on the Attention Switching Task. The x axis is trials and the y axis is switch cost reaction time (s) over midpoint and endpoint. Columns denote the least squares means. Error bars denote the SE of the least squares means.** $p < .01$

5.8.4.1.1.2 Switch cost accuracy

One outlying observation was removed from the final model. Baseline switch cost accuracy was a significant covariate, $F(1,127) = 26.81$, $p < .001$, such that performance at baseline was positively associated with performance at midpoint and endpoint. IQ was a marginally significant covariate, $F(1,45) = 3.88$, $p = .055$, such that those with lower IQ scores were more accurate. Age and gender were not significant covariates and removed from the final model. There were significant baseline*condition*week, $F(1,127) = 3.97$, $p = .049$, gender*condition*week, $F(4,41) = 3.17$, $p = .023$, and baseline*condition, $F(1,127) = 5.28$, $p = .023$, interactions.

The baseline*condition*week interaction is presented in Figure 5.14(A) and 5.14(B). Inspection of post hoc comparisons revealed those in the active condition (Figure 5.14(A)) were significantly less accurate (-2.55 ± 1.55) compared to those in the placebo condition (Figure 5.14(B); 4.97 ± 1.36; $t(41) = -3.66$, $p = .004$) at midpoint at a baseline switch cost accuracy of 10. Also at a

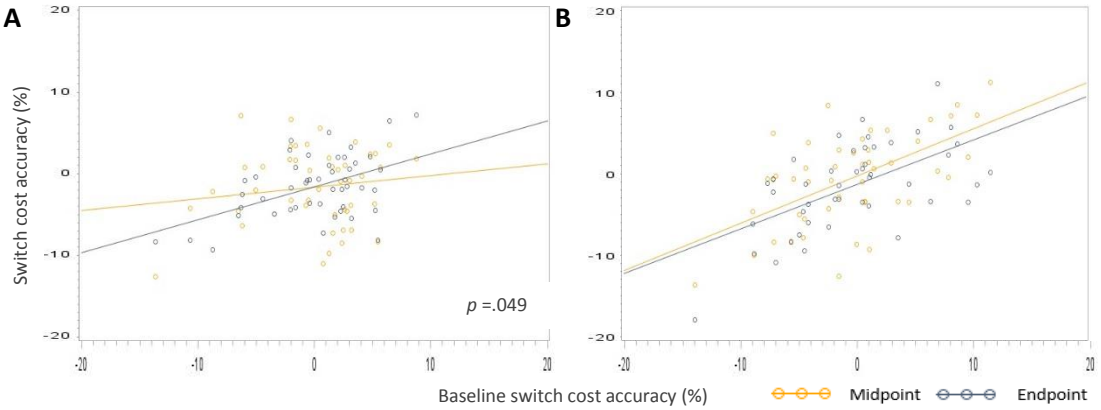
baseline switch cost accuracy of 10, those in the active condition at midpoint (-2.55 ± 1.55) were significantly less accurate than those in the placebo condition at endpoint (3.69 ± 1.40; $t(41) = -2.99$, $p = .023$), and there was a trend towards those in the active condition demonstrating less accuracy at midpoint (-2.55 ± 1.55) relative to endpoint (2.05 ± 1.55; $t(41) = -2.50$, $p = .074$). No other post hoc comparisons were significant.

Figure 5.14(C) and 5.14(D) present the gender*condition*week interaction. Figure 5.14(C) indicates that females in the active condition demonstrated better performance at midpoint, whereas males in the same condition showed greater switch cost accuracy at endpoint. In the placebo condition (Figure 5.14(D)), both females and males performed better at midpoint relative to endpoint. Inspection of post hoc comparisons revealed that males in the active condition (-5.17 ± 1.22) demonstrated marginally significantly poorer performance compared to females in the placebo condition (-0.59 ± 0.77 $t(41) = -3.19$, $p = .050$) at midpoint. Further, females in the active condition (-0.17 ± 0.78) showed significantly greater cost switch accuracy compared to males in the same condition (-5.17 ± 1.22; $t(41) = 3.43$, $p = .027$) at midpoint. No other post hoc comparisons were significant.

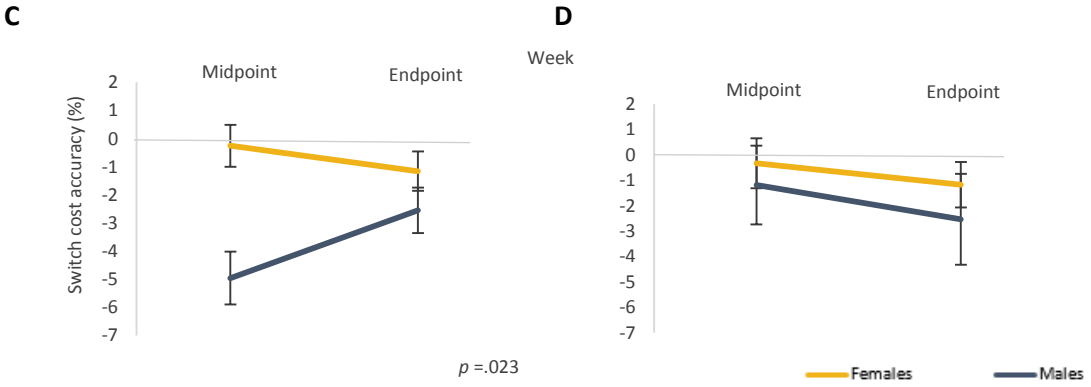
Figure 5.14(E) shows the interaction between baseline and condition. Inspection of post hoc comparisons revealed those in the active condition showed significantly poorer switch cost accuracy (-0.25 ± 1.24) compared to those in the placebo condition (4.33 ± 1.12; $t(45) = -2.73$, $p = .009$) at a baseline switch cost accuracy of 10. This was a trend at a baseline switch cost accuracy of 0 (-2.21 ± 0.60 vs. -0.76 ± 0.61; $t(45) = -1.69$, $p = .098$). No other post hoc comparisons were significant.

There was also a trend towards a main effect of condition, $F(1,45) = 2.85$, $p = .098$, and a significant main effect of trial, $F(1,47) = 28.65$, $p < .001$. The main effect of trial (switch costs vs. repeat costs) is presented in Figure 5.14(F). Accuracy switch costs (-2.91 ± 0.50) were significantly higher (less accurate) compared to repeat costs (-0.16 ± 0.50) across both conditions. There was no significant trial*condition interaction, and this term was removed from the final model. Trial*condition*week was also nonsignificant, $F(3,43) = 0.92$, $p = .439$.

Interaction between baseline*condition*week on Attention Switching Task: Switch cost accuracy



Interaction between gender*condition*week on Attention Switching Task: Switch cost accuracy



Interaction between baseline*condition on Attention Switching Task: Switch cost accuracy

Main effect of trial on Attention Switching Task: Switch cost accuracy

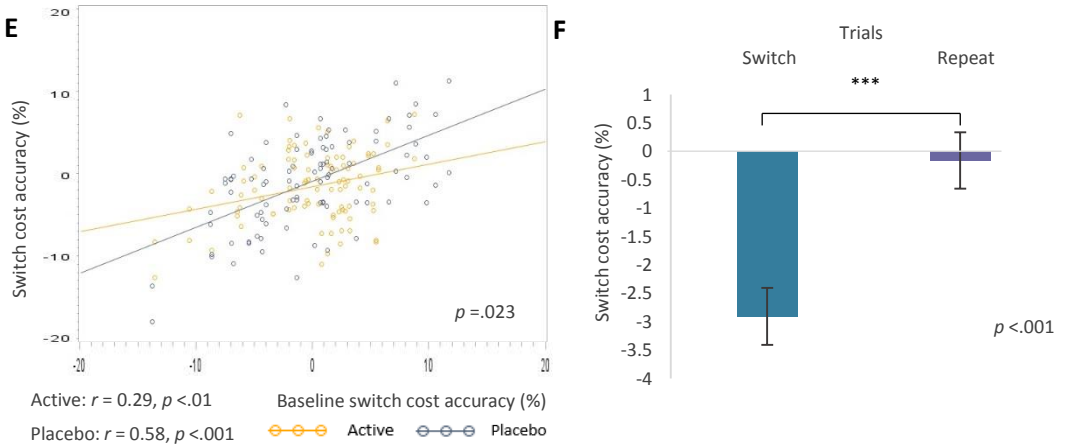


Figure 5.14 Switch cost accuracy on the Attention Switching Task. (A-B) The x axis represents baseline switch cost accuracy (%) and the y axis is switch cost accuracy (%) over subsequent test points. Regression lines show relationship between x and y by week. (C-D) The x axis represents

week and the y axis is switch cost accuracy (%) over midpoint and endpoint. Lines denote the mean. Error bars denote the SE of the mean. (E) The x axis represents baseline switch cost accuracy (%) and the y axis is switch cost accuracy (%) over subsequent test points. Regression lines show relationship between x and y by condition. (F) The x axis represents trials and the y axis is switch cost accuracy (%) over midpoint and endpoint. Columns denote the least squares means. Error bars denote the SE of the least squares means. *** p <.001. (A & C) Is the active condition, (B & D) is the placebo condition.

5.8.4.1.1.3 Target accuracy

One outlying observation was removed from the final model. Baseline performance was a significant covariate, $F(1,218) = 85.67$, p <.001, which was positively correlated with accuracy on later test points. Age was also a significant covariate, $F(1,45) = 5.70$, $p = .021$, such that older participants were less accurate. IQ and gender were not significant covariates and were removed from the final model. There was also no significant main effect of condition, $F(1,45) = 0.65$, $p = .426$, nor a significant baseline*condition interaction, and this term was removed from the final model. No higher order interactions other than IQ*condition*week ($F(4,218) = 3.52$, $p = .008$) were significant.

Figure 5.15(A) and 5.15(B) present how performance differed as a function of IQ, condition and week. Figure 5.15(A) shows those in the active condition were more accurate at endpoint compared to midpoint irrespective of IQ. Figure 5.15(B) presents a clear interaction such that at lower IQ scores, those in the placebo condition performed better at midpoint relative to endpoint, whereas at higher IQ scores, performance was superior at endpoint than at midpoint. Inspection of post hoc comparisons revealed at an IQ score of 100, those in the active condition (18.24 ± 1.33) were significantly more accurate compared to those in the placebo condition (13.50 ± 1.09; $t(41) = 2.76$, $p = .041$) at endpoint. This same relationship was marginally significant at an IQ score of 90 (19.48 ± 2.07 vs. 12.62 ± 1.58; $t(41) = 2.63$, $p = .056$) and 110 (17.01 ± 0.70 vs. 14.38 ± 0.69; $t(41) = 2.66$, $p = .052$). Moreover, those in the active condition were significantly more accurate (17.01 ± 0.70) at endpoint than those in the placebo condition at midpoint (13.98 ± 0.68; $t(41) = 3.10$, $p = .018$) at an IQ score of 110. This was marginally significant at an IQ score of 120 (15.77 ± 0.69 vs. 13.46 ± 0.60; $t(41) = 2.53$, $p = .070$). There was a trend towards those in the active condition performing more accurately (16.32 ± 0.71) compared to those in the placebo condition (13.98 ± 0.68; $t(41) = 2.39$, $p = .096$) at midpoint at an IQ of 110 and higher. Finally, those in the placebo condition were significantly less accurate at midpoint (13.46 ± 0.60) relative to endpoint (15.27 ± 0.62; $t(41) = -3.77$, $p = .003$) at an IQ score of 120. No other post hoc comparisons were significant.

There was a significant interaction between condition and week, $F(1,41) = 5.15$, $p = .029$, shown in Figure 5.15(C). Inspection of post hoc comparisons revealed those in the active condition (15.91 ± 0.58) were significantly more accurate compared to those in the placebo condition at midpoint (13.67 ± 0.57; $t(41) = 2.77$, $p = .040$). Further, those in the active condition were significantly more accurate at endpoint (16.27 ± 0.58) relative to those in the placebo condition at midpoint (13.67 ± 0.57; $t(41) = 3.20$, $p = .014$). Finally, those in the placebo condition were significantly less accurate at midpoint (13.67 ± 0.57) compared to endpoint (14.91 ± 0.59; $t(41) = -2.72$, $p = .045$). No other post hoc comparisons were significant.

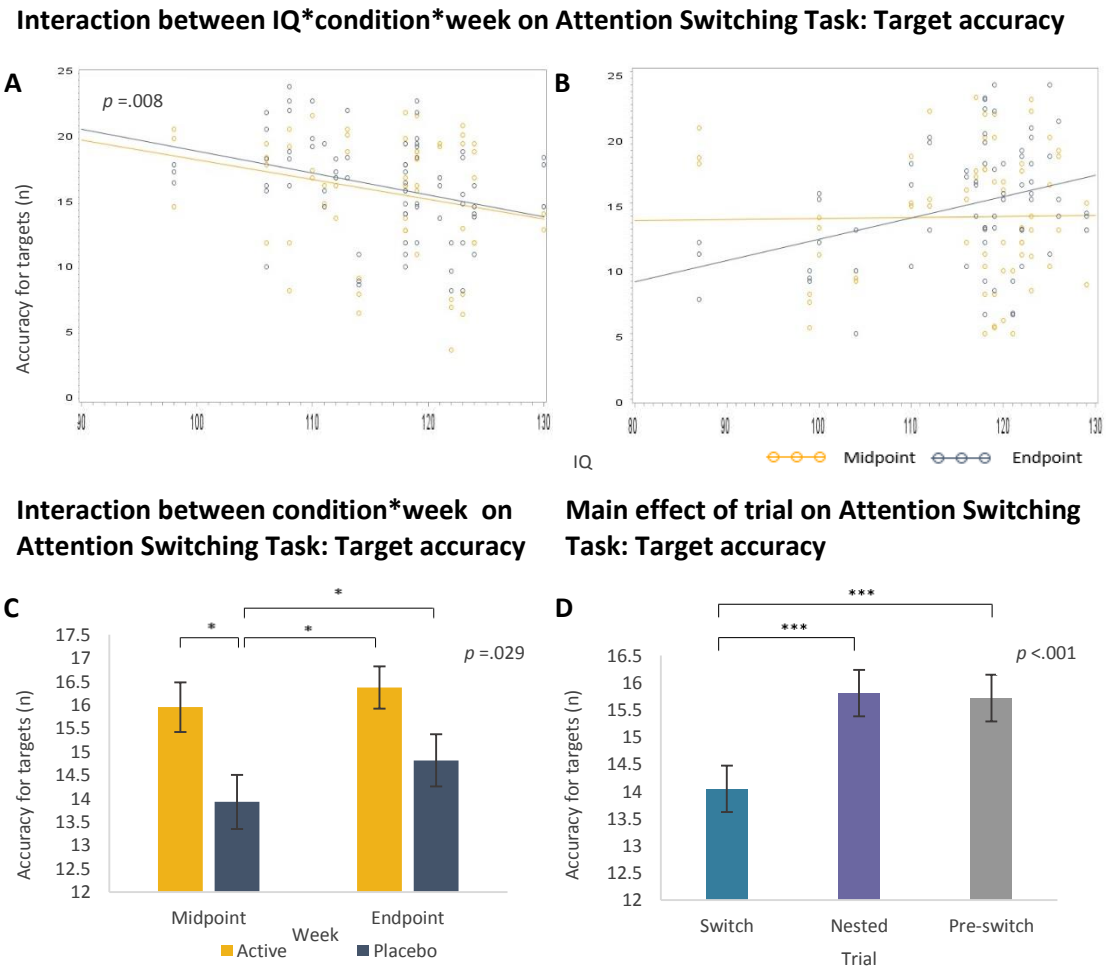


Figure 5.15 Target accuracy on the Attention Switching Task. (A-B) The x axis represents IQ and the y axis is accuracy (n) for targets over midpoint and endpoint. Regression lines show relationship between x and y by week. (C) The x axis represents week and the y axis is accuracy

(n) for targets over midpoint and endpoint. Columns denote the mean. Error bars denote the SE of the mean. (D) The x axis represents trial type and the y axis is accuracy (n) for targets over midpoint and endpoint. Columns denote the least squares means. Error bars denote the SE of the least squares means. * p <.05, *** p <.001. (A) Is the active condition, (B) is the placebo condition.

There was also a significant main effect of trial, $F(2,92) = 16.20$, p <.001, Figure 5.15 (D). As expected, irrespective of condition, there was a performance decrement for switch trials. Whereas performance was similar across nested and pre-switch trials. Inspection of post hoc comparisons revealed that performance was significantly less accurate on switch trials (14.05 ± 0.43) compared to nested trials (15.81 ± 0.43; $t(92) = -5.10$, p <.001) and pre-switch trials (15.72 ± 0.43; $t(92) = -4.77$, p <.001). There was no significant trial*condition interaction, $F(2,92) = 1.27$, $p = .285$. A main effect of week was also significant, $F(1,41) = 5.37$, $p = .026$, such that participants were less accurate at midpoint (14.79 ± 0.41) than at endpoint (15.59 ± 0.41).

5.8.4.1.1.4 Target reaction time

No outlying observations were removed from the final model. Accuracy was a significant covariate, $F(1,220) = 1075.44$, p <.001, such that reaction time increased with greater accuracy. Baseline, age, IQ and gender were all nonsignificant covariates and removed from the final model. There was no significant main effect of condition, $F(1,46) = 0.65$, $p = .424$. There was a significant interaction between baseline*condition*week, $F(1,220) = 4.09$, $p = .045$, baseline*condition, $F(1,220) = 5.02$, $p = .026$ and baseline*week, $F(1,220) = 4.73$, $p = .031$.

Figure 5.16(A) and 5.16(B) show the relationship between baseline performance, condition and week, which appears to be driven by the difference in performance between midpoint and endpoint in both conditions at slower baseline reaction time. Inspection of post hoc comparisons revealed those in the active condition were marginally significantly slower (11.61 ± 0.40) compared to those in the placebo condition at midpoint (10.22 ± 0.40; $t(41) = 2.56$, p = .065) at a baseline reaction time of 1. This became a trend at a baseline reaction time of 2 (11.48 ± 0.36 vs. 10.26 ± 0.37; $t(41) = 2.47$, $p = .079$). Moreover, those in the active condition at midpoint (11.61 ± 0.40) were significantly slower than those in the placebo condition at endpoint (9.95 ± 0.41; $t(41) = 2.99$, $p = .024$) at a baseline reaction time of 1 to 4, inclusive (faster baseline reaction time). This was marginally significant at a baseline reaction time of 5 (11.08 ± 0.26 vs. 10.11 ± 0.28; $t(41) = 2.63$, $p = .056$) and a trend at a baseline reaction time of 6 (10.95 ± 0.23 vs. 10.15 ± 0.25; $t(41) = 2.41$, $p = .090$). At a baseline reaction time of 10, there was a trend for the active condition to respond more quickly at midpoint (10.43 ± 0.18) than at endpoint (10.82 ±

0.19; $t(41) = -2.49$, $p = .077$). This was significant at a baseline reaction time of 11 and higher (slower baseline reaction time) (10.30 ± 0.20 vs. 10.81 ± 0.20; $t(41) = -3.03$, $p = .021$). No other post hoc comparisons were significant.

The interaction between baseline reaction time and condition is shown below in Figure 5.16(C). This clearly shows those in the placebo condition demonstrated faster reaction time relative to those in the active condition at quicker baseline reaction time. The convergence of the regression lines suggests that performance by condition was similar at poorer baseline performance. Inspection of post hoc comparisons revealed those in the active condition were significantly slower (11.26 ± 0.35) compared to those in the placebo condition (10.09 ± 0.35; $t(46) = 2.47$, $p = .017$) at a baseline reaction time of 1 to 6, inclusive (faster baseline reaction time). This was marginally significant at a baseline reaction time of 7 (10.84 ± 0.18 vs. 10.31 ± 0.20; $t(46) = 1.98$, $p = .054$) and a trend at a baseline reaction time of 8 (slower baseline reaction time) (10.77 ± 0.17 vs. 10.34 ± 0.18; $t(46) = 1.70$, $p = .095$). No other post hoc comparisons were significant.

There was a significant main effect of trial, $F(2,94) = 12.99$, $p < .001$. Figure 5.16(D) shows the difference in reaction time between each trial type. As expected, reaction time was markedly increased on switch trials but similar on nested and pre-switch trials. Inspection of post hoc comparisons revealed reaction time was significantly slower on switch trials (10.91 ± 0.14) compared to nested trials (10.37 ± 0.14; $t(94) = 4.27$, $p < .001$) and pre-switch trials (10.31 ± 0.14; $t(94) = 4.67$, $p < .001$). Trial*condition and trial*condition*week interactions were not significant and both terms were removed from the final model. As with target accuracy, there was also a significant main effect of week, $F(1,41) = 5.83$, $p = .020$, however, post hoc comparisons did not reveal a significant difference.

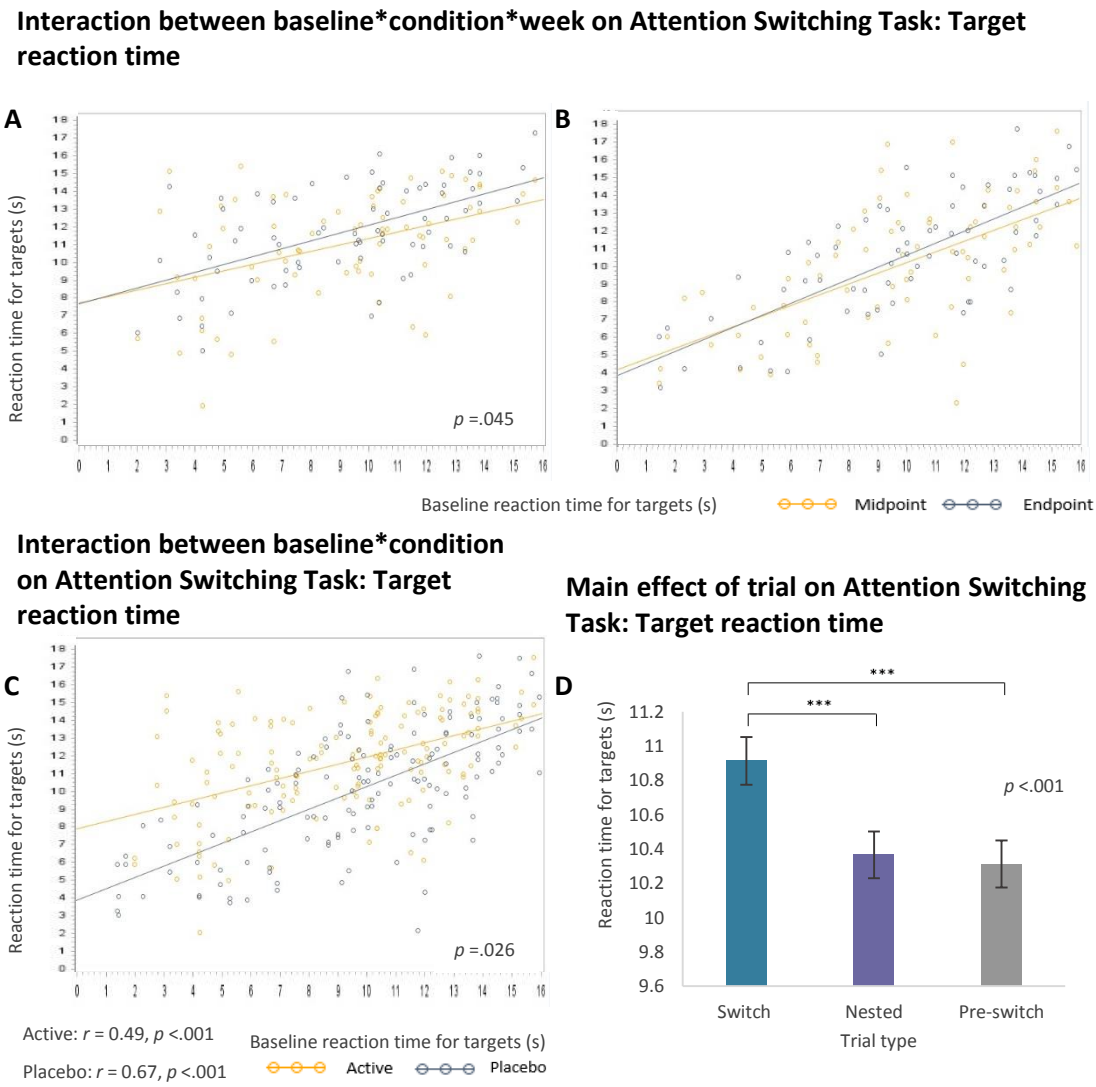


Figure 5.16 Target reaction time on the Attention Switching Task. (A-B) The x axis represents baseline reaction time (s) for targets and the y axis is reaction time (s) for targets over subsequent test points. Regression lines show relationship between x and y by week. (C) The x axis represents baseline reaction time (s) for targets and the y axis is reaction time (s) for targets over subsequent test points. Regression lines show relationship between x and y by condition. (D) The x axis represents trial type and the y axis is reaction time (s) for targets over midpoint and endpoint. Columns denote the least squares means. Error bars denote the SE of the least squares means. *** p <.001. (A) Is the active condition, (B) is the placebo condition.

5.8.4.1.2 Rapid Visual Information Processing Task (RVIP)

5.8.4.1.2.1 Hits

No outlying observations were removed from the final model. The number of hits identified at baseline was a significant covariate, $F(1,43) = 137.44$, p <.001, which was positively associated with performance on later test occasions. Age and IQ were also significant covariates, $F(1,43)$ =

4.94, p = .032 and $F(1,43)$ = 7.82, p = .008, respectively. The number of hits decreased with age but increased with higher IQ. Gender was not a significant covariate and was removed from the final model. There was also no significant main effect of condition, $F(1,43)$ = 0.46, p = .502, and no significant baseline*condition interaction, and this term was removed from the model. No higher order interactions were significant other than a trend for IQ*condition*week, ($F(3,35)$ = 2.45, p = .080).

5.8.4.1.2.2 False alarms

Four outlying observations were removed from the final model. The number of false alarms at baseline was a significant covariate, $F(1,41)$ = 61.25, p <.001, which was positively correlated with false alarm rate at midpoint and endpoint. Age was also a significant covariate, $F(1,41)$ = 6.30, p = .016, such that greater false alarms were committed with age. IQ and gender were not significant covariates and were removed from the final model. No higher order interactions were significant. There was a significant baseline*condition interaction and a trend towards a baseline*week interaction, $F(1,41)$ = 7.46, p = .009 and $F(1,38)$ = 3.08, p = .087, respectively.

Figure 5.17 shows the interaction between baseline performance and condition, and indicates that with superior baseline performance, those in the placebo condition committed less false alarms compared to those in the active condition. However, at poorer baseline performance, the opposite is demonstrated, such that those in the active condition committed less false alarms relative to those in the placebo condition. Consistent with this, inspection of post hoc comparisons revealed those in the active condition (13.98 ± 1.63) committed significantly more false alarms compared to those in the placebo condition (8.85 ± 1.82; $t(41)$ = 2.10 p = .042) if their baseline false alarm rate was 10. Conversely, at a baseline false alarm rate of 40 and above (poorer baseline performance), those in the active condition (27.53 ± 2.57) committed significantly less false alarms than those in the placebo condition (36.59 ± 3.61; $t(41)$ = -2.06, p = .046). No other post hoc comparisons were significant.

There was also a significant main effect of condition, $F(1,41)$ = 7.22, p = .010, such that a greater number of false alarms were committed by those in the active condition (17.51 ± 1.43) compared to those in the placebo condition (16.08 ± 1.49), however, post hoc tests did not reveal a significant difference.

Interaction between baseline*condition on Rapid Visual Information Processing Task (RVIP): False alarms

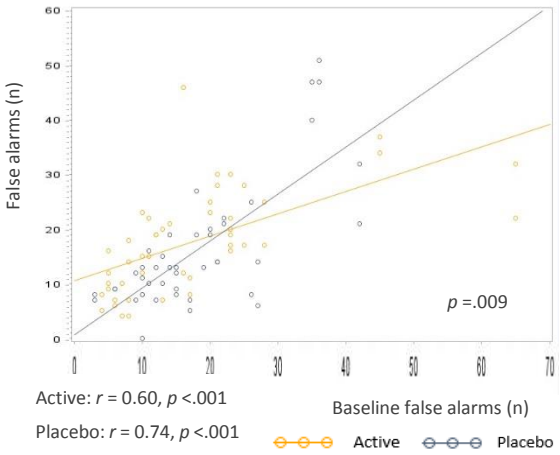


Figure 5.17 False alarms on the Rapid Visual Information Processing Task (RVIP). The x axis represents baseline number of false alarms (n) and the y axis is number of false alarms (n) over subsequent test points. Regression lines show relationship between x and y by condition.

5.8.4.1.2.3 Reaction time for hits

Nine outlying observations were removed from the final model. Baseline reaction time for hits was a significant covariate, $F(1,42) = 29.43$, $p < .001$, this being positively related to with performance at midpoint and endpoint. IQ was a significant covariate, $F(1,42) = 4.52$, $p = .039$, with response latencies being greater for those with higher IQ scores. Trial and the number of hits achieved (total correct) were also significant covariates, $F(1,1628) = 5.34$, $p = .021$ and $F(1,1628) = 10.07$, $p = .002$, respectively, with performance fluctuating across each. Gender was marginally significant, $F(1,42) = 3.80$, $p = .058$, with females (0.67 ± 0.01) responding more quickly compared to males (0.72 ± 0.02). Age was not a significant covariate and was removed from the final model. There was no significant main effect of condition, $F(1,42) = 1.88$, $p = .178$ and no significant baseline*condition interaction, $F(1,42) = 2.16$, $p = .149$. No higher order interactions were significant. There was a significant baseline*week interaction, $F(1,1628) = 5.29$, $p = .022$, and a significant main effect of week, $F(1,41) = 4.66$, $p = .037$, however, post hoc tests did not reveal a significant difference.

5.8.4.1.3 N-back Task

5.8.4.1.3.1 Target accuracy

One outlying observation was removed from the final model. Baseline target accuracy was a significant covariate, $F(1,45) = 96.29$, $p < .001$, and was positively associated with performance

at subsequent test sessions. Age, IQ and gender were not significant covariates and were removed from the final model. No higher order interactions were significant nor was baseline*condition, and this term was removed from the final model. There was a marginally significant baseline*week interaction, $F(1,33) = 3.68$, $p = .064$. Additionally, there was a trend towards a main effect of condition, $F(1,45) = 3.36$, $p = .073$. Those in the active condition (60.18 ± 2.67) obtained greater target accuracy compared to those in the placebo condition (58.07 ± 2.71).

5.8.4.1.3.2 Total accuracy

Three outlying observations were removed from the final model. Baseline total accuracy was a significant covariate, $F(1,45) = 89.13$, $p < .001$, such that baseline performance was positively related to performance at later test points. Age, IQ and gender were not significant covariates and were removed from the final model. No higher order interactions were significant nor was baseline*condition, and this term was removed from the final model. There was a significant baseline*week interaction, $F(1,31) = 4.94$, $p = .034$, and a significant main effect of condition, $F(1,45) = 4.66$, $p = .036$. The main effect of condition is shown in Figure 5.18, which indicates that those in the active condition (56.83 ± 2.76) demonstrated significantly greater total accuracy compared to those in the placebo condition (52.97 ± 2.77).

Main effect of condition on N-back Task: Total accuracy

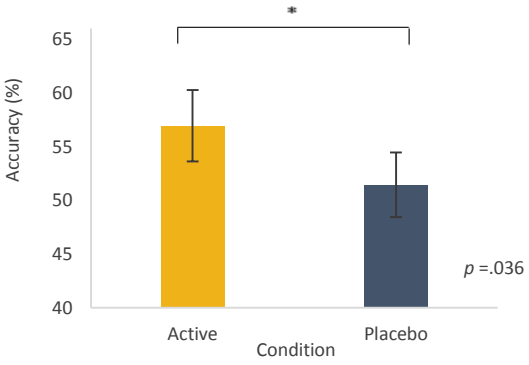


Figure 5.18 Total accuracy on the N-back Task. The x axis is condition and the y axis is total accuracy (%) over midpoint and endpoint. Columns denote the mean. Error bars denote the SE of the mean.* p <.05

5.8.4.1.3.3 Reaction time for targets

No outlying observations were removed from the final model. Baseline performance was a significant covariate, $F(1,43) = 111.90$, p <.001, such that this was positively associated with performance at midpoint and endpoint. Age, IQ, gender, trial and the number of targets achieved (total correct) were not significant and were removed from the final model. No higher order interactions were significant nor was baseline*condition, $F(1,43) = 1.50$, $p = .228$. There was a marginally significant main effect of condition, $F(1,43) = 3.60$, $p = .064$, such that those in the active condition (0.727 ± 0.00) demonstrated slower response latencies compared to those in the placebo condition (0.726 ± 0.00).

5.8.4.1.3.4 Reaction time for nontargets

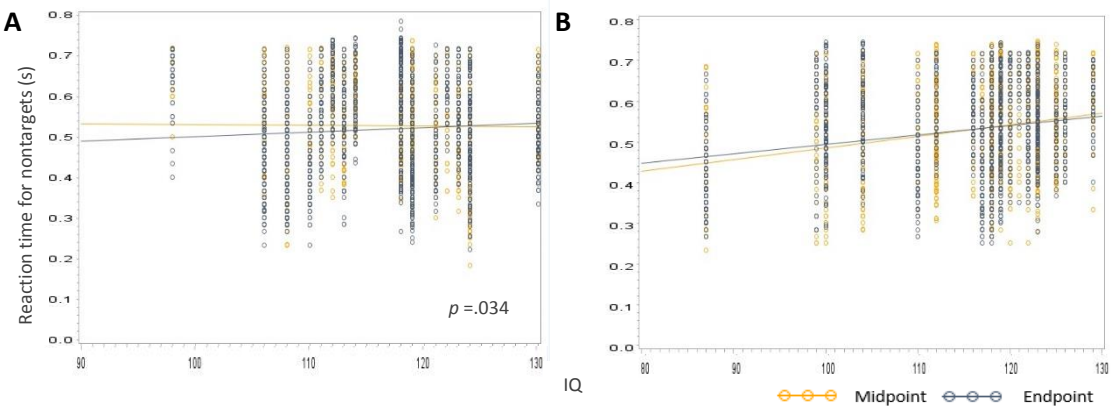
Thirty-eight outlying observations were removed from the final model. Baseline reaction time for nontargets was a significant covariate, $F(1,42) = 18.63$, p <.001, with this being positively correlated with performance on subsequent test occasions. Trial and the number of targets achieved (total correct) were significant covariates, $F(1,9423) = 15.42$, p <.001 and $F(1,9423) = 108.22$, p <.001, respectively, with performance fluctuating across the trials. IQ was also a significant covariate, $F(1,42) = 4.61$, $p = .038$, such that reaction time for nontargets increased with higher IQ scores. Gender was not a significant covariate, $F(1,42) = 2.28$, $p = .139$, and nor was age, which was removed from the final model. There was no significant main effect of condition, $F(1,42) = 0.02$, $p = .878$. IQ*condition*week and age*condition*week were significant, $F(3,9423) = 2.88$, $p = .034$, and $F(4,9423) = 5.04$, $p = .001$, respectively. There was also

a significant baseline*condition interaction, $F(1,42) = 14.35$, $p = .001$, and a significant interaction between condition and week, $F(1,45) = 4.82$, $p = .033$.

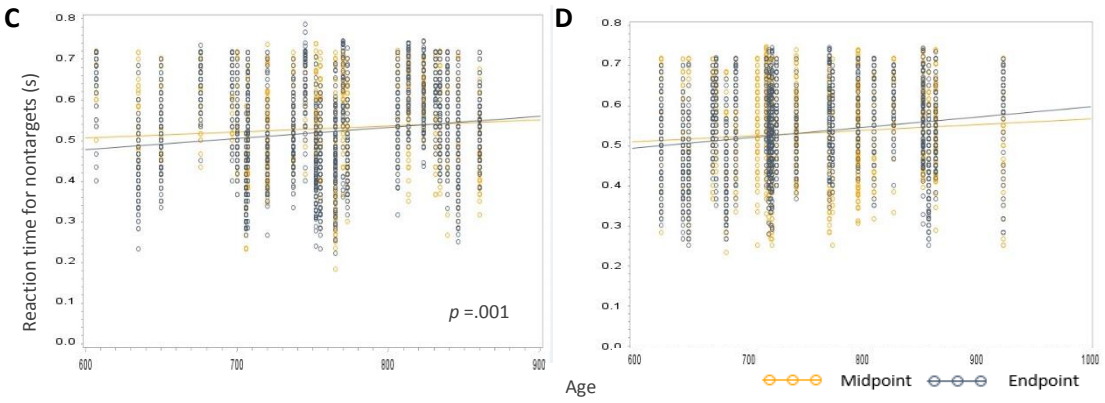
Figure 5.19(A) and 5.19(B) shows the relationship between IQ, condition and week. In the active condition (Figure 5.19(A)), participants with lower IQ scores tended to react to nontargets more slowly at midpoint compared to endpoint, conversely, those with higher IQ scores responded similarly across both weeks. However, in the placebo condition (Figure 5.19(B)), those with lower IQ scores tended to show faster reaction time at midpoint, however, again, those with higher IQ scores responded similarly across both test sessions. Inspection of post hoc comparisons revealed a trend towards those in the active condition (0.51 ± 0.01) demonstrating faster reaction time compared to those in the placebo condition (0.54 ± 0.01; $t(45) = -2.40$, $p = .091$) at endpoint and an IQ score of 120. Also at an IQ score of 120, there was a trend towards those in the active condition at endpoint (0.51 ± 0.01) responding more quickly to nontargets relative to those in the placebo condition at midpoint (0.54 ± 0.01; $t(45) = -2.41$, $p = .089$). This same pattern of performance was marginally significant at an IQ score of 130 (0.52 ± 0.02 vs. 0.56 ± 0.01; $t(45) = -2.53$, $p = .068$). Finally, those in the active condition reacted significantly more slowly at midpoint (0.53 ± 0.03) than at endpoint (0.50 ± 0.03; $t(45) = 2.72$, $p = .044$) at an IQ score of 90 to 120, inclusive. No other post hoc comparisons were significant.

Figure 5.19(C) and 5.19(D) present the relationship between age, condition and week. Irrespective of condition, younger participants demonstrated faster reaction time for nontargets at endpoint compared to midpoint, whilst older participants showed faster performance at midpoint. Inspection of post hoc comparisons revealed significant within condition differences in performance only. Specifically, at 700 months of age (approximately 58 years of age), those in the active condition responded significantly slower at midpoint (0.52 ± 0.01) than at endpoint (0.50 ± 0.01; $t(45) = 5.48$, $p < .001$). At 900 months of age (75 years of age), those in the placebo condition reacted significantly faster at midpoint (0.53 ± 0.01) compared to endpoint (0.55 ± 0.01; $t(45) = -3.06$, $p = .019$). No other post hoc comparisons were significant.

Interaction between IQ*condition*week on N-back Task: Reaction time for nontargets



Interaction between age*condition*week on N-back Task: Reaction time for nontargets



Interaction between baseline*condition on N-back Task: Reaction time for nontargets

Interaction between condition*week on N-back Task: Reaction time for nontargets

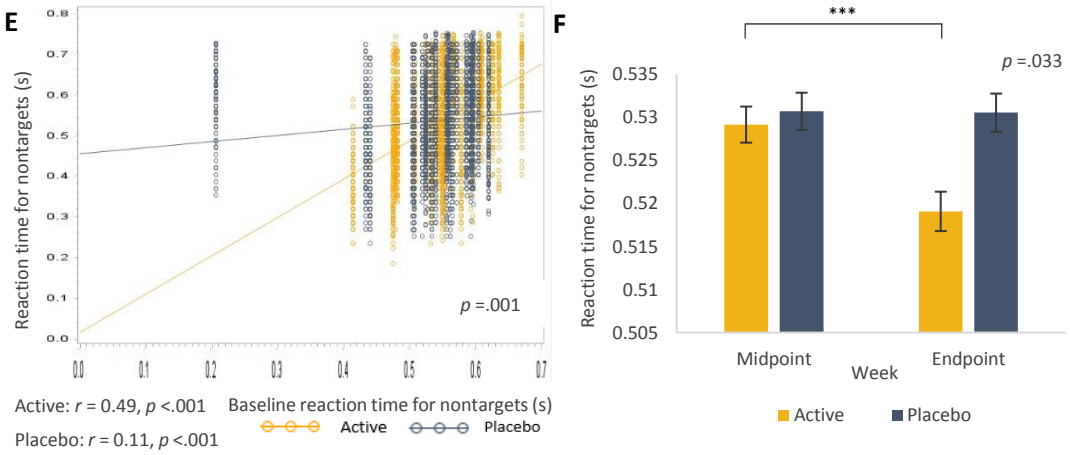


Figure 5.19 Reaction time for nontargets on the N-back Task. (A-B) The x axis is IQ and the y axis is reaction time (s) over midpoint and endpoint. (C-D) The x axis represents age (months) and the y axis is reaction time (s) over midpoint and endpoint. (A-D) Regression lines show

relationship between x and y by week. (E) The x axis is baseline reaction time (s) and the y axis is the reaction time (s) over subsequent test points. Regression lines show relationship between x and y by condition. (F) the x axis is week and the y axis is reaction time (s) over midpoint and endpoint. Columns denote the mean. Error bars denote the SE of the mean. *** p <.001. (A & C) Is the active condition, (B & D) is the placebo condition.

The relationship between baseline and condition is shown in Figure 5.19(E), which indicates those in the active condition demonstrated faster reaction time at quicker baseline performance compared to those in the placebo condition. Only at particularly slow baseline performance is this pattern of performance reversed, such that those in the placebo condition show faster reaction latencies compared to those in the active condition. Consistent with this, post hoc comparisons revealed those in the active condition demonstrated significantly faster reaction time for nontarget trials compared to those in the placebo condition if their baseline reaction time for nontargets was 0.5 or below (0.50 ± 0.01 vs. 0.53 ± 0.01; $t(42) = -2.73$, $p = .009$). However, at a baseline reaction time for nontargets of 0.6, those in the active condition (0.55 ± 0.01) responded marginally significantly more slowly to nontargets compared to those in the placebo condition (0.53 ± 0.01; $t(42) = 1.98$, $p = .054$). No other post hoc comparisons were significant.

The significant condition*week interaction is depicted in Figure 5.19(F), which shows the relationship between condition and week in which those in the active condition show faster reaction time for nontargets at endpoint than at midpoint, whereas there was no difference in the placebo condition over time. Inspection of post hoc comparisons revealed those in the active condition demonstrated significantly slower reaction time at midpoint (0.53 ± 0.01) compared to endpoint (0.51 ± 0.01; $t(45) = 4.98$, p <.001). No other post hoc comparisons were significant. Lastly, there was a significant main effect of week, $F(1,45) = 11.38$, $p = .002$, with performance being significantly slower at midpoint (0.53 ± 0.01) relative to endpoint (0.52 ± 0.01).

5.8.4.2 Measures of memory performance

Table 5.9 provides a summary of the means (± SE) across measures of memory performance for active and placebo conditions.

Table 5.9 Mean (± SE) on measures of memory performance (Pattern Separation Task, Face-Name Associative Memory and Visual Verbal Learning Test) by condition and week

Outcome	Baseline (*week 0*)[a] Mean ± SE	Midpoint (*week 6*)[a] Mean ± SE	Endpoint (*week 12*)[b] Mean ± SE	Active vs. placebo
Pattern Separation Task: Pattern separation score (n)				
Active	-3.96 ± 1.14	-3.33 ± 1.22	-4.17 ± 1.06	$F(1,45) = 5.08$, $p = .029$
Placebo	-3.96 ± 0.90	-3.58 ± 0.89	-2.43 ± 0.81	
Pattern Separation Task: Recognition score (n)				
Active	2.46 ± 0.45	2.75 ± 0.56	2.96 ± 0.88	$F(1,44) = 0.17$, $p = .685$
Placebo	2.54 ± 0.47	2.96 ± 0.56	3.87 ± 0.59	
Face-Name Associative Memory Exam: Immediate recall (n)				
Active	5.46 ± 0.55	6.13 ± 0.75	5.63 ± 0.92	$F(1,43) = 1.76$, $p = .192$
Placebo	4.38 ± 0.62	5.29 ± 0.69	5.26 ± 0.64	
Face-Name Associative Memory Exam: Delayed recall (n)				
Active	4.83 ± 0.62	4.83 ± 0.65	4.38 ± 0.80	$F(1,42) = 0.04$, $p = .837$
Placebo	3.50 ± 0.57	3.92 ± 0.75	4.26 ± 0.63	
Visual Verbal Learning Test (VVLT): Rate of learning (n)				
Active (trial A1)	7.83 ± 0.60	8.21 ± 0.43	8.58 ± 0.52	$F(1,43) = 2.25$, $p = .141$
Placebo (trial A1)	7.75 ± 0.40	7.83 ± 0.45	8.04 ± 0.42	
Active (trial A2)	10.13 ± 0.59	11.04 ± 0.41	11.58 ± 0.44	
Placebo (trial A2)	10.38 ± 0.49	10.71 ± 0.49	11.00 ± 0.54	
Active (trial A3)	11.50 ± 0.50	12.29 ± 0.44	12.92 ± 0.52	

Outcome	Baseline (*week 0*)[a] Mean ± SE	Midpoint (*week 6*)[a] Mean ± SE	Endpoint (*week 12*)[b] Mean ± SE	Active vs. placebo
Placebo (trial A3)	11.21 ± 0.51	12.04 ± 0.42	12.30 ± 0.48	
Visual Verbal Learning Test (VVLT): New learning (n)				
Active	2.83 ± 0.58	6.96 ± 0.52	7.29 ± 0.56	$F(1,44) = 0.44$, $p = .512$
Placebo	6.08 ± 0.54	6.20 ± 0.55	5.52 ± 0.58	
Visual Verbal Learning Test (VVLT): Retroactive interference (n)				
Active	3.00 ± 0.49	2.54 ± 0.43	3.67 ± 0.47	$F(1,40) = 2.86$, $p = .098$
Placebo	2.79 ± 0.32	2.92 ± 0.40	2.39 ± 0.37	
Visual Verbal Learning Test (VVLT): Proactive interference (n)				
Active	0.83 ± 0.53	1.25 ± 0.46	1.29 ± 0.33	$F(1,42) = 0.60$, $p = .442$
Placebo	1.67 ± 0.48	1.63 ± 0.42	2.52 ± 0.54	
Visual Verbal Learning Test (VVLT): Delayed recall (n)				
Active	8.14 ± 0.64	8.96 ± 0.58	9.33 ± 0.70	$F(1,43) = 6.27$, $p = .016$
Placebo	7.83 ± 0.75	9.00 ± 0.74	9.09 ± 0.62	

Notes. [a]n=48; [b]n=47; n: the number of trials; s: seconds. For more information on cognitive outcomes, please refer to Table 5.3.

5.8.4.2.1 Pattern Separation Task

5.8.4.2.1.1 Pattern separation score

No outlying observations were removed from the final model. Baseline performance was a significant covariate, $F(1,45) = 47.15$, $p < .001$, with this being positively related to pattern separation performance at subsequent test sessions. Age, IQ, and gender were all nonsignificant covariates and were removed from the final model. All higher order interactions were also nonsignificant, as was baseline*condition, and this term was removed from the final model. There was a significant main effect of condition, $F(1,45) = 5.08$, $p = .029$, shown in Figure 5.20, which indicates those in the active condition (-3.74 ± 0.66) achieved significantly lower pattern separation scores compared to those in the placebo condition (-2.66 ± 0.67).

Main effect of condition on Pattern Separation Task: Pattern separation score

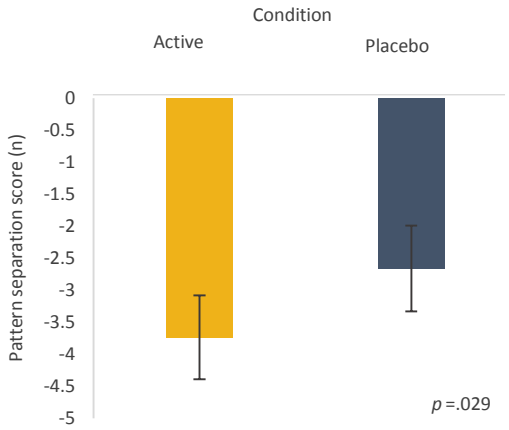


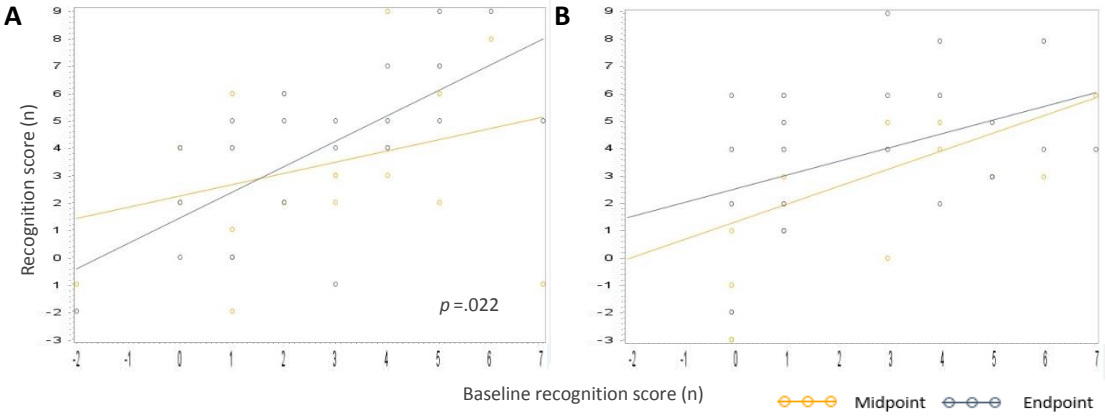
Figure 5.20 Pattern separation score on Pattern Separation Task. The x axis represents condition and the y axis is the pattern separation score (n) over midpoint and endpoint. Columns denote the least squares means. Error bars denote the SE of the least squares means.

5.8.4.2.1.2 Recognition score

One outlying observation was removed from the final model. Baseline recognition score was significant covariate, $F(1,44) = 21.91$, $p < .001$, and was positively associated with performance at midpoint and endpoint. Age, IQ and gender were all nonsignificant covariates and removed from the final model. There was no significant main effect of condition, $F(1,44) = 0.17$, $p = .685$, nor was there a significant interaction between baseline*condition, $F(1,44) = 0.69$, $p = .410$. There were significant baseline*condition*week, $F(1,38) = 5.73$, $p = .022$, and gender*condition*week, $F(4,38) = 4.99$, $p = .003$, interactions.

Figure 5.21(A) and 5.21(B) show the relationship between baseline performance and week by condition. This interaction appears to be a function of the difference in performance demonstrated at midpoint and endpoint by those in the active condition at better baseline performance. Inspection of post hoc comparisons revealed a trend towards those in the active condition obtaining higher recognition scores (7.33 ± 0.96) compared to those in the placebo condition (4.00 ± 0.99; $t(38) = 2.42$, $p = .090$) at endpoint if their baseline recognition score was 6. No other post hoc comparisons were significant.

Interaction between baseline*condition*week on Pattern Separation Task: Recogniton score



Interaction between gender*condition*week on Pattern Separation Task: Recogniton score

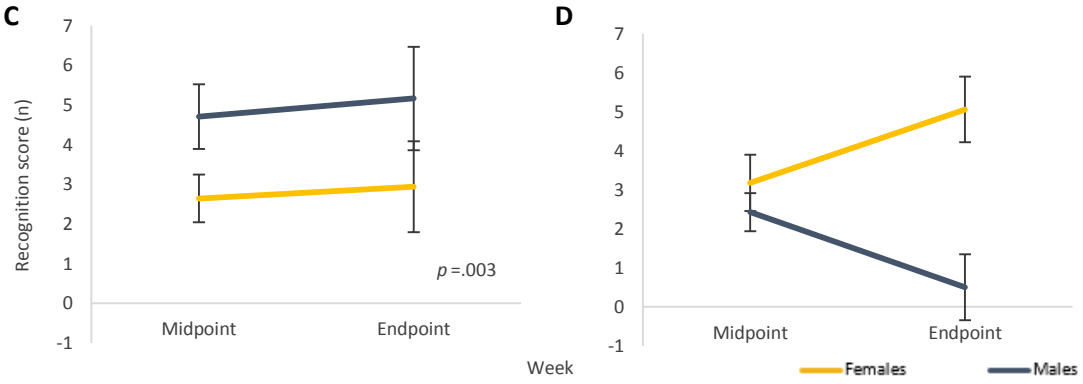


Figure 5.21 Recognition score on the Pattern Separation Task. (A-B) The x axis represents baseline recognition score (n) and the y axis is recognition score (n) over subsequent test points. Regression lines show relationship between x and y by week. (C-D) The x axis represents week and the y axis is recognition score (n) over midpoint and endpoint. Lines denote the mean. Error bars denote the SE of the mean. (A & C) Is the active condition, (B & D) is the placebo condition.

Figure 5.21(C) and 5.21(D) present the relationship between gender, condition and week. In the active condition (Figure 5.21(C)), males performed better than females on both test occasions (as shown by the parallel lines). Whilst in the placebo condition (Figure 5.21 (D)), females obtained a higher recognition score at endpoint relative to midpoint, whereas males performed better at midpoint than at endpoint. Inspection of post hoc comparisons revealed a trend towards males in the active condition (4.97 ± 0.96) demonstrating better performance compared to males in the placebo condition at endpoint (0.87 ± 0.97; t(38) = 3.00, p = .081). Females in the placebo condition (4.94 ± 0.58) illustrated significantly higher recognition performance relative to males in the same condition at endpoint (0.87 ± 0.97; t(38) = 3.55, p =

.021). There was also a trend towards females in the placebo condition achieving a lower recognition score at midpoint (2.92 ± 0.58) compared to endpoint (4.94 ± 0.58; $t(38) = -2.97$, $p = .086$). No other post hoc comparisons were significant.

5.8.4.2.2 Face-Name Associative Memory Exam

5.8.4.2.2.1 Immediate recall

No outlying observations were removed from the final model. The number of names recalled immediately at baseline was a significant covariate, $F(1,43) = 53.27$, $p < .001$, such that this was positively related to immediate recall at midpoint and endpoint. Age was also a significant covariate, $F(1,43) = 5.37$, $p = .025$, with older participants recalling fewer names correctly compared to younger participants. IQ and gender were not significant covariates and were removed from the final model. There was no significant main effect of condition, $F(1,43) = 1.76$, $p = .192$; baseline*condition was also nonsignificant, $F(1,43) = 0.68$, $p = .414$. There was a significant baseline*condition*week interaction, $F(1,39) = 5.39$, $p = .026$, however, post hoc comparisons did not reveal any significant differences. There was also a significant condition*week interaction, $F(1,39) = 4.83$, $p = .034$, such that participants in the active condition demonstrated poorer immediate recall compared to those in the placebo condition at midpoint (5.25 ± 0.54 vs. 5.92 ± 0.58) and endpoint (4.77 ± 0.54 vs. 5.58 ± 0.60), however, post hoc comparisons did not reveal any significant differences.

5.8.4.2.2.2 Delayed recall

No outlying observations were removed from the final model. The number of names recalled after a delay at baseline was a significant covariate, $F(1,42) = 70.94$, $p < .001$ and was positively correlated with performance at subsequent test sessions. Age was also a significant covariate, $F(1,42) = 4.67$, $p = .036$, such that performance became poorer with age. IQ was not a significant covariate, $F(1,42) = 0.00$, $p = .957$, nor was gender, and this term was removed from the final model. There was no significant main effect of condition, $F(1,42) = 0.04$, $p = .837$ and baseline*condition was also not significant, $F(1,42) = 0.11$, $p = .736$. There was a significant baseline*condition*week interaction, $F(2,40) = 6.23$, $p = .004$, and IQ*condition*week was marginally significant, $F(3,40) = 2.69$, $p = .059$.

The baseline*condition*week interaction is shown in Figure 5.22. Figure 5.22(A) shows that at poorer baseline performance, participants in the active condition recalled more items correctly following a delay at midpoint, whilst those that demonstrated better delayed recall at baseline,

recalled more items successfully at endpoint. The opposite pattern of performance was shown by those in the placebo condition (Figure 5.22(B)). Inspection of post hoc comparisons revealed a trend towards those in the active condition (0.17 ± 0.74) recalling less items correctly following a delay compared to those in the placebo condition (2.48 ± 0.58; $t(40) = -2.52$, $p = .072$) at endpoint at a baseline delayed recall score of 1 and 2 (1.27 ± 0.61 vs. 3.16 ± 0.49; $t(40) = -2.47$, $p = .081$). Participants within the active condition recalled significantly more items after a delay at midpoint (2.10 ± 0.74) compared to endpoint (0.17 ± 0.74; $t(40) = 2.89$, $p = .030$) at a baseline delayed recall score of 1 and 2 (2.81 ± 0.61 vs. 1.27 ± 0.61; $t(40) = 2.78$, $p = .040$). This pattern of performance became a trend at a baseline delayed recall score of 3 (3.53 ± 0.50 vs. 2.37 ± 0.50; $t(40) = 2.50$, $p = .074$). No other post hoc comparisons were significant.

There was also a significant main effect of week, $F(1,40) = 6.72$, $p = .013$, however, post hoc comparisons did not reveal a significant difference.

Interaction between baseline*condition*week on Face-Name Associative Memory Exam: Delayed recall

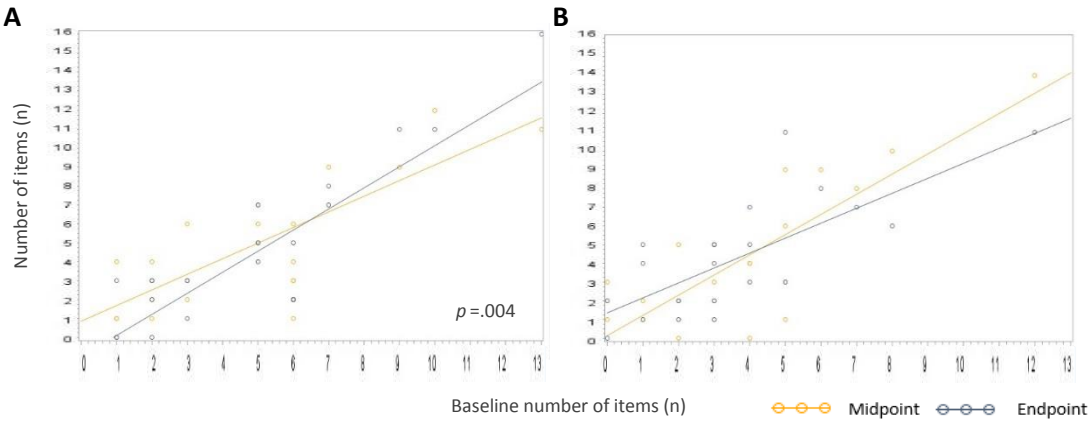


Figure 5.22 Delayed recall on the Face-Name Associative Memory Exam. The x axis represents baseline number of items correctly recalled (n) and the y axis is the number of items correctly recalled (n) over subsequent test points. Regression lines show relationship between x and y by week. (A) Is the active condition, (B) is the placebo condition.

5.8.4.2.3 Visual Verbal Learning Test (VVLT)

5.8.4.2.3.1 Rate of learning (word list A)

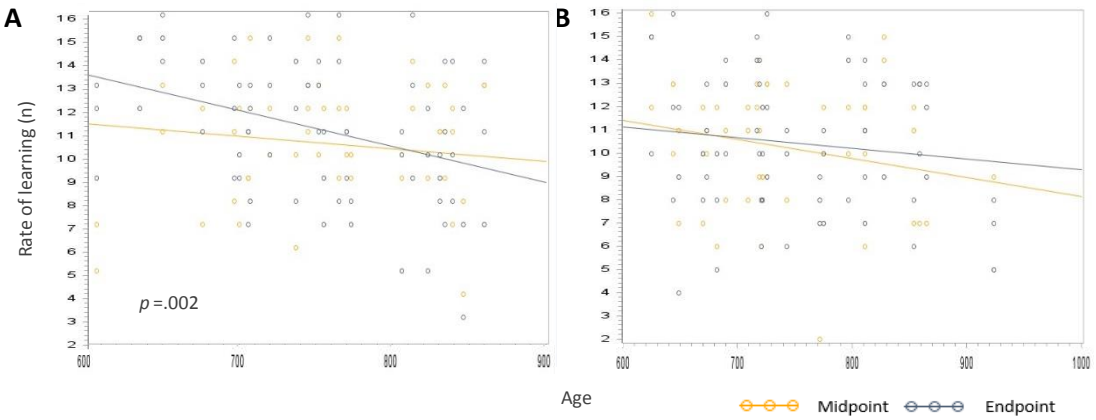
One outlying observation was removed from the final model. Baseline performance was a significant covariate, $F(1,225) = 14.07$, $p < .001$, such that rate of learning at baseline was positively associated with performance at later test sessions. Age and IQ were significant covariates, $F(1,43) = 6.13$, $p = .017$ and $F(1,43) = 11.73$, $p = .001$, respectively. Rate of learning showed an age-related decline and those with higher IQ scores achieved greater rate of learning

compared to those with lower IQ scores. Gender was also a significant covariate, $F(1,43) = 5.77$, $p = .021$, such that females (10.86 ± 0.23) performed better than males (9.82 ± 0.36). There was no significant main effect of condition, $F(1,43) = 2.25$, $p = .141$ and no significant baseline*condition interaction, and this term was removed from the final model. No higher order interactions other than age*condition*week, $F(3,225) = 5.10$, $p = .002$, were significant.

Figure 5.23(A) and 5.23(B) show how performance varied as a function of age, condition and week. In the active condition (Figure 5.23(A)), younger participants demonstrated better performance at endpoint compared to midpoint, whereas, older participants showed a greater rate of learning at midpoint relative to endpoint. Performance differences by week in the placebo condition are less discernible for younger participants. Older participants in the placebo condition (Figure 5.23(B)) performed better at endpoint compared to midpoint. Inspection of post hoc comparisons revealed those in the active condition at endpoint (11.75 ± 0.39) demonstrated significantly better performance compared to those in the placebo condition at midpoint (10.26 ± 0.39; $t(42) = 2.69$, $p = .048$) and at endpoint (10.12 ± 0.40; $t(42) = 2.90$, $p = .029$) at 700 months of age (approximately 58 years of age). Within condition at this same age, participants in the active condition demonstrated significantly poorer performance at midpoint (10.63 ± 0.39) than at endpoint (11.75 ± 0.39; $t(42) = -3.97$, $p = .002$). No other post hoc comparisons were significant.

There was also a significant condition*week interaction, $F(1,42) = 13.31$, $p < .001$. Those in the active condition achieved a greater rate of learning on both test occasions relative to those in the placebo condition, particularly at endpoint. Moreover, participants in the active condition showed the greatest improvement in performance over time. Consistent with this, inspection of post hoc comparisons revealed participants in the active condition demonstrated marginally significantly poorer performance at midpoint (10.35 ± 0.32) compared to their performance at endpoint (10.96 ± 0.32; $t(42) = -2.56$, $p = .065$). No other post hoc comparisons were significant.

Interaction between age*condition*week on Visual Verbal Learning Test (VVLT): Rate of learning



Main effect of trial on Visual Verbal Learning Test (VVLT): Rate of learning

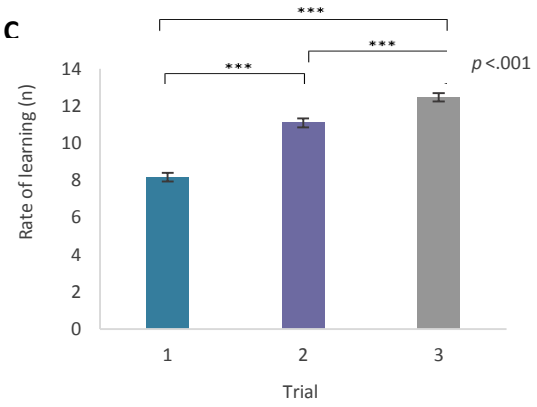


Figure 5.23 Rate of learning on the Visual Verbal Learning Test (VVLT). (A-B) The x axis is age (months) and the y axis the rate of learning (n) over midpoint and endpoint. Regression lines show relationship between x and y by week. (C) The x axis represents trial and the y axis is the rate of learning (n) over midpoint and endpoint. Columns denote the mean. Error bars denote the SE of the mean. *** p <.001. (A) Is the active condition, (B) is the placebo condition.

There was also a significant main effect of trial, $F(2,94) = 81.73$, p <.001, shown in Figure 5.23(C), which shows incremental learning over the first three trials of the measure. Inspection of post hoc comparisons revealed, irrespective of condition, that participants recalled significantly less items correctly for trial 1 (8.38 ± 0.27) compared to trial 2 (10.77 ± 0.24; $t(94) = -10.27$, p <.001). Moreover, participants recalled significantly less items correctly for trial 1 (8.38 ± 0.27) than for trial 3 (11.88 ± 0.25; $t(94) = -12.66$, p <.001). Lastly, participants also recalled significantly less items correctly for trial 2 (10.77 ± 0.24) compared to trial 3 (11.88 ± 0.25; $t(94) = -5.66$, p <.001). There was no significant trial*condition interaction, and this term was removed from the final model.

5.8.4.2.3.2 New learning (word list B)

No outlying observations were removed from the final model. Baseline performance was a significant covariate, $F(1,44) = 33.11$, $p < .001$, and was positively related to performance on subsequent test sessions. Gender was not a significant covariate, $F(1,44) = 0.45$, $p = .505$. Age and IQ were also not significant covariates and were removed from the final model. There was no significant main effect of condition, $F(1,44) = 0.44$, $p = .512$, and no significant interaction between baseline*condition, and this term was removed from the final model. No higher order interactions were significant. There was a significant condition*week interaction, $F(1,42) = 5.09$, $p = .029$, however, post hoc comparisons did not reveal any significant differences.

5.8.4.2.3.3 Retroactive interference (Trial A3-Trial A4)

Two outlying observations were removed from the final model. Age was a significant covariate, $F(1,40) = 7.55$, $p = .009$, such that performance got worse with age. Gender was also a significant covariate, $F(1,40) = 7.48$, $p = .009$, with females (3.04 ± 0.21) performing inferior to males (1.89 ± 0.35). IQ was not a significant covariate, $F(1,40) = 0.31$, $p = .582$, nor was baseline performance, which was removed from the final model. No higher order interactions were significant. There were significant baseline*condition and condition*week interactions, $F(2,40) = 6.60$, $p = .003$ and $F(1,41) = 5.53$, $p = .024$, respectively.

Figure 5.24(A) shows the baseline*condition interaction, such that at superior baseline performance, those in the active condition display less retroactive interference compared to those in the placebo condition, whereas the opposite is seen at poorer baseline performance. Inspection of post hoc comparisons revealed a trend towards those in the active condition (1.40 ± 0.44) performing better than those in the placebo condition (2.71 ± 0.65; $t(40) = -1.69$, $p = .098$) if their baseline retroactive interference score was 0. Whereas at a baseline retroactive interference score of 5 and above, those in the active condition (3.34 ± 0.34) performed less well relative to those in the placebo condition (2.19 ± 0.44; $t(40) = 2.12$, $p = .041$).

The interaction between condition and week is shown in Figure 5.24(B). Inspection of post hoc comparisons revealed the performance by those in the active condition at midpoint (1.66 ± 0.40) was significantly better compared to their performance at endpoint (3.39 ± 0.38; $t(41) = -3.10$, $p = .018$). No other post hoc comparisons were significant.

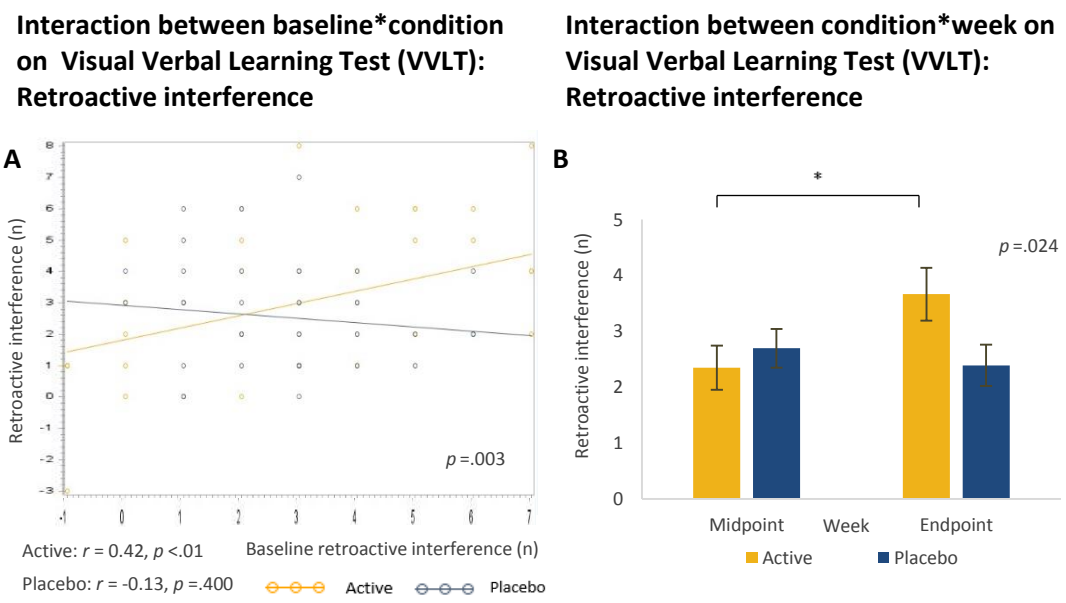


Figure 5.24 Retroactive interference on the Visual Verbal Learning Test (VVLT). (A) The x axis represents baseline retroactive interference (n) and the y axis is retroactive interference (n) over subsequent test points. Regression lines show relationship between x and y by condition. (B) The x axis is week and the y axis is retroactive interference (n) over midpoint and endpoint. Columns denote the mean. Error bars denote the SE of the mean. * p <.05.

There was also a trend towards a main effect of condition, $F(1,40) = 2.86$, $p = .098$, such that participants in the placebo condition (2.41 ± 0.28) demonstrated less retroactive interference compared to those in the active condition (2.53 ± 0.27), and a marginally significant main effect of week, $F(1,41) = 3.89$, $p = .055$, with performance being better at midpoint (2.07 ± 0.28) than at endpoint (2.86 ± 0.28).

5.8.4.2.3.4 Proactive interference (Trial A1-Trial B1)

Two outlying observations were removed from the final model. Baseline, age and IQ were not significant covariates, $F(1,42) = 1.15$, $p = .290$, $F(1,42) = 0.32$, $p = .577$ and $F(1,42) = 0.43$, $p = .516$, respectively. Gender was also nonsignificant and was removed from the final model. There was no significant main effect of condition, $F(1,42) = 0.60$, $p = .442$. There was a marginally significant baseline*condition interaction, $F(1,42) = 3.56$, $p = .066$. There was also a significant baseline*week interaction, $F(1,38) = 4.15$, $p = .049$. No higher order interactions were significant other than a marginally significant interaction for gender*condition*week, $F(4,38) = 2.56$, $p = .054$.

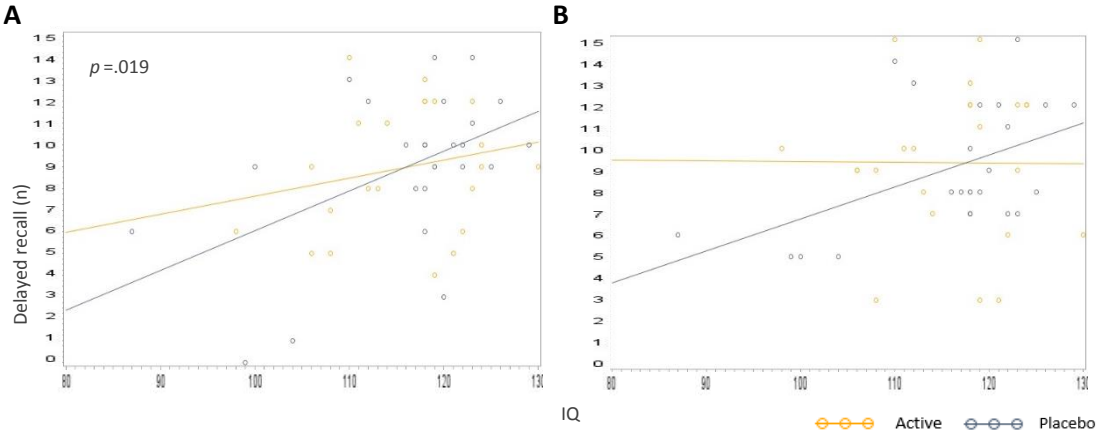
5.8.4.2.3.5 Delayed recall

No outlying observations were removed from the final model. Baseline performance was a significant covariate, $F(1,43) = 39.85$, $p < .001$, such that this was positively associated with performance at later test sessions. Gender was not a significant covariate, $F(1,43) = 2.38$, $p = .130$. Age and IQ were also nonsignificant and were removed from the final model. There was a marginally significant baseline*condition interaction, $F(1,43) = 3.68$, $p = .062$. No higher order interactions were significant other than IQ*condition*week, $F(4,38) = 3.37$, $p = .019$, which is shown in Figure 5.25 (A - midpoint) and 5.25(B - endpoint).

On both test occasions, below an IQ score of 110, participants in the active condition correctly recalled more items following a delay relative to those in the placebo condition, whilst above this IQ score, better performance was demonstrated by participants in the placebo condition. Inspection of post hoc comparisons revealed those in the active condition (10.82 ± 1.13) correctly recalled significantly more items following a delay compared to those in the placebo condition (6.56 ± 1.01; $t(38) = 2.80$, $p = .038$) at endpoint at an IQ score of 100. This became a trend at an IQ score of 110 (9.88 ± 0.61 vs. 7.76 ± 0.64; $t(38) = 2.39$, $p = .096$). Moreover, those in the active condition (10.82 ± 1.13) recalled significantly more words correctly following a delay at endpoint compared to those in the placebo condition at midpoint at an IQ score of 100 (6.12 ± 1.01; $t(38) = 3.10$, $p = .018$). This pattern of performance was a trend at an IQ score of 110 (9.88 ± 0.61 vs. 7.74 ± 0.63; $t(38) = 2.43$, $p = .089$). No other post hoc comparisons were significant.

Lastly, there was a main effect of condition, $F(1,43) = 6.27$, $p = .016$, as shown in Figure 5.25(C). Participants in active condition (9.07 ± 0.44) obtained a greater delayed recall score than those in the placebo condition (8.58 ± 0.46).

Interaction between IQ*condition*week on Visual Verbal Learning Test (VVLT): Delayed recall



Main effect of condition on Visual Verbal Learning Test (VVLT): Delayed recall

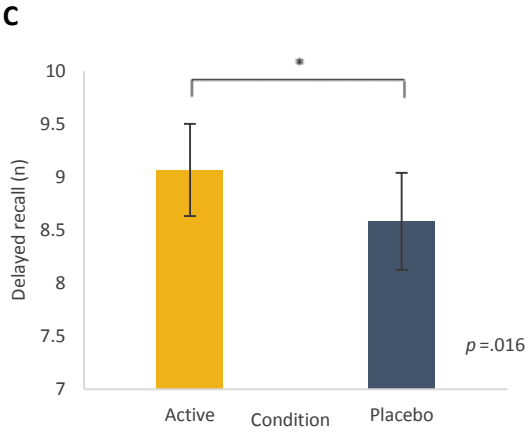


Figure 5.25 Delayed recall on the Visual Verbal Learning Test (VVLT). (A-B) The x axis represents IQ score and the y axis is delayed recall (n). Regression lines show relationship between x and y by condition. (C) The x axis is condition and the y axis is delayed recall (n) over midpoint and endpoint. Columns denote the mean. Error bars denote the SE of the mean.* p <.05. (A) Is midpoint, (B) is endpoint.

5.8.5 Chronic Effect of intervention on subjective evaluation of cognitive failures

5.8.5.1 Cognitive Failures Questionnaire

Two outlying observations were removed from the final model. Subjective evaluation of cognitive failures at baseline was a significant covariate, $F(1,44) = 77.74$, $p < .001$, such that self-ratings at baseline were positively associated with evaluations at subsequent test points. Age, IQ and gender were not significant covariates and were removed from the final model. There was no significant main effect of condition, $F(1,44) = 1.21$, $p = .277$ and no significant interaction between baseline and condition, $F(1,44) = 0.53$, $p = .473$. There were significant baseline*condition*week, $F(1,41) = 5.16$, $p = .028$, baseline*week, $F(1,41) = 4.89$, $p = .033$ and condition*week, $F(1,41) = 4.89$, $p = .033$, interactions.

The baseline*condition*week interaction is shown in Figure 5.26(A) and 5.26(B). In the active condition (5.26(A)), those who rated themselves as low for cognitive failures at baseline subsequently rated themselves as experiencing greater cognitive failures at endpoint relative to midpoint. With increasing baseline rating, greater cognitive failures were experienced at midpoint compared to endpoint. In the placebo condition (Figure 5.26(B)), self-perceptions of cognitive failures experienced were similar across both test points. Inspection of post hoc comparisons revealed a trend towards those in the active condition experiencing greater cognitive failures at midpoint (0.56 ± 0.04) compared to endpoint (0.47 ± 0.03; $t(41) = 2.47$, $p = .080$) at a baseline rating of 0.6. This became significant at a baseline rating of 0.7 and above (greater cognitive failure experienced) (0.65 ± 0.05 vs. 0.51 ± 0.04; $t(41) = 2.72$, $p = .045$). No other post hoc comparisons were significant.

In view of the significant condition*week interaction, participants in the active condition rated themselves as experiencing greater cognitive failures compared to those in the placebo group at both midpoint (0.43 ± 0.03 vs. 0.39 ± 0.03) and endpoint (0.40 ± 0.03 vs. 0.36 ± 0.03). However, post hoc comparisons did not reveal any significant differences.

Interaction between baseline*condition*week on Cognitive Failures Questionnaire

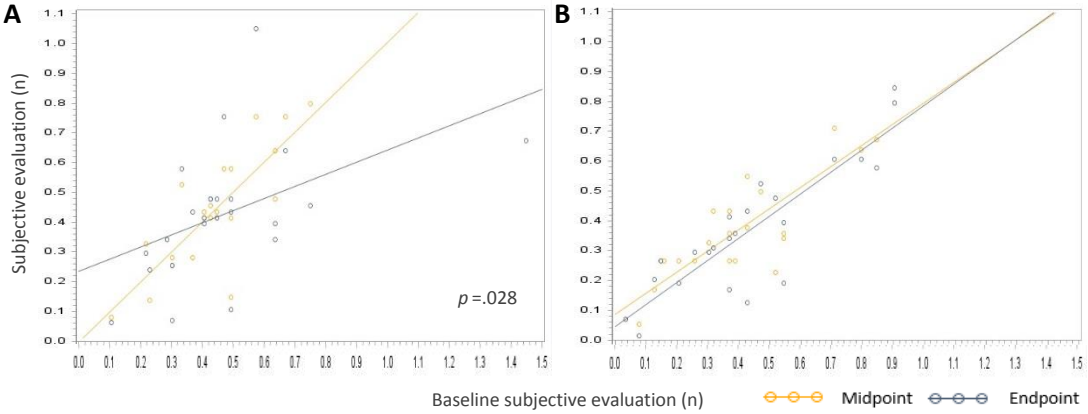


Figure 5.26 Self-reported cognitive failures. The x axis is baseline subjective evaluation (n) and the y axis is subjective evaluation (n) over subsequent test points. Regression lines show relationship between x and y by week. (A) Is the active condition, (B) is the placebo condition.

5.8.6 Summary of findings

5.8.6.1 Effects of acute GPL with SM supplementation on cognitive performance in middle-aged and older adults with a subjective memory complaints

A tabulated summary of the effect of the acute intervention on cognitive performance is shown in Table 5.10.

Table 5.10 Tabulated summary of cognitive performance outcomes following acute supplementation

	Main effects		Covariates						
Cognitive outcome	**Condition**	**Trial**	**Baseline**	**Age**	**IQ**	**Gender**	**Trial**	**Total correct**	**Accuracy**
Attention Switching Task									
Switch cost reaction time	S (p = .008)	S (p = .013)	NS	NS	-	T (p = .093)	/	/	S (p <.001)
Switch cost accuracy	NS	S (p = .021)	S (p <.001)	S (p = .038)	NS	NS	/	/	/
Target accuracy	NS	NS	S (p <.001)	-	-	-	/	/	/
Target reaction time	S (p = .041)	S (p = .004)	NS	-	-	-	/	/	S (p <.001)
Rapid Visual Information Processing Task									
Hits	S (p = .012)	/	S (p <.001)	-	-	-	/	/	/
False alarms	NS	/	S (p <.001)	-	S (p = .002)	NS	/	/	/
Reaction time for hits	S (p = .004)	/	S (p <.001)	S (p <.001)	-	S (p = .005)	-	S (p <.001)	/

	Main effects		Covariates						
Cognitive outcome	**Condition**	**Trial**	**Baseline**	**Age**	**IQ**	**Gender**	**Trial**	**Total correct**	**Accuracy**
N-back Task									
Target accuracy	S (p = .009)	/	S (p <.001)	-	S (p = .010)	T (p = .096)	/	/	/
Total accuracy	MS (p = .066)	/	S (p <.001)	-	S (p = .010)	NS	/	/	/
Reaction time for targets	NS	/	S (p <.001)	-	NS	T (p = .095)	S (p = .022)	S (p = .009)	/
Reaction time for nontargets	S (p <.001)	/	S (p <.001)	S (p = .049)	S (p <.001)	S (p <.001)	S (p = .027)	S (p <.001)	/
Pattern Separation Task									
Pattern separation score	NS	/	S (p <.001)	-	-	T (p = .088)	/	/	/
Recognition score	S (p = .038)	/	S (p <.001)	-	-	NS	/	/	/
Face-Name Associative Memory Exam									
Immediate recall	NS	/	S (p = .002)	-	-	NS	/	/	/
Delayed recall	MS (p = .064)	/	S (p <.001)	-	NS	-	/	/	/
Visual Verbal Learning Test									
Rate of learning	NS	S (p <.001)	S (p <.001)	S (p = .008)	S (p <.001)	-	/	/	/
New learning	NS	/	S (p <.001)	S (p = .044)	-	NS	/	/	/
Retroactive interference	NS	/	NS	-	-	NS	/	/	/

	Main effects		Covariates						
Cognitive outcome	**Condition**	**Trial**	**Baseline**	**Age**	**IQ**	**Gender**	**Trial**	**Total correct**	**Accuracy**
Proactive interference	NS	/	NS	S (p = .040)	-	NS	/	/	/
Delayed recall	MS (p = .053)	/	S (p <.001)	S (p = .018)	-	NS	/	/	/

Table 5.10 continued.

	Interaction terms	
Cognitive outcome	**Baseline*condition**	**Trial*condition**
Attention Switching Task		
Switch cost reaction time	MS (p = .052)	NS
Switch cost accuracy	-	NS
Target accuracy	-	NS
Target reaction time	S (p = .014)	NS
Rapid Visual Information Processing Task		
Hits	-	/
False alarms	NS	/
Reaction time for hits	S (p = .001)	/

	Interaction terms	
Cognitive outcome	**Baseline*condition**	**Trial*condition**
Pattern Separation Task		
Pattern separation score	-	/
Recognition score	S (p = .006)	/
Face-Name Associative Memory Exam		
Immediate recall	-	/
Delayed recall	-	/
Visual Verbal Learning Test		
Rate of learning	-	NS
New learning	NS	/

	Interaction terms	
Cognitive outcome	**Baseline*condition**	**Trial*condition**
N-back Task		
Target accuracy	S (p = .012)	/
Total accuracy	MS (p = .057)	/
Reaction time for targets	NS	/
Reaction time for nontargets	S (p <.001)	/

	Interaction terms	
Cognitive outcome	**Baseline*condition**	**Trial*condition**
Retroactive interference	NS	/
Proactive interference	-	/
Delayed recall	NS	/

Notes. Accuracy: target accuracy used in target reaction time analysis / switch cost accuracy used in switch cost reaction time analysis; MS: Marginally significant, NS: Nonsignificant, S: Significant. Total correct: number of correct trials; T; Trend; – indicates term removed from the final model for best fit, / indicates this was not entered into the model.

A summary of significant, marginally significant, and trends for covariates, main effects and interactions following acute supplementation is provided below:

Baseline performance as a covariate by cognitive measure outcome:

Cognitive performance at baseline (time 1, pre-first dose) was positively related to performance at time 2 (+90 minutes post-time 1 and first dose) on sixteen out of twenty cognitive measure outcomes assessing memory performance (Pattern Separation Task: Pattern separation score and recognition score; Visual Verbal Learning Test: Rate of learning, new learning, delayed recall; Face-Name Associative Memory Exam: Immediate and delayed recall), and executive function (Rapid visual Information Processing: hits, false alarms and reaction time for hits; N-back: Target accuracy, total accuracy, reaction time for targets and reaction time for nontargets; Attention Switching Task: target accuracy and switch cost accuracy).

Age, IQ and gender of participants as covariates by cognitive measure outcome:

Despite participants not differing statistically on age, IQ and gender at study entry, these demographic characteristics were significant covariates on a number of outcomes. Age was significant on seven outcomes, such that age-related decline (poorer performance with increased age) for verbal memory outcomes (Visual Verbal Learning Test: Rate of learning, new learning, proactive interference and delayed recall) and for reaction time on executive function measures (Rapid Visual Information Processing: reaction time for hits and N-back: reaction time for nontargets) was observed. Conversely, accuracy switch costs improved with increasing age on a measure of executive function (Attention Switching Task), however, this was a weak linear correlation with considerable variation. IQ was a significant covariate on 5 outcomes, with improved accuracy being associated with higher IQ scores for verbal memory (Visual Verbal Learning Test: Rate of learning) and for executive function (Rapid Visual Information Processing: false alarms and N-back: target accuracy and total accuracy). However, higher IQ was also associated with longer reaction time for nontargets on the N-back task, perhaps representing a speed accuracy trade off. Gender was a significant factor on two outcomes and showed a trend on four. Nevertheless, there was no clear indication of either gender demonstrating significantly superior performance for accuracy or reaction time or in any specific cognitive domain. With respect to executive function, females showed significantly faster reaction time than males for hits on the Rapid Visual Information Processing, whilst males demonstrated significantly faster response latencies to that of females for nontargets on the N-back task. Moreover, there were

trends towards females responding faster than males for switch costs on the Attention Switching Task and towards targets on the N-back task. There were also trends towards females showing greater target accuracy to males on the N-back task. On the Pattern Separation Task, males scored higher than females (pattern separation score).

Main effect of condition by cognitive measure outcome:

Condition (active vs placebo) differed significantly for three executive function outcomes. Those in the active condition achieved significantly more hits on the Rapid Visual Information Processing Task and demonstrated significantly faster performance for nontargets on the N-back task compared to those in the placebo condition. Notably, this main effect of condition on the N-back task was qualified by a baseline*condition interaction, such that the significantly faster reaction time shown by those in the active condition was observed only for those with faster baseline reaction times. At slower baseline response time, participants in the active condition were significantly slower than those in the placebo condition. Condition also significantly affected performance on the Attention Switching Task whereby those in the placebo condition were significantly faster for switch costs compared to those in the active condition. This again was qualified by a marginally significant baseline*condition interaction, which indicated that those in the active condition demonstrated significantly slower reaction time relative to those in the placebo condition at faster baseline performance. Furthermore, those in the placebo condition achieved marginally greater total accuracy (N-back Task) than their counterparts in the active condition. There was a corresponding marginally significant baseline*condition interaction, such that at poorer baseline performance, participants in the placebo condition performed better than those in the active condition. Conversely, the opposite pattern of performance was shown at greater baseline total accuracy; an advantage shown by those in the active condition. On a measure assessing memory performance, participants in the placebo condition recalled marginally more items correctly following a delay (Face-Name Associative Memory Exam and Visual Verbal Learning Test). There was also a trend towards those in the active condition demonstrating slower reaction latencies compared to participants in the placebo condition for hits on the Rapid Visual Information Processing Task. Again, there was a corresponding baseline*condition interaction that indicated that those in the active condition responded significantly faster than those in the placebo condition at faster baseline reaction time, whereas at slower baseline performance, participants in the placebo condition demonstrated significantly faster responses than those in the active condition.

Interaction between baseline performance and condition by cognitive measure outcome:
Significant baseline*condition interactions (in the absence of a main effect of condition) were further found on one measure assessing memory performance and two measures sensitive to executive performance. With respect to the recognition score outcome of the Pattern Separation Task, at superior baseline performance, those in the active condition achieved significantly higher recognition scores compared to those in the placebo condition. On the N-back task, those in the active condition demonstrated significantly poorer target accuracy compared to those in the placebo condition at poorer baseline performance. Lastly, on the Attention Switching Task (target reaction time), at quicker baseline target reaction time, participants in the active condition responded slower than those in the placebo condition, however, at poorer baseline performance, participants in the active condition demonstrated faster responses to those in the placebo condition.

Overall, it appears that whilst there were some benefits of an acute dose of the active supplement, these benefits were mainly demonstrated in participants with higher levels of performance at baseline.

5.8.6.2 Effects of chronic GPL with SM supplementation on cognitive performance and subjective evaluation of cognitive failures in middle-aged and older adults with a subjective memory complaints

A tabulated summary of the effect of the chronic intervention on cognitive performance is shown in Table 5.11.

Table 5.11 Tabulated summary of cognitive performance outcomes following chronic supplementation

	Main effects			Covariates						
Cognitive outcome	**Condition**	**Week**	**Trial**	**Baseline**	**Age**	**IQ**	**Gender**	**Trial**	**Total correct**	**Accuracy**
Attention Switching Task										
Switch cost reaction time	NS	NS	S (p = .001)	NS	-	-	NS	/	/	S (p <.001)
Switch cost accuracy	T (p = .098)	NS	S (p <.001)	S (p <.001)	-	MS (p = .055)	-	/	/	/
Target accuracy	NS	S (p = .026)	S (p <.001)	S (p <.001)	S (p = .021)	-	-	/	/	/
Target reaction time	NS	S (p = .020)	S (p <.001)	-	-	-	-	/	/	S (p <.001)
Rapid Visual Information Processing Task										
Hits	NS	NS	/	S (p <.001)	S (p = .032)	S (p = .008)	-	/	/	/
False alarms	S (p = .010)	NS	/	S (p <.001)	S (p = .016)	-	-	/	/	/
Reaction time for hits	NS	S (p = .037)	/	S (p <.001)	-	S (p = .039)	MS (p = .058)	S (p = .021)	S (p = .002)	/

	Main effects			Covariates						
Cognitive outcome	**Condition**	**Week**	**Trial**	**Baseline**	**Age**	**IQ**	**Gender**	**Trial**	**Total correct**	**Accuracy**
N-back Task										
Target accuracy	T (p = .073)	NS	/	S (p <.001)	-	-	-	/	/	/
Total accuracy	S (p = .036)	NS	/	S (p <.001)	-	-	-	/	/	/
Reaction time for targets	MS (p = .064)	NS	/	S (p <.001)	-	-	-	-	-	/
Reaction time for nontargets	NS	S (p = .002)	/	S (p <.001)	-	S (p = .038)	NS	S (p <.001)	S (p <.001)	/
Pattern Separation Task										
Pattern separation score	S (p = .029)	NS	/	S (p <.001)	-	-	-	/	/	/
Recognition score	NS	NS	/	S (p <.001)	-	-	-	/	/	/
Face-Name Associative Memory Exam										
Immediate recall	NS	NS	/	S (p <.001)	S (p = .025)	-	-	/	/	/
Delayed recall	NS	S (p = .013)	/	S (p <.001)	S (p = .036)	NS	-	/	/	/
Visual Verbal Learning Test										
Rate of learning	NS	NS	S (p <.001)	S (p <.001)	S (p = .017)	S (p = .001)	S (p = .021)	/	/	/
New learning	NS	NS	/	S (p <.001)	-	-	NS	/	/	/

	Main effects			Covariates						
Cognitive outcome	**Condition**	**Week**	**Trial**	**Baseline**	**Age**	**IQ**	**Gender**	**Trial**	**Total correct**	**Accuracy**
Retroactive interference	T (p = .098)	MS (p = .055)	/	-	S (p = .009)	NS	S (p = .009)	/	/	/
Proactive interference	NS	NS	/	NS	NS	NS	-	/	/	/
Delayed recall	S (p = .016)	NS	/	S (p <.001)	-	-	NS	/	/	/

Table 5.11 continued.

	Interaction terms								
Cognitive outcome	**Cond* week**	**Baseline* condition**	**Baseline* week**	**Trial* condition**	**Baseline* condition* week**	**Trial* condition* week**	**Age* condition* week**	**IQ* condition* week**	**Gender* condition* week**
Attention Switching Task									
Switch cost reaction time	S (p = .026)	-	S (p = .046)	-	NS	-	-	NS	NS
Switch cost accuracy	NS	S (p = .023)	NS	-	S (p = .049)	NS	-	-	S (p = .023)
Target accuracy	S (p = .029)	-	-	NS	-	-	NS	S (p = .008)	NS
Target reaction time	NS	S (p = .026)	S (p = .031)	-	S (p = .045)	-	-	NS	NS

	Interaction terms								
Cognitive outcome	**Cond* week**	**Baseline* condition**	**Baseline* week**	**Trial* condition**	**Baseline* condition* week**	**Trial* condition* week**	**Age* condition* week**	**IQ* condition* week**	**Gender* condition* week**
Rapid Visual Information Processing Task									
Hits	NS	-	-	/	-	/	NS	T (p = .080)	NS
False alarms	NS	S (p = .009)	T (p = .087)	/	-	/	-	-	NS
Reaction time for hits	NS	NS	S (p = .022)	/	-	/	-	-	NS
N-back Task									
Target accuracy	NS	-	MS (p = .064)	/	NS	/	NS	-	NS
Total accuracy	NS	-	S (p = .034)	/	NS	/	NS	-	NS
Reaction time for targets	NS	NS	-	/	-	/	NS	NS	-
Reaction time for nontargets	S (p = .033)	S (p = .001)	-	/	-	/	S (p = .001)	S (p = .034)	-
Pattern Separation Task									
Pattern separation score	NS	-	-	/	-	/	NS	-	NS
Recognition score	NS	NS	NS	/	S (p = .022)	/	-	-	S (p = .003)
Face-Name Associative Memory Exam									
Immediate recall	S (p = .034)	NS	NS	/	S (p = .026)	/	-	-	NS
Delayed recall	NS	NS	-	/	S (p = .004)	/	-	MS (p = .059)	-

	Interaction terms								
Cognitive outcome	**Cond* week**	**Baseline* condition**	**Baseline* week**	**Trial* condition**	**Baseline* condition* week**	**Trial* condition* week**	**Age* condition* week**	**IQ* condition* week**	**Gender* condition* week**
Visual Verbal Learning Test									
Rate of learning	S (p <.001)	-	-	-	-	-	S (p = .002)	-	NS
New learning	S (p = .029)	-	-	/	-	/	-	-	NS
Retroactive interference	S (p = .024)	S (p = .003)	-	/	-	/	-	-	NS
Proactive interference	NS	MS (p = .066)	S (p = .049)	/	-	/	-	-	MS (p = .054)
Delayed recall	NS	MS (p = .062)	-	/	-	/	-	S (p = .019)	NS

Notes. Accuracy: target accuracy used in target reaction time analysis / switch cost accuracy used in switch cost reaction time analysis; MS: Marginally significant, NS: Nonsignificant, S: Significant. Total correct: number of correct trials; T; Trend; – indicates term removed from the final model for best fit, / indicates this was not entered into the model.

A summary of significant, marginally significant, and trends for covariates, main effects and interactions following chronic supplementation is provided below:

Baseline performance as a covariate by cognitive measure outcome:

Consistent with the analysis of the cognitive measure data obtained following acute supplement administration, cognitive performance at baseline was a significant covariate and positively associated with subsequent performance on sixteen out of twenty cognitive measure outcomes. Of those that assessed memory performance, these included Pattern Separation Task (pattern separation score and recognition score), Face-Name Associative Memory Exam (immediate and delayed recall) and the Visual Verbal Learning Test (rate of learning, new learning and delayed recall). Of those that assessed executive function, these included the Rapid Visual Information Processing Task (hits, false alarms, reaction time for hits), N-back (target and total accuracy, and reaction time for targets and nontargets) and the Attention Switching Task (target accuracy and switch cost accuracy). In addition, baseline evaluations on the Cognitive Failures Questionnaire also predicted subsequent evaluations.

Age, IQ and gender of participants as covariates by cognitive measure outcome:

Age was significant on seven cognitive measure outcomes, with performance showing age-related decline on outcome measures including the Face-Name Associative Memory Exam (immediate and delayed recall), Visual Verbal Learning Test (rate of learning and retroactive interference), Rapid Visual Information Processing Task(hits and false alarms) and on the Attention Switching Task (target accuracy). IQ was significant on four cognitive outcomes and marginally significant on one other. A higher IQ significantly predicted more accurate performance on the Visual Verbal Learning Test (rate of learning) and Rapid Visual Information Processing Task (hits). However, as with the findings from the analysis of the cognitive data following acute supplementation, IQ was a significant covariate for reaction time performance, such that response latencies were greater for those with higher IQ scores on Rapid Visual Information Processing Task (reaction time for hits) and the N-back task (reaction time for nontargets). Again, this may represent a speed accuracy trade off with respect to the Rapid Visual Information Processing measure, whereas the longer response times shown on the N-back task may represent more cautious executive decision making. IQ was further marginally significant on the Attention Switching Task (switch cost accuracy), with those with lower IQ scores demonstrating greater switch cost accuracy, however, this was represented by a weak

correlation. Gender was significant on two outcomes assessing memory performance and marginally significant on one outcome assessing executive function. As with the findings derived from the analysis of cognitive data following acute analysis, there is no clear indication of either gender showing a significant advantage. Specifically, females demonstrated significantly better performance than males on the rate of learning outcome of the Visual Verbal Learning Test, whilst males showed significantly less retroactive interference compared to females on the same measure. Consistent with post-acute supplementation performance, females responded faster than males for hits on the Rapid Visual Information Processing Task, however, this was only a marginally significant difference.

Main effect of condition by cognitive measure outcome:

Significant or marginally significant main effects of condition or trends towards an effect of condition were typically qualified by a corresponding baseline*condition and baseline*condition*week interactions, further highlighting the association between baseline performance and subsequent performance. Exceptions to this occurred for the Pattern Separation Task (pattern separation score): those in the active condition demonstrated significantly poorer performance to those in the placebo condition; N-back (target accuracy): trend towards participants in the active condition showing greater accuracy to those in the placebo condition; N-back (total accuracy): significant difference in favour of those in the active condition; N-back (reaction time for targets): those in the active condition demonstrated marginally significantly slower response times compared to those in the placebo condition.

Participants in the active condition demonstrated significantly superior performance to those in the placebo condition on the Visual Verbal Learning Test for delayed recall, however, this main effect of condition was qualified by a marginally significant baseline*condition interaction and a significant IQ*week*condition interaction. Performance (pooled across midpoint and endpoint) was superior in the active condition but only at higher levels of baseline performance. Furthermore, there was a stronger effect of condition in favour of the active condition at endpoint in those with an IQ score of 100 (with a trend for those with an IQ score of 110). There was a trend towards superior performance on the Visual Verbal Learning Test for retroactive interference (lower retroactive interference) demonstrated by those in the placebo condition relative to the active condition. However, this was qualified by significant baseline*condition and condition*week interactions. Notably, at better baseline performance (lower retroactive interference), there was a trend towards participants in the active condition performing better than those in the placebo condition. However, participants in the active condition demonstrated

significantly greater retroactive interference to those in the placebo condition at poorer baseline performance. In addition, those in the active condition performed better at midpoint than at endpoint. Participants in the placebo condition also showed superior performance to those in the active condition on the Rapid Visual Information Processing Task (false alarms: significant). However, this was qualified by a significant baseline*condition interaction, such that participants in the active condition committed significantly more false alarms at better baseline performance but significantly less at poorer baseline performance to those in the placebo condition. Post hoc comparisons were inconclusive in respect of the main effect of condition. Participants in the placebo condition further showed superior performance to those in the active condition on the Attention Switching Task (switch cost accuracy: trend). This trend towards a benefit for placebo was qualified by baseline*condition*week (where those in the placebo condition demonstrated better performance compared to those in the active condition at midpoint depending on their baseline performance) and gender*condition* week (the effect of week depended upon gender in the active condition but not the placebo condition) interactions.

Two-way and higher order interactions featuring participants' demographics in the absence of a significant main effect of condition by cognitive measure outcome:

For the Pattern Separation Task (recognition score), there were significant baseline*condition*week and gender*condition*week interactions. On the Face-Name Associative Memory Exam (delayed recall), there was a significant baseline*condition*week interaction and a marginally significant IQ*condition*week interaction. For the Visual Verbal Learning Test (rate of learning), there were significant condition*week and age*condition*week interactions. On the same cognitive measure (proactive interference), there were marginally significant baseline*condition and gender*condition*week interactions. For the N-back task (reaction time for nontargets), there were significant baseline*condition, condition*week, IQ*condition*week and Age*condition*week interactions. On the Attention Switching Task, there were significant condition*week and IQ*condition*week interactions for target accuracy; significant baseline*condition and baseline*condition*week interactions for target reaction time; significant baseline*condition*week, gender*condition*week and baseline*condition interactions, however, there was also a trend towards a main effect of condition for switch cost accuracy, and a significant condition*week interaction for switch cost reaction time. Post hoc comparisons were inconclusive for the condition*week interaction for switch cost reaction time.

Interaction between baseline self-ratings, condition and week on cognitive failures:

There was a significant baseline*condition*week interaction for the Cognitive Failures Questionnaire. These data indicated an interaction between baseline evaluations and subsequent evaluations (at midpoint and endpoint) in the active condition but not in the placebo condition. Specifically, participants in the active condition reported experiencing greater cognitive failures at midpoint compared to endpoint if they had reported more cognitive failures at baseline.

5.9 Discussion

5.9.1 Overview of findings

The primary aim of the present study was to examine whether acute (cognitive assessment +90 minutes post-first dose) and/or chronic (12 week) supplementation of Lacprodan® PL-20 led to an improvement in cognitive function, specifically memory and executive function in adults aged 50 years and over with a SMC. A supplementary research question concerned whether Lacprodan® PL-20 would result in a reduction in the frequency of cognitive failures experienced by those who participated in the study. It was hypothesised that Lacprodan® PL-20 supplementation would lead to improvements in cognitive function demonstrated by superior performance on the cognitive measures compared to those in the placebo condition. In addition, it was expected that improvements in cognitive function would lead to a decrease in the prevalence of cognitive failures. As with Study 1, the findings following both the acute and chronic administration of Lacprodan® PL-20 suggest limited efficacy of the product to promote improvements to cognitive function. Following acute administration, there was a significant difference in performance by condition on the primary outcome (Attention Switching Task: Switch cost reaction time), however, this was in favour of the placebo condition. Regarding the secondary outcomes, a benefit of Lacprodan® PL-20 supplementation was seen on the Rapid Visual Information Processing measure (Hits) and on the N-back Task (reaction time for nontargets). This main effect of condition for reaction time for nontargets was qualified by a baseline*condition interaction, such that those in the active condition were significantly faster than participants in the placebo condition at faster baseline performance, whilst participants in the placebo condition were significantly faster than those in the active condition at slower baseline reaction time. With respect to the findings following chronic supplementation, on the primary outcome, there was no significant/marginally significant/trend towards a main effect of

condition. There was a significant interaction between condition and week, however, post hoc comparisons were inconclusive. Performance differences in favour of those in the active condition as shown on secondary outcomes include superior performance to those in the placebo condition on the N-back Task including greater target accuracy (trend) and total accuracy (significant). However, participants in the active condition also demonstrated marginally significantly slower reaction times for targets relative to those in the placebo condition, suggesting a speed-accuracy trade-off. For those in the active condition who reported experiencing greater cognitive failures at baseline, there was a reduction in the extent of these from midpoint to endpoint. However, no differences were found between the two conditions and evaluations by those in the placebo group were similar across both weeks.

Cognitive performance between conditions tended to differ as a function of baseline performance, as indicated by the many baseline*condition interactions (as well as the less frequent baseline*condition*week interactions). Consistent with this, cognitive performance at baseline was typically found to be a significant predictor of subsequent performance observed on later test occasions. The findings based on the cognitive measure data obtained following both acute and chronic supplementation suggest that treatment benefits of Lacprodan® PL-20 were mainly demonstrated in participants with higher levels of performance at baseline. Other studies that supplemented similar samples (middle-aged/older adults with SMCs) with GPLs (Crook et al., 1991; Moré et al., 2014; Vakhapova et al., 2010) also reported that their study findings differed as a function of baseline performance. Similarly, baseline evaluation of cognitive failures was found to be a significant covariate. Baseline performance differences can undermine efforts to explore treatment effects. This is also the case for other baseline characteristics, such as age, IQ and gender. Despite attempting to control for the influence of these variables by using stratified randomisation when allocating participants to condition (block randomisation in which level of education was used as a proxy for IQ, see section 5.6.2), demographic variables were found to be significant predictors of performance. That said, it could be argued that level of education was a poor proxy for IQ in that for adults over 50 years of age, going on to further education when they were younger may have been dependent upon their family situation and whether enrolling in higher education was financially viable. To reduce differences that exist between the conditions, individuals' cognitive measure performance could be assessed, and their demographic details collected, prior to study enrolment for the purpose of tightening inclusion criteria. For example, with respect to IQ, IQ*condition*week significant interactions were found on the Verbal Visual Learning Test (delayed recall), the N-back Task

(reaction time for nontargets) and the Attention Switching Task (target accuracy) following the chronic analysis. Overall, those in the active group tended to demonstrate greater accuracy and faster reaction time than their counterparts in the placebo condition, typically at endpoint, if they had IQ scores ranging from 90 to 120. These results suggest that participant recruitment in similar studies might be best focussed on those with average (100) IQ and the range confined to +/- 1 SD i.e. 85 to 115.

5.9.2 Possible explanations for the limited significant treatment effects on cognition performance following acute and chronic supplementation with Lacprodan® PL-20

5.9.2.1 Study quality and statistical approach

Considering the strengths and weaknesses of the current study, many of the strengths of Study 1 (section 4.8.2) apply to the present study. The study design conformed to a randomised, double-blind, placebo-controlled study design and supplement products were matched for taste and appearance and packaged in identical sachets except for a 3-digit code used for identification. Researchers remained blind at the time of data analysis and were unblinded only following completion of data analysis. The outcome data from all measures utilised in the present study was analysed and reported following the administration of all measures that were planned to be utilised in the study *a priori*. It can therefore be concluded that the study demonstrates a low risk of bias and can be classed as being of good quality in accordance with the Cochrane risk of bias tool for randomized clinical trials (Higgins et al., 2011). Moreover, including an opportunity for participants to become familiar with the measures during the screening appointment and using parallel versions of these on all test occasions reduces the extent of practice effects (Beglinger et al., 2005; Bell et al., 2018). The statistical approach employed in the analysis of the data obtained following acute and chronic administration was also consistent with Study 1 with respect to controlling for baseline data and participant demographic characteristics (see section 4.8.2.2. for the merits of this approach).

As with Study 1, an *a posteriori* calculation was performed for both the acute and chronic interventions. Based on the number of participants included in each analysis, observed difference in LS-means and variability (standard deviation) on the primary outcome, an *a posteriori* calculation determined the power to detect a difference between the active and placebo conditions was 0.722 and 0.05 in respect of the acute and chronic interventions,

respectively. Additionally, a sample size of 61 and 1,445,954 would be required to achieve power of 0.8 in respect of the acute and chronic interventions, respectively. It can therefore be concluded that both parts of the study were underpowered due to an insufficient number of participants.

5.9.2.2 Duration of intervention

In view of the duration of intervention, particularly the acute administration but also the duration of the chronic administration of Lacprodan® PL-20, the length of treatment may have not been sufficient to induce alterations at the level of the cell membrane of adequate strength to effect a positive impact on cognitive function (see section 4.8.2.3). However, three randomised, double-blind, placebo-controlled design studies that recruited a similar sample to that used in the present study did report a benefit of GPL supplementation. Crook and colleagues (1991) reported a benefit of PS sourced from bovine cortex after 3 weeks on a Name-face association measure and after 12 weeks on the Wechsler Memory Scale Logical Memory Subtest (Form A). Vakhapova et al. (2010) found a favourable effect of PS-DHA/EPA after 15 weeks of consumption on immediate verbal recall of a word list, whilst a composite of PS + PA for 3 months led to positive treatment effects on the information, visual memory and memorising numbers components of the Wechsler Memory Scale (Moré et al., 2014). However, despite the apparent similarity in the sample (adults with a SMC), there are significant differences between these studies and the current one discussed under 5.9.3, which might explain the discrepancy in research findings. Importantly, Moré et al. (2014) also conducted kinetic analysis of soy-PS in 8 overnight-fasted healthy volunteers and reported the level of PS in serum peaked 90 minutes post single ingestion of 5 capsules each containing 100 mg PS + 80 PA. This finding is promising, as it provides support towards PS potentially being available for uptake into the brain 90 minutes after oral consumption, which informed the timing between first dose and cognitive testing in the present study. Crucially, just as with PL-DHA in that it is not clear how quickly or to what extent brain DHA levels respond to alterations in plasma PL-DHA (Lacombe et al., 2018), it is currently unknown how GPLs present in the brain correspond to those available in serum. Thus, it should not be assumed that concentrations of GPLs within the brain have a direct relationship with GPLs available within serum or that we can infer the timing or rate of uptake.

5.9.2.3 Measurement of cognitive performance

As discussed previously (see section 5.4), the selection of cognitive measures for use in the current study was informed by the findings of similar studies that have reported positive treatment effects following GPL supplementation. Evidence concerning which cognitive domains are typically compromised with increasing age and the facilitation and protection of cognitive function by GPLs was reviewed (see sections 5.1 and 2.7, respectively). It was important to select measures that were sensitive and salient to the study sample i.e. those that assessed memory function. As such, the selection of measures to assess cognitive function was considered and well-informed.

5.9.2.4 Compliance

Supplements were consumed by participants in their homes for convenience in this free-living study. Compliance with the study protocol required participants to consume the supplement daily. Compliance was measured using a consumption diary and the return of supplement sachets. Supplement consumption was also discussed face-to-face on each test occasion. However, unlike in Study 1, no direct measurement of compliance could be taken. Records concerning participant consumption may therefore not reflect the actual frequency or pattern of supplement intake across experimental conditions.

5.9.3 Current findings in the context of previous empirical evidence

The findings of the current study are not consistent with previous studies that have explored the potential advantage of GPL intake to cognitive performance in adults with SMCs and observed positive treatment effects (Crook et al., 1991; Moré et al., 2014; Vakhapova et al., 2010). The difference in study findings may reflect a difference in analytical approach, the potential for practice effects, study inclusion criteria and GPL composition. Moré et al. (2014) failed to control for potential confounding variables, such as baseline cognitive performance and demographic variables in the analysis of their data. Failing to account for confounding variables in an analysis threatens the validity of inferences made about cause and effect (Pourhoseingholi, Baghestani, & Vahedi, 2012). Similarly, although Crook et al. (1991) included baseline performance data as a covariate in their analysis, they failed to account for IQ. Vakhapova and colleagues (2010) controlled for baseline performance and cognitive function (MMSE score; participant demographics were removed from the final model due to being nonsignificant), however, the reported improvements in performance on the RAVLT, which enabled them to conclude a benefit of PS-DHA/EPA may have been undermined by practice effects. Indeed, practice effects

have been reported on measures assessing immediate and delayed recall of word lists (Bell et al., 2018). The validity of subjective memory complaints reported by participants in the current study were not assessed using, for example, a questionnaire or a neuropsychological measure. Both Vakhapova et al. (2010) and Crook et al. (1991) used a memory complaint questionnaire and required individuals to score above a cut-off to be eligible to participate. Eligibility also depended upon an individual's performance on a neuropsychological battery (Vakhapova et al., 2010) or on a standardised memory assessment (Crook et al., 1991), which was used to ratify the complaint. In the absence of a formal assessment of memory function in the present study, it is impossible to know whether the participants have experienced similar prior declines in memory and therefore would meet the same eligibility criteria. As such, comparing the findings of the current study with the findings of either of the two previous studies may be erroneous. Moreover, the composition of the GPL supplement provided by both Vakhapova et al. (2010) and Crook et al. (1991) contained DHA (PS-DHA and PS sourced from bovine cortex, respectively) unlike Lacprodan® PL-20, which is sourced from bovine milk. This again makes it difficult to draw comparisons between the studies, given PS enriched with DHA is known to promote cognitive function in multiple ways (see section 3.5.1).

5.10 Conclusion

A randomised, double-blind, placebo-controlled, parallel groups study was undertaken to examine whether providing Lacprodan® PL-20 as a single dose (acute) and chronically (12 weeks) would promote cognitive performance. The analysis of the study data adjusted for baseline cognitive performance/subjective evaluation of cognitive failures, age, IQ and gender. The findings provided limited evidence as to the efficacy of the active supplement on cognitive performance and benefits were mainly demonstrated in participants with higher levels of performance at baseline. The degree of self-reported cognitive failures reduced over time in those that received the active supplement and reported experiencing them to a greater extent at baseline, however, no between condition differences were found. These findings are not consistent with other studies that have supplemented similar experimental samples with GPLs. This difference in the study findings may reflect heterogeneity in analytical approach, study eligibility criteria and supplement composition.

6. General discussion

This chapter provides a summary of the key findings of this thesis in relation to the original thesis aims presented in Chapter 1. The implications of the findings are discussed in the context of previous research, limitations of the work undertaken and recommendations for future research are outlined.

Due to the bioactive properties of GPLs, particularly with respect to their potential to support cognition discussed in Chapter 2, a number of intervention studies have explored the benefits of acute and chronic administration to cognitive performance. In some cases, the quality of these supplementation studies has been lacking with regard to the study design and/or the analytical procedure. Importantly, the work undertaken for this thesis aimed to address the limited existing empirical findings concerning GPL supplementation in children and adults with a SMC. Supplementing Lacprodan® PL-20 at both ends of the lifespan enabled the examination of its potential to facilitate cognitive performance during periods of potential vulnerability. That is, childhood is a critical time for cognitive development, as demonstrated by the continued development of the prefrontal cortex (Giedd, 2004) and corresponding cognitive functions, such as memory consolidation (Preston & Eichenbaum, 2013), working memory (Lara & Wallis, 2015) and attentional control (Rossi et al., 2009). Conversely, later life is associated with decline in cognitive function, illustrated for example, by reduced memory function (Harada et al., 2013; Nyberg, 2017; Tromp et al., 2015). During this time, the function of cellular membranes may be compromised, as a consequence of increasing age and factors such as oxidative stress (Nicolson, 2016; Nicolson & Ash, 2014, 2017). Adults with SMCs may be at a stage prior to MCI in the eventual development of AD (Reisberg & Gauthier, 2008; Saykin et al., 2006). Supplementing adults with a SMC but no objective cognitive impairment possibly confers the greatest likelihood of detecting an effect if the particular nutritional intervention can confer a benefit compared to adults with cognitive impairment, such as MCI (Miquel et al., 2018).

6.1 Key findings

The systematic research review (Chapter 3) identified ten intervention studies, which met the inclusion criteria. Seven of these studies supplemented GPLs, whilst the remaining three administered a composite supplement, Lacprodan® PL-20, that was used in the two empirical studies reported in this thesis. In brief, the review identified a benefit of PS, PS-DHA/EPA and Lacprodan® PL-20 supplementation, which was mainly evident for the enhancement of memory

performance. Moreover, the review highlighted the absence of empirical research which examined the effect of GPLs on cognitive performance in children or adolescents. The quality of the studies reviewed varied. Controlling for covariates known to correlate with cognitive performance (age, IQ, gender) and baseline performance to provide a more precise estimate of treatment effect(s) was rarely performed. In three studies exploring the extent to which PLs buffer the effects of acute stress on cognitive performance (Hellhammer et al., 2010; Parker et al., 2011; Schubert et al., 2011), there was no measurement of cognitive performance taken prior to the intervention (so an absence of a true baseline). Across the studies that reported the effects of chronic supplement administration (≥ 2 week), only the minority reported monitoring supplement intake (3/8; Boyle et al., 2019; Cheatham et al., 2012; Vakhapova et al., 2010). Other methodological issues include evidence of practice effects in at least one study (Baumeister et al., 2008), small sample sizes (n=8-16) (Baumeister et al., 2008; Harris et al., 1983; Rosadini et al., 1990), and none of the included studies provided justification for the cognitive measures employed in terms of their sensitivity to identify nutrient-induced changes in cognitive function.

The aim of Study 1 (n=70; Chapter 4) was to address the absence of empirical research exploring the potential of GPLs to effect cognitive performance in school-aged children using a suitable randomised controlled design and carefully selected measures of cognitive function. This study aimed to examine the relationship between Lacprodan® PL-20 supplementation for 6 weeks and performance on six cognitive measures assessing four cognitive domains as well as subjective evaluations of appetite, mood, motivation and mental alertness. The impact of Lacprodan® PL-20 supplementation on cognitive performance was limited to a trend towards those in the active condition correctly responding to marginally more trials and reaching a higher span on a measure of working memory (Spatial Span). Performance on cognitive measures was found to vary as a function of baseline performance, which often interacted with condition and week. Performance at baseline was positively correlated with later performance at midpoint (week 3) and endpoint (week 12) on all but two of the cognitive measures irrespective of condition. Participants' demographic characteristics including age, IQ and gender were also found to be associated with performance on the cognitive outcomes. Better performance was demonstrated by older participants. Having a higher IQ was also related to improved performance on most outcomes, however, on a measure sensitive to visuospatial memory, those with a higher IQ were generally slower. Females showed superior accuracy when their motor skills were assessed and superior performance on a measure of verbal memory. The advantage demonstrated by females on the verbal memory measure is unsurprising given

females tended to score more highly compared to males on the WASI vocabulary subtest (Wechsler, 1999) at baseline (reviewed in section 4.7.1). However, males exhibited faster reaction and movement time relative to females. Higher order interactions were also found that varied by age, IQ and gender; nonetheless, there was no consistent relationship with Lacprodan® PL-20 supplementation. Baseline subjective ratings were also positively associated with subsequent self-ratings. Moreover, there was a trend towards participants in the placebo condition rather than those in the active condition to rate themselves as more cheerful. For ease of distraction, of those who perceived themselves as less easily distracted at baseline, participants in the placebo condition reported being significantly less easily distracted relative to those in the active condition. Whilst at lower-to-mid and higher baseline subjective ratings, those in the placebo condition perceived themselves to be significantly higher on a scale of bad temperedness compared to participants in the active condition at midpoint. Nevertheless, at the same baseline subjective ratings, those in the placebo condition also perceived themselves as significantly more bad tempered at midpoint vs. endpoint, whilst there was a trend towards participants in the active condition reporting feeling less bad tempered at midpoint relative to endpoint. There was a trend for perceived frustration to vary by condition and week, such that those in the placebo condition appeared to feel increasingly frustrated over time, whereas those in the active condition became less frustrated from midpoint to endpoint.

Study 2 (n=50) built on the existing empirical literature and examined the effect of GPL plus SM supplementation in middle-aged and older adults with SMCs. In addition, this study also sought to add to the limited knowledge of PL effects on cognitive performance following acute administration. Specifically, Study 2 aimed to explore whether consumption of Lacprodan® PL-20 after a single dose (acute) and chronically would lead to gains in cognitive performance measures of memory and executive function assessed at after 6 (midpoint) and 12 weeks (endpoint) of administration. Performance gains shown by those in the active condition following both acute and chronic intake of Lacprodan® PL-20 were again minimal. In the absence of a corresponding baseline*condition or baseline*condition*week interaction(s), performance gains included significantly more hits on the Rapid Visual Information Processing Task (acute) and greater target accuracy (trend) and total accuracy (significant) on the N-back Task (chronic) were observed. However, as participants in the active condition were marginally significantly slower when responding to targets on the N-back Task (chronic), the accuracy performance advantage may indicate a speed-accuracy trade-off. Similarly, there was a trend towards those in the active condition demonstrating slower reaction time for hits on the Rapid Visual Information Processing Task (acute), but a corresponding baseline*condition interaction

indicated that this was only at slower baseline performance. Cognitive performance differences between conditions were found to vary depending on baseline performance and to a lesser extent, performance at baseline and week, both following acute (no baseline*condition*week interactions were requested for the acute analysis, see section 5.7) and chronic supplementation. Importantly, it was typically found that any benefit of Lacprodan® PL-20 was mainly demonstrated by participants with higher levels of performance at baseline. Following both acute and chronic administration of Lacprodan® PL-20, baseline performance was a significant predictor of later performance for the majority of cognitive outcomes. Age, IQ and gender were also related to performance. Following both acute and chronic supplementation, where age was a significant covariate, performance showed age-related decline except on the Attention Switching Task, such that switch cost accuracy improved with age (following acute administration only). However, this correlation was weak and there was substantial variation in performance. This along with the fact that it was not observed following chronic supplementation suggests the relationship should not be accepted at face value. A higher IQ significantly predicted greater performance except for reaction time outcomes on the N-back Task (acute and chronic) and Rapid Visual Information Processing Task (chronic), such that those with lower IQ scores showed faster response times. This may represent a speed accuracy trade-off and/or more cautious decision making (executive function). In addition, those with a lower IQ score also showed marginally significant greater switch cost accuracy (chronic), although again, this was only a weak correlation. Gender was also a significant covariate, with males and females demonstrating superior performance across several measures, both for accuracy and response latencies (acute and chronic). A consistent finding (acute and chronic) includes females demonstrating faster reaction time for hits on the Rapid Visual Information Processing Task. Of the studies reviewed in Chapter 3 that recruited a similar sample to the present study, Vakhapova et al. (2010) entered participant demographics in their initial model, however, these were subsequently removed, as they were found not to be significant. The benefit of Lacprodan® PL-20 consumption on frequency of cognitive slips was also examined across the 12 weeks. Those in the active condition who reported experiencing greater cognitive failures at baseline reported a reduction in the extent of these over time. Importantly, this reported moderation was in the absence of any consistent improvement shown on the cognitive measures. This reflects the notion that SMCs may not correspond with actual performance on cognitive tests, suggesting that those who present with a SMC may reflect a proportion of the population sometimes referred to as the “worried well”. These are often high functioning individuals accustomed to relying on their cognitive capacity who notice very quickly when this

seems to lapse, even though their day to day functioning is ostensibly unimpaired. Conversely, however, it has been reported that SMCs may represent subtle changes in resting-state functional connectivity that are not detected by neuropsychological tests (Kawagoe, Onoda, & Yamaguchi, 2019). There was no difference in the subjective evaluations on the CFQ at baseline between conditions. As this finding applies only to those who reported greater cognitive failures at baseline, it may be that they are particularly concerned about their memory function/observant of such slips and were invested in the study, the combination of which led to a placebo effect.

Both Study 1 and Study 2 were underpowered (power of <0.8) due to an insufficient number of participants (see sections 4.8.2.1 and 5.9.2.1. for *a posteriori* estimations). For Study 1, it was calculated that 58 participants were required per treatment arm, whilst with respect to Study 2, it was estimated that 27 participants were required per treatment arm. Given the observed difference in LS-means and the observed standard deviation, in order to achieve a power of 0.8, a sample size of 25,118, 6281 and 1571 would be required at baseline, midpoint and endpoint, respectively, for Study 1, and a sample size of 61 and 1,445,954 would be required in respect of the acute and chronic interventions, respectively.

The use of multiple cognitive measures sensitive to a wide range of cognitive functions afforded a comprehensive assessment of cognitive performance in both studies. However, it is acknowledged that including multiple cognitive tests with multiple outcomes increases the risk of false positives i.e. Type 1 error rate, and while post hoc tests control for this, it is likely that some of the effects reported are type 1 errors.

6.1.1 Influence of participant baseline characteristics on cognitive performance

Taken together, the findings of both studies indicate that participants' demographic characteristics and baseline performance on the cognitive measures had a greater impact on cognitive performance than the active supplement. As expected, in Study 1, better performance was demonstrated by older children. This is consistent with the literature on cognitive development during childhood that indicates that childhood is characterised as a period of brain maturation, this being associated with increasing sophistication of cognitive function (see section 4.2). Conversely, cognitive performance reduced with increasing age in the adults who participated in Study 2 for all but one outcome, such that age was a significant covariate. These findings reflect empirical work that has identified age-related decrements in cognitive function (see sections 5.1 and 5.2). In both studies, IQ was also a significant predictor of performance of

participants. This is a common finding in the assessment of cognitive function, that is, that IQ accounts for a high proportion of the variance (Mohn et al., 2014). Furthermore, the positive relationship between IQ and performance (with the exception of a few outcomes) is in accordance with the observation that individuals with higher IQ scores typically perform better on measures assessing mental abilities (Checa & Fernández-Berrocal, 2015; Haier, 2014). With regard to gender, the performance advantage demonstrated by males in Study 1 aligns with previous research suggesting gender differences in visual processing emerge from 4-7 years of age, with males being beneficiaries (Palejwala & Fine, 2015). The superior verbal memory performance demonstrated by females in Study 1 has previously been discussed in relation to baseline performance on the WASI vocabulary subtest (Wechsler, 1999). In Study 2, there were three trends following the acute analysis towards females outperforming males on measures of executive function. Of particular interest concerns reaction time for targets and target accuracy on the N-back Task. However, given males were significantly faster at identifying nontargets compared to females on the same task, the trends towards superior performance, particularly towards reaction time for targets, as shown by females should be considered within the wider context. The common finding that cognitive performance by condition varied as a function of performance at baseline across both studies is especially important. As reported in Chapter 3, of the eight studies included in the systematic review that assessed participants at baseline, only three of these controlled for baseline performance. In cases where this does not happen, estimates of main effects will be less precise (O' Connell et al., 2017), meaning researchers may misinterpret their findings. All in all, it is critical that future intervention studies (and research more widely) controls for possible confounding or causally prior variables in the analysis of their data.

6.2 Recommendations for the composition of GPL species and corresponding fatty acid profiles to affect cognitive performance in future research

6.2.1 Composition of the active supplement used in both studies in this thesis

As described in section 4.5.3.1, Lacprodan® PL-20 is a bovine milk-derived PL-enriched protein concentrate (Sokol, Ulven, Færgeman, & Ejsing, 2015). Shotgun lipidomics analysis using MS/MS^{ALL} of Lacprodan® PL-20 identified 6 lipid classes along with their FA composition (Sokol et al., 2015). Polyunsaturated FAs including AA and DPA present in Lacprodan® PL-20 were mainly identified as PE, PS and PI species and the molar abundance was identified as <5 mol%

across all lipid classes evaluated (Sokol et al., 2015). A breakdown of the PL constituents of Lacprodan® PL-20 and their relative FAs attached is listed in Table 6.1.

Table 6.1 Composition of molecular PL species in Lacprodan® PL-20 (Sokol et al., 2015)

Lipid classes	Molar abundance (mol%)	Molecular FA composite (most abundant species)[a]
PE	35	PE 18:1[b] /18:1[c] (34 mol%)
PC	18	PC 16:0[d] /18:1[c] (23 mol%)
PS	9	PS 18:0[e] /18:1[c] (38 mol%)
PI	5	PI 18:0 /18:1[c] (39 mol%)
Sphingomyelin	8	-

Notes. [a]Triglycerides tended to have saturated and short-chain FA moieties determined by MS/MS^{ALL} analysis; [b]Oleic acid; [c]*sn*-1 location / *sn*-2 location; [d]Palmitic acid; [e]Stearic acid.

One suggestion for the limited efficacy of Lacprodan® PL-20 in promoting improvements in cognitive performance relates to its lipid composition. Importantly, Lacprodan® PL-20 is intended for infant formula. Infant formula tends to mimic the nutritional composition of breast milk as far as possible (Martin, Ling, & Blackburn, 2016). Specifically, the composition of mature breast milk is used as a guide (Wiedeman et al., 2018). Notably, in mature breast milk, approximately half of the FAs are saturated FAs, with palmitic acid (16:0) contributing a large proportion of this (Delplanque, Gibson, Koletzko, Lapillonne, & Strandvik, 2015; Koletzko, 2017). Stearic acid (18:0) also features as a major saturated FA, whereas oleic acid (18:1) is the predominant monounsaturated FA (Bravi et al., 2016). It is therefore apparent that Lacprodan® PL-20 is well matched to mature breast milk in respect of its FA profile. The similarity between Lacprodan® PL-20 and breast milk is further supported by work that has quantified PL classes present in human milk. That is, the molecular abundance of GPLs in Lacprodan® PL-20 is in accordance with the GPL concentrations identified in human milk (Giuffrida et al., 2013).

6.2.2 Suitability of the active supplement for use in children and adults for cognitive performance enhancement

Lacprodan® PL-20 may be a suitable alternative to breast milk to support the cognitive development of infants. Evidence in support of this comes from a study that supplemented neonatal piglets with either 0%, 0.8% or 2.5% Lacprodan® PL-20 from postnatal day 2 – 28. Those supplemented Lacprodan® PL-20 demonstrated greater accuracy and faster reaction time on a spatial T-maze task. Moreover, changes in phosphatidylcholine-related metabolites in the hippocampus were found to reflect diet (Liu et al., 2014). Nonetheless, this does not mean that

this same GPL and FA profile is likely to confer benefits for brain or cognitive development or enhance cognitive function in children or prevent cognitive decline/improve cognitive function in middle-aged and older adults. For instance, palmitic acid is required by infants to promote growth and meet metabolic energy needs (Innis, 2016). However, in adults with chronic disease, palmitic acid has received much attention due to the reports of adverse health effects (Innis, 2016). Essentially, palmitic acid is the most abundant saturated FA in the body and can be obtained from diet where it is present in high quantities in meats and dairy products (Schommer, Marwarha, Nagamoto-Combs, & Ghribi, 2018), however, palmitic acid can also be synthesised de novo (Guest, Garg, Bilgin, & Grant, 2013). Importantly, as palmitic acid can be synthesised endogenously, the regulated tissue content of palmitic acid is not sensitive to alterations in intake (Carta, Murru, Banni, & Manca, 2017). Disruption of the homeostatic balance of palmitic acid associated with uncontrolled de novo synthesis is implicated in and activated by physiopathological conditions including neurodegenerative disease and chronic nutritional imbalance (Carta et al., 2017). The consequences of this include systemic inflammatory response and metabolic dysregulation leading to insulin resistance and dysregulation in fat deposition. Specifically, palmitic acid has been observed to induce TLR4 receptor activation in hypothalamic microglia and stimulate the release of cytokines, leading to inflammation and neuronal stress (Valdearcos et al., 2014). Consistent with this, in a recent review, palmitic acid was implicated in inflammatory responses, since it is a toll-like receptor agonist, whilst it's metabolic products lead to increased ROS generation, which in turn strengthens TLR4-induced signalling (Korbecki & Bajdak-Rusinek, 2019). Moreover, both palmitic acid and stearic acid have been observed to impair glucose metabolism and mitochondrial function (Hirabara, Curi, & Maechler, 2010). In Parkinson's disease, both saturated FAs have been found to be pathologically augmented in lipid rafts (Mesa-Herrera, Taoro-González, Valdés-Baizabal, Diaz, & Marín, 2019). Oleic acid, on the other hand, has received attention for its neuroprotective effects (Mazza et al., 2018) in the context of the Mediterranean diet (MedDi), where it contributes to the monounsaturated FA content in the form of extra-virgin olive oil (Trichopoulou, 2007). Higher adherence to the MedDi has been reviewed favourably in respect of cognition in that it has been associated with a reduced risk of cognitive impairment (Psaltopoulou et al., 2013; Singh et al., 2014; Wu & Sun, 2017), better cognitive function and lower rates of cognitive decline (Lourida et al., 2013). However, oleic acid is one of a number of bioactive constituents of the diet (Omar, 2019), whilst polyphenolic compounds (Hornedo-Ortega et al., 2018), PUFAs, antioxidants, vitamins and minerals (Knight, Bryan, & Murphy, 2016) have also been cited.

6.2.3 GPL and FA composition recommendations for future supplementation studies

In view of the above and in consideration of the points raised previously in sections 2.6, 2.7.6.2 and 4.3, a better FA profile to be supplemented would include FAs for which there is sensitivity with respect to dietary intake and which show promise in terms of potential effects on cognitive function. Suitable candidates include DHA and EPA. It is acknowledged that to date, empirical work that has supplemented either or both long-chain polyunsaturated fatty acids (LC-PUFA) in combination have not revealed overwhelmingly favourable findings in terms of enhancing or maintaining cognitive function in children or in adults. This was the case for the 9 RCTs reviewed in Chapter 4 (section 4.3.1) that supplemented school-aged children. Elsewhere, conflicting findings have been reported as to the benefits of DHA supplementation on cognitive function across the lifespan (Derbyshire, 2018; Ghasemi Fard et al., 2019). However, there is plenty of evidence to suggest that DHA and EPA are involved in various processes that support cognitive function. In addition to those discussed previously (see sections 2.7.6.2 and 4.3), DHA as well as EPA are known to slow the degradation of neural tissue (Bazan, 2007; Calon et al., 2004) and improve the endothelial function (Wang et al., 2012), thereby potentially impacting cerebral blood flow (CBF) (O'Donovan et al., 2019). Notably, CBF has been classed as a sensitive physiological marker of cerebrovascular function and has been suggested to be one of the mechanisms by which polyphenols contribute to improvements in cognitive functioning (Joris, Mensink, Adam, & Liu, 2018). This mechanism could also apply to DHA and/or EPA. DHA and EPA also reduce morbidity and mortality from cardiovascular disease (Massaro, Scoditti, Carluccio, & De Caterina, 2008), which in turn reduces the risk of subsequent neurodegeneration (Ng, Turek, & Hakim, 2013). The inconsistent findings reported following omega-3 PUFA supplementation might be accounted for by the variations in basal levels (Derbyshire, 2018; Ghasemi Fard et al., 2019; Ostermann & Schebb, 2017), where the larger the distance between basal PUFA status and the "change threshold", the greater the effect of supplementation (Keenan et al., 2012). In addition, other factors that contribute to variation in findings include issues in study design and analysis, inadequate intervention duration, inappropriate dosage of treatments, age or gender bias within conditions, and inadequate power due to small samples (Ghasemifard, Turchini, & Sinclair, 2014).

In respect of the GPL molar abundance, it could be argued that it would be advantageous to increase the relative abundance of both PI and PS. As discussed earlier in this thesis (see section 2.7), a derivative of PI, $PI(3,4,5)P_3$, supports the uptake of glucose, LTP and LTD, whereas

$PI(4,5)P_2$ also supports LTP, is involved in neuronal function via its regulation of voltage-gated potassium and calcium channels and preparation of synaptic vesicles, and is implicated in PKC activation. To date, the use of PI as a single supplement has not been explored. With regard to PS, one of the conclusions of the systematic research review (Chapter 3) was that PS may offer the potential to facilitate cognitive performance. Of the five studies that supplemented PS (Baumeister et al., 2008; Crook et al., 1991; Parker et al., 2011; Rosadini et al., 1990; Vakhapova et al., 2010), two of these did not report positive treatment effects, one acute (Rosadini et al., 1990) and one chronic (Baumeister et al., 2008). It was proposed that that this lack of a benefit was due to the supplement dose being too low (Rosadini et al., 1990), practice effects (Baumeister et al., 2008) and inadequate sample size leading to a lack of power to detect an effect (Baumeister et al., 2008; Rosadini et al., 1990). In brief, PS may contribute to improvements in cognitive performance due to its role in PKC activation, therefore having an indirect role in synaptic remodelling and neurotrophic activity in the hippocampus (see section 2.7.3). Furthermore, PS in the brain is enriched with DHA, sufficient quantities of which aid neuronal communication (see section 3.5.1).

In keeping with previous GPL studies which have reported cognitive performance enhancements following GPL administration, one of which supplemented PS with DHA and EPA (Vakhapova et al., 2010), the other PS sourced from bovine cortex (high DHA content) (Crook et al., 1991), esterification of PS and PI to DHA and EPA respectively may further make both GPLs ideal candidates for augmenting cognitive performance. After all, in the brain, PS is enriched with DHA while PI is enriched with EPA (Chen et al., 2009). This remains speculative, however, since no studies have examined this combination.

6.3 Limitations of the studies presented in this thesis

6.3.1 Study placebo product

A placebo group offers the opportunity to include a condition in which there is maximal treatment separation from the experimental condition (Castro, 2007). However, this is dependent upon the placebo being a pharmacologically inert substance (Gupta & Verma, 2013). The placebo in Study 2 contained maltodextrin as an ingredient to ensure comparable viscosity between treatment products and therefore minimise the risk of unblinding the research team and participants. Maltodextrins comprise a class of carbohydrates and are applied in a range of foods (Hofman, Buul, & Brouns, 2016). A recent study reported feeding piglets a formula

containing either digestible maltodextrin or lactose as the main carbohydrate source (28% total nutrient composition) from 1 – 9 weeks of age. The piglets fed the maltodextrin-based formula demonstrated improved long-term spatial memory compared to the piglets fed the lactose-based formula at 12 – 17 weeks of age, 3 – 6 weeks post-treatment termination (Clouard, Le Bourgot, Respondek, Bolhuis, & Gerrits, 2018). Moreover, a 6.4% maltodextrin solution used every 10 minutes during 65 minutes of moderately high-intensity exercise as a mouth-rinse solution was found to attenuate exercise-induced decline in performance on the Stroop test, a measure of executive function, in young adults. It was suggested that this result was a function of the maltodextrin mouth-rinse inhibiting excessive release of the stress hormones, norepinephrine and epinephrine (Konishi et al., 2017). Indeed, executive function performance can be disrupted by high levels of norepinephrine release (Clark & Noudoost, 2014). Conversely, ad libitum access for 17 days to a maltodextrin solution (10.4% w/v maltodextrin powder vs. 10% w/v sucrose) was observed to produce impaired performance on a location recognition task in rats following of access to maltodextrin (Kendig, Lin, Beilharz, Rooney, & Boakes, 2014). Taken together, the findings from these studies suggest that maltodextrin is a nutritionally active ingredient that can affect cognitive function. By implication, the placebo product supplemented in the present study may have unintentionally influenced the cognitive performance of those in the placebo condition and confounded the study findings. This reflects the wider issue concerning the selection of an appropriate placebo in food and nutrition research, and highlights that the lack of nutritionally inert food components mean that ingredients which could affect a response may be included in placebo foods that therefore undermine the experimental comparison (Harris & Johns, 2011).

6.3.2 Compliance

Supplement consumption was directly monitored in Study 1 (Chapter 4) enabling accurate measurement of intake. In Study 2 (Chapter 5), supplements were consumed by participants in their homes for convenience and compliance was checked by use of a consumption diary and the collection of empty/part empty supplement sachets. Hence, records concerning participant consumption used in Study 2 may not be a true representation of supplement intake. Biological measures offer another option for monitoring compliance. Two studies reviewed in the systematic research review (Chapter 3) that supplemented PC evaluated supplement intake by determining blood choline concentrations, which was found to be raised following supplement consumption (Cheatham et al., 2012; Harris et al., 1983). It is acknowledged that had a biological measure been used in Study 2 instead of the diary system, it is probable that this would have

resulted in a different conclusion regarding participant compliance and as consequence, which participants would have been eligible for inclusion in the PP analysis. However, the practicalities of this type of compliance monitoring are considerable and can result in greater participant attrition or lower rates of recruitment in the first place.

6.3.3 Serial cognitive testing and order effects

The order of administration of each of the cognitive measures in both studies reported in this thesis remained the same on each test occasion. Both studies employed a verbal memory measure comprised of immediate and delayed recall components, with a fixed time frame between each. By following the same order of administration, the period between each component could be consistent. This in addition to following a repeated measures design in both studies inevitably increased the possibility of observing performance gains due to familiarity with the experimental procedure, the research staff and the cognitive measures employed. To reduce the potential for practice effects to interfere with any true effect(s) of treatment, test practice versions were administered in both studies prior to baseline (familiarisation). It has been proposed that practice effects are strongest between the first two study visits (Bell et al., 2018). Therefore, including a familiarisation stage aimed to reduce the degree to which such effects confounded study findings. In a further effort to minimise their possibility, cognitive measures were counterbalanced on each test occasion. Despite these efforts, there was evidence of an effect of practice. For example, in Study 2, this was most evident on measures of executive function (both accuracy and reaction time).

6.3.4 Optimal duration of intervention

The ideal period for supplementation with GPLs leading to membrane lipid replacement in the brain and the potential subsequent promotion of cognitive function is currently unknown. Based upon the plasma half-life of unesterified DHA, Section 4.8.2.3 suggested that GPL supplementation should take place over a period of months. As with PS-DHA, there is a lack of knowledge concerning how long it takes for GPLs to reach the brain and in what quantities they are taken up. A further complication concerns exogenous PLs being unprotected from oxidation and degradation during digestion, mainly in the small intestine (Nicolson & Ash, 2017). As a consequence, it is likely that there will be a loss in the availability of undamaged PLs for uptake, which further adds to the length of intervention required. The use of fructooligosaccharides and antioxidants can be used to protect GPLs from phospholipases, acidity and bile salts (Hendry, 1993; Vereyken, Chupin, Demel, Smeekens, & De Kruijff, 2001), thus promoting PL integrity.

6.4 Conclusion

Overall, the findings from the PL intervention studies presented in this thesis add to the existing heterogeneous evidence of the potential for dietary PLs to moderate cognitive performance. Effects were not greater or more consistent in children as opposed to adults with a SMC, despite the use of different cognitive test batteries, each of which had demonstrated sensitivity to the effects of nutritional interventions in these samples previously. Differences in the types of PLs administered in these and previous studies and the specific measures of cognitive function employed may underpin some of the heterogeneity in the results seen. The overriding influence of baseline as a predictor of subsequent performance, which accounted for a large proportion of the variance across many of the outcome measures in the studies reported in this thesis is an important factor in the failure to demonstrate any effect of the GPL intervention. Controlling statistically for divergent baseline performance revealed no significant, consequential effects of PL intake. The importance of a potentially active placebo in Study 2 cannot be underestimated.

Similarly, the relative cognitive capacity of the adults sampled in whom only a subjective memory complaint was reported may have meant that the sample was not likely to respond to the PL intervention. However, where effects were shown, these were in those adults in the normal range of IQ (within 1 SD of the population mean). The potential protective effects of GPL (Chapter 2) intake may be limited to individuals who are perhaps more likely to be compromised cognitively than the relatively high functioning older adults recruited to the study presented in this thesis. More generally, observed modulatory effects of dietary interventions on cognitive performance are often small and inconsistently reported (Mcdaniel, Maier, & Einstein, 2003). The sensitivity of such manipulations, if they are bioactive, may only be demonstrated in sub-groups with specific nutritional or cognitive vulnerabilities yet sub-group analysis has been criticised as fishing (Pocock et al., 2002).

Equally, the possibility that PLs in the form/composition of Lacprodan® PL-20 have no effect on cognitive function is a plausible hypothesis, which is supported by the results of the studies presented in this thesis. The hypothesis that PL intake may have potential protective effects in other groups of participants remains to be tested. There are, for example, suggestive data for effects of cognitive function under conditions of stress (Boyle et al., 2019; Hellhammer et al., 2010; Parker et al., 2011; Schubert et al., 2011), although there are a number of limitations with these findings. Moreover, there is strong physiological mechanistic data (explored in Chapter 2), which suggests that certain GPLs and FAs in other combinations or concentrations could

confer beneficial or protective effects on cognition in the developing child or the ageing adult. Future research could profitably explore the effects of these on sensitive measures of cognitive function in vulnerable samples of individuals.

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Appendix 1. Nomenclature, chain length and degree of saturation of fatty acids referred to throughout the thesis

Fatty acid	Type	Chain length (number of carbon atoms)	Degree of saturation (number of double bonds)
Alpha-linolenic acid (ALA)	Polyunsaturated (omega-3)	18	3
Arachidonic acid (AA)	Polyunsaturated (omega-6)	20	4
Docosahexaenoic acid (DHA)	Polyunsaturated (omega-3)	22	6
Docosapentaenoic acid (DPA)	Polyunsaturated (omega-3)	22	5
Eicosapentaenoic acid (EPA)	Polyunsaturated (omega-3)	20	5
Lignoceric acid	Saturated	24	0
Linoleic acid (LA)	Polyunsaturated (omega-6)	18	2
Nervonic acid	Monounsaturated (omega-9)	24	1
Oleic acid	Monounsaturated (omega-9)	18	1
Palmitic acid	Saturated	16	0
Stearic acid	Saturated	18	0

Appendix 2. Cognitive search terms, limits and tags used in the systematic research review

CINAHL	
Search terms	**Search strategy**
Cognition	Keyword search
Cognition	MeSH search
Memory	Keyword search
Memory / false memory / memory, short term	MeSH search (memory as main term plus narrower terms)
Attention	Keyword search
Attention	MeSH search
Visual-spatial	Keyword search
Visualspatial	Keyword search
Visuo-spatial	Keyword search
Visuospatial	Keyword search
Spatial perception	MeSH search
Recall	Keyword search
Recall	Comes under memory
Recognition	Keyword search
Recognition (psychology)	MeSH search
Problem solving	Keyword search
Problem solving	MeSH search
Reaction time	Keyword search
Reaction time	MeSH search
Response time	Keyword search
Response time	Comes under reaction time
Vigilance	Keyword search
Arousal	MeSH search
Executive function	Keyword search
Executive function	MeSH search
Psychomotor	Keyword search
Psychomotor performance/ motor skill	MeSH search (psychomotor performance as main term plus broader term)
Sphingolipid	Keyword search
Acidic glycosphingolipid	Keyword search
Ganglioside	Keyword search
Phosphosphingolipid	Keyword search
Sphingomyelin	Keyword search
Glycerophospholipid	Keyword search
Phosphatidylethanolamine	Keyword search
Phosphatidylcholine	Keyword search
Phosphatidylcholines	MeSH search
Phosphatidylserine	Keyword search
Phosphatidylinositol	Keyword search

Embase Classic + Embase 1947		
Search terms	**Search strategy**	**MeSH synonyms**
Cogniti$	Multiple search (.mp)	
Cognition	MeSH search	*Cognitive accessibility* *Cognitive balance* *Cognitive dissonance* *Cognitive function* *Cognitive structure* *Cognitive symptoms* *Cognitive task* *Cognitive thinking* *Neurobehavioural manifestations* *Volition*
Memory	Multiple search (.mp)	
Memory/ or associative memory/ or auditory memory/ or autobiographical memory/ or declarative memory/ or episodic memory/ or explicit memory/ or false memory/ or implicit memory/ or long term memory/ or memory consolidation/ or olfactory memory/ or procedural memory/ or prospective memory/ or recall/ or recognition/ or reference memory/ or repetition priming/ or retrospective memory/ or semantic memory/ or sensory memory/ or short term memory/ or spatial memory/ or tactile memory/ or verbal memory/ or visual memory/ or word list recall/ or word recognition/ or working memory/	MeSH search (memory as main term plus narrower terms)	*Item recall* *Memory function* *Nonspatial memory* *Remembering* *Reminiscence*
Attention	Multiple search (.mp)	
Attention/ or alertness/ or awareness/ or consciousness/ or distractibility/ or mental concentration/ or selective attention/	MeSH search (Attention as main term plus narrower terms)	*Attentiveness*
Visual-spatial	Multiple search (.mp)	
Visualspatial	Multiple search (.mp)	
Depth perception	MeSH search	*Depth discrimination* *Perception, depth* *Perception, space* *Perception, visuospatial* *Space perception* *Spatial perception* *Vision disparity* *Visuospatial perception*
Visuo-spatial	Multiple search (.mp)	

Visuospatial	Multiple search (.mp)	
Recall	Multiple search (.mp)	
Recall	MeSH search	*Free recall* *Mental recall* *Recall phenomenon*
Recognition	Multiple search (.mp)	
Recognition	MeSH search	*Form recognition* *Recognition (psychology)*
Problem solving	Multiple search (.mp)	
Problem solving	MeSH search	
Reaction time	Multiple search (.mp)	
Reaction time	MeSH search	*Time reactivity* *Time, reaction*
Response time	Multiple search (.mp)	
Response time	MeSH search	*Choice reaction time* *Reaction time, choice*
Vigilance	Multiple search (.mp)	
Alertness	MeSH search	*Mental alertness* *Psychical alertness* *Task, vigilance* *Vigilance* *Vigilance task*
Executive function	Multiple search (.mp)	
Executive function	MeSH search	*Cognitive control* *Executive control*
Psychomotor	Multiple search (.mp)	
Psychomotor performance/ or psychomotor activity	MeSH search (psychomotor performance as main term plus broader term)	*Psychomotor skill* *Psychomotor task* *Psychic activity* *Psychomotor function*
Sphingolipid	Multiple search (.mp)	
Sphingolipids	Multiple search (.mp)	
Sphingolipid	MeSH search	
Acidic glycosphingolipid	Multiple search (.mp)	
Acidic glycosphingolipids	Multiple search (.mp)	
Glycosphingolipid	MeSH search	
Ganglioside	Multiple search (.mp)	
Gangliosides	Multiple search (.mp)	
Ganglioside	MeSH search	
Phosphosphingolipid	Multiple search (.mp)	
Phosphosphingolipids	Multiple search (.mp)	
Sphingomyelin	Multiple search (.mp)	
Sphingomyelins	Multiple search (.mp)	
Sphingomyelin	MeSH search	
Glycerophospholipid	Multiple search (.mp)	
Glycerophospholipids	Multiple search (.mp)	
Glycerophospholipid	MeSH search	

Phosphatidylethanolamine	Multiple search (.mp)	
Phosphatidylethanolamines	Multiple search (.mp)	
Phosphatidylethanolamine	MeSH search	
Phosphatidylcholine	Multiple search (.mp)	
Phosphatidylcholines	Multiple search (.mp)	
Phosphatidylcholine	MeSH search	
Phosphatidylserine	Multiple search (.mp)	
Phosphatidylserines	Multiple search (.mp)	
Phosphatidylserine	MeSH search	
Phosphatidylinositol	Multiple search (.mp)	
Phosphatidylinositols	Multiple search (.mp)	
Phosphatidylinositol	MeSH search	

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Search terms	**Search strategy**
Cogniti*	Multiple search (.mp)
Cognition	MeSH search
Memory	Multiple search (.mp)
Memory/ or deja vu/ or memory, episodic/ or memory, long-term/ or memory consolidation/ or memory, short-term/ or mental recall/ or "recognition (psychology)"/ or repetition priming/ or "retention (psychology)"/ or spatial memory/	MeSH search (memory as main term plus narrower terms)
Attention	Multiple search (.mp)
Attention	MeSH search
Visualspatial	Multiple search (.mp)
Visual spatial	Multiple search (.mp)
Space perception	MeSH search
Visuospatial	Multiple search (.mp)
Visuo spatial	Multiple search (.mp)
Recall	Multiple search (.mp)
Recognition	Multiple search (.mp)
Problem solving	Multiple search (.mp)
Problem solving/ or heuristics/	MeSH search (problem solving as main term plus narrower term)
Reaction time	Multiple search (.mp)
Reaction time	MeSH search
Response time	Multiple search (.mp)
Vigilance	Multiple search (.mp)
Arousal	MeSH search
Executive function	Multiple search (.mp)
Executive function	MeSH search
Psychomotor	Multiple search (.mp)
Psychomotor performance/ or motor skills/	MeSH search

	(psychomotor performance as main term plus narrower term)
Sphingolipid	Multiple search (.mp)
Sphingolipids	Multiple search (.mp)
Sphingolipids	MeSH search
Acidic glycosphingolipid	Multiple search (.mp)
Acidic glycosphingolipids	Multiple search (.mp)
Acidic glycosphingolipids	MeSH search
Ganglioside	Multiple search (.mp)
Gangliosides	Multiple search (.mp)
Gangliosides	MeSH search
Phosphosphingolipid	Multiple search (.mp)
Phosphosphingolipids	Multiple search (.mp)
Sphingomyelin	Multiple search (.mp)
Sphingomyelins	Multiple search (.mp)
Sphingomyelins	MeSH search
Glycerophospholipid	Multiple search (.mp)
Glycerophospholipids	Multiple search (.mp)
Glycerophospholipids	MeSH search
Phosphatidylethanolamine	Multiple search (.mp)
Phosphatidylethanolamines	Multiple search (.mp)
Phosphatidylethanolamines	MeSH search
Phosphatidylcholine	Multiple search (.mp)
Phosphatidylcholines	Multiple search (.mp)
Phosphatidylcholines	MeSH search
Phosphatidylserine	Multiple search (.mp)
Phosphatidylserines	Multiple search (.mp)
Phosphatidylserines	MeSH search
Phosphatidylinositol	Multiple search (.mp)
Phosphatidylinositols	Multiple search (.mp)
Phosphatidylinositols	MeSH search

PubMed	
Search terms	**Search strategy**
Cogniti*	Title/Abstract (TIAB)
Cognition	MeSH:noexp
Memory	Title/Abstract (TIAB)
Memory	MeSH search
Attention	Title/Abstract (TIAB)
Attention	MeSH search
Visual-spatial	Title/Abstract (TIAB)
Visualspatial	Title/Abstract (TIAB)
Visuo-spatial	Title/Abstract (TIAB)
Visuospatial	Title/Abstract (TIAB)
Spatial Processing	MeSH search
Recall	Title/Abstract (TIAB)
Mental Recall	MeSH search
Recognition	Title/Abstract (TIAB)
Problem solving	Title/Abstract (TIAB)
Problem solving	MeSH search
Reaction time	Title/Abstract (TIAB)
Reaction time	MeSH search
Response time	Title/Abstract (TIAB)
Vigilance	Title/Abstract (TIAB)
Arousal	MeSH search
Executive function	Title/Abstract (TIAB)
Executive function	MeSH search
Psychomotor	Title/Abstract (TIAB)
Psychomotor Performance	MeSH search
Sphingolipid	Title/Abstract (TIAB)
Sphingolipids	Title/Abstract (TIAB)
Sphingolipids	MeSH:noexp
Acidic glycosphingolipid	Title/Abstract (TIAB)
Acidic glycosphingolipids	Title/Abstract (TIAB)
Acidic glycosphingolipids	MeSH:noexp
Ganglioside	Title/Abstract (TIAB)
Gangliosides	Title/Abstract (TIAB)
Gangliosides	MeSH:noexp
Phosphosphingolipid	Title/Abstract (TIAB)
Phosphosphingolipids	Title/Abstract (TIAB)
Sphingomyelin	Title/Abstract (TIAB)
Sphingomyelins	Title/Abstract (TIAB)
Sphingomyelins	MeSH search
Glycerophospholipid	Title/Abstract (TIAB)
Glycerophospholipids	Title/Abstract (TIAB)
Glycerophospholipids	MeSH:noexp
Phosphatidylethanolamine	Title/Abstract (TIAB)
Phosphatidylethanolamines	Title/Abstract (TIAB)

Phosphatidylethanolamines	MeSH search
Phosphatidylcholine	Title/Abstract (TIAB)
Phosphatidylcholines	Title/Abstract (TIAB)
Phosphatidylcholines	MeSH:noexp
Phosphatidylserine	Title/Abstract (TIAB)
Phosphatidylserines	Title/Abstract (TIAB)
Phosphatidylserines	MeSH search
Phosphatidylinositol	Title/Abstract (TIAB)
Phosphatidylinositols	Title/Abstract (TIAB)
Phosphatidylinositols	MeSH:noexp

Appendix 3. Table 3.A Summary of studies included in the systematic review

Reference	Ingredient	Sample characteristics	Design and intervention	Cognitive measures (battery and sub tests)	Reported findings	Subgroup/Further analyses	Effect summary
Cheatham et al. (2012)	Nutrasal PhosChol supplement : 750 mg choline/d as PC	Healthy pregnant women (n=99). All mothers intended to breastfeed for ≥ 90 days. Actual: all infants were breast fed for a minimum of 45 days. Age (21 - 41 years) at conception - PhosChol condition: 30.2 ± 3.8, placebo condition: 30.8 ± 4.9. Infants:- PhosChol condition	Mothers kept a 3-day food diary immediately prior to 30-week gestation & 45-day postpartum visits - recorded all food and drink consumed on two typical weekdays, and 1 day at the weekend. Levels of choline intake and betaine were estimated. Each day (out of three) analysed individually, then averaged per ppt. Total choline intake = av. amount ingested per day + level in the supplement (750mg/0mg) x	(a) Short-term visuospatial memory delayed response task. Administered twice per test point. Toy chosen by infant was hidden in wells - either 2-wells or 3 embedded within a table. Six trials for each with delays of 3,9,15 secs ordered 3, 9, 15, 3, 9, 15. Outcome = selection of correct well signified by infant reach/visual regard if infant reached with both hands. (b) Long-term episodic memory - deferred imitation (infant required to model an action demonstrated by a researcher after a 15 minute delay). Infant expected to imitate	Food diary: only significant difference between the two conditions at each of the 2 diary completion points - experimental condition had a greater betaine intake ($p < .05$) at 30- week gestation. Data reduction Short-term visuospatial memory task: identified ≥ 4 trials required for a reliable data point. Where infants had completed ≤ 3 trials, data regarded as missing. % correct calculated - correct responses / number of trials where the infant had made a response (out of 12). Loss of 39-46 ppt scores on second administration of task (at each test point) suggested	None	x

Reference	Ingredient	Sample characteristics	Design and intervention	Cognitive measures (battery and sub tests)	Reported findings	Subgroup/Further analyses	Effect summary
		(n=49). Length of gestation (weeks): 39.5 ± 1.6. Placebo condition (n=50). Length of gestation (weeks): 39.3 ± 1.4. Significant difference in BMI and weight between the two mothers in the two conditions at conception, with those in the control condition being heavier and having a higher BMI (p< .05).	ppts compliance rate. Significant difference found in betaine intake at 30-week gestation between the two conditions (p < .05). Single-centre RDBPC parallel group study. Intervention from 18-week gestation - 90 days postpartum, 6 per day. (1) Experimental condition: Nutrasal gel capsules containing choline (750 mg/d), with each capsule containing 833 mg PC. (2) Control	action demonstrated by researcher following delay. Four x object-action sets per test point. Infant explored object for 30 secs followed by researcher modelled target action 3 times within 30 secs x 4 objects = 4 minutes per test point. After 15-minute delay, each object represented individually- infant freely explored for 30 secs. Outcome = if target action was displayed by infant (% correct within age) (c) Language development using the MacArthur-Bates Short Form Vocabulary Checklist: level I, number of words spoken from a pre-identified list of 89	infants either tired, bored or frustrated with task on second administration. Therefore, only % correct scores from first administration at each test point average together were used. Long-term episodic memory: Paired sample t-test showed no differences between the scores from the two test points within each age (10/12 months), so scores averaged together = % correct within age computed. Age effects were found:- significant improvement in performance from 10 - 12 months on the short-term visuospatial memory task, the long-term memory task and words spoken (all p < 0.0001).		

Reference	Ingredient	Sample characteristics	Design and intervention	Cognitive measures (battery and sub tests)	Reported findings	Subgroup/Further analyses	Effect summary
			condition: Corn oil gel capsules. All infants tested two times 7-10 days apart at 10 and 12 months (4 test points). This was scheduled in line with estimated due date.	words selected from the vocabulary checklist of the MacArthur-Bates Communicative Development Inventory (CDI): Words and Gestures. (d) Composite index of global development. Mullen Scales of Early Learning, AGS edition to assess motor, language and cognitive development. Administration of fine motor & visual reception was at the first test point, gross motor & expressive + receptive language at the second test point at each age.	No significant difference in the performance of the two conditions at either 10 or 12 months. Marginal significance was found for an effect of condition for the percentage correct on the Long-Term Episodic Memory Task (p = .056) at 12-months, with those in the placebo condition demonstrating better performance. Finding did not remain when betaine intake at 30-week gestation was added to the analysis (covariate) - significantly higher in the PhosChol condition ($p <$ .05). Stepwise regression analyses (backward elimination): betaine intake at 30-week gestation was a negative		

Reference	Ingredient	Sample characteristics	Design and intervention	Cognitive measures (battery and sub tests)	Reported findings	Subgroup/Further analyses	Effect summary
					predictor of short-term visuospatial memory delayed response performance at 10 months when task was novel (p = .061). Betaine intake at 45 days postpartum was a significant positive predictor short-term visuospatial memory delayed response task performance when the task was novel (*p* = .058), words spoken (*p* = .017) and global development (composite measure) (*p* = .026) at 12 months. Choline intake at 45 days postpartum - same patterns of prediction.		
Harris et al. (1983)	Lecithin (PC)	Healthy adults (n=9); Male: 3. Age: 22-55; 39.9 ± 10.3 years.	Single-centre RDBPC cross-over (oral dose; acute supplementation). Ppts randomly allocated to: (1) Aqueous	(a) Categorised serial learning task. Parallel versions - a list of 10 items of either animals, cities or vegetables were memorised. Outcome = the number	There was no significant difference in the performance between the two conditions. The number of errors made on the uncategorised word recognition task was also	None	x

Reference	Ingredient	Sample characteristics	Design and intervention	Cognitive measures (battery and sub tests)	Reported findings	Subgroup/Further analyses	Effect summary
			flavoured suspension of 20g lecithin. (2) Equal volume of a taste- and appearance - matched placebo containing sugar, corn oil, flour, water gelatine & peppermint flavour. Three testing sessions. The first = practice. Second and third took place 5 hours post-supplement. Experimental sessions were at least 48 hours apart. Used parallel versions for cognitive measures.	of trials required for 3 sequential correct recitations of the list during learning stage and number of words recalled in the correct order 30 minutes post learning stage. The following were presented in random order: (b) A test of retrieval from long-term memory. Ppts had to produce in 1 minute as many nouns as possible from a set category - colours, sports, flowers, boys' names, fruits. Outcome = number of items averaged across two trials. (c) Paired associates learning task. Ppts had to memorise 5 sets of words pairs. Used high and low-imagery forms.	analysed. Again, no main effect of treatment was found. Higher mean choline level in plasma was observed post-lecithin supplementation. No relationship between extent of choline increase and cognitive performance.		

Reference	Ingredient	Sample characteristics	Design and intervention	Cognitive measures (battery and sub tests)	Reported findings	Subgroup/Further analyses	Effect summary
			Blood was taken at the same time of testing to measure choline levels.	Outcome = number of trials required to learn the sets if high and low-imagery words used for target & stimulus. (d) Uncategorised word recognition task. Ppts had to recognise 15 target words from a list of 60 words. Score adjusted for guessing. (e) Digit/symbol subtask of WAIS. Outcome = psychomotor speed.			
Rosadini et al. (1990)	PS	Healthy male adults (n=8). Age: 21-28; 24.5 ± 1.7 years.	Single-centre RDBPC cross-over study (acute supplementation). 25 mg, 50 mg, 75 mg of PS or matching placebo administered intravenously (3 minute infusion) according to a	(a) EEG recordings (in baseline conditions, continuously during infusion and 10, 30, 90, 180 and 360 minutes after PS/placebo administration) were in 3-min segments, in resting conditions and with the eyes closed; Following each post-	No significant differences were identified for any of the cognitive measures.	None	x

Reference	Ingredient	Sample characteristics	Design and intervention	Cognitive measures (battery and sub tests)	Reported findings	Subgroup/Further analyses	Effect summary
			balanced order (Latin-square design) provided by the manufacturer. Sessions at weekly intervals began after a normal night's sleep and at least 2 hours after a light standardized meal at 14.00.	supplement EEG recording (@10, 30, 90, 180 and 360 minutes post-PS/placebo administration): (b) Short-Term Retention Test for sequences of letters; (c) Backward Digit Span Test; (d) Immediate Retention Test for sequences of colours.			
Baumeister et al. (2008)	PS from soy	Healthy males (n=16). Age: 25 ± 3 years PS condition (n=8): Age 24 ± 3 years. Control condition (n=8): Age 26 ± 2.	Single-centre RDBPC parallel group study. Ppts took 1 x nutritional bar per day for 6 weeks:- (1) 35 g IQ PLUS brain bar containing 200 mg soy-based PS; (2) 35 g placebo bar (0 mg PS).	(a) Stroop word-colour interference (computerised): Congruent, incongruent and neutral conditions. Outcomes: RT. (b) D2 concentration test: Within a pre-set time, ppts have to cross out specific letters that are arranged in rows. Two types of error can occur	Ppts increased their performance over time (in both conditions) but there were no significant between-condition differences or any interactions between condition x trial x time.	None	x

Reference	Ingredient	Sample characteristics	Design and intervention	Cognitive measures (battery and sub tests)	Reported findings	Subgroup/Further analyses	Effect summary
			Bars equal on kcal, protein, carbohydrate, fat and vitamins (1.4 mg vitamin B1, 1.4 mg vitamin B6, 42 mg vitamin C, 4.6 mg vitamin E, 2.8 mg niacin, 4.2 mg pantothenic acid). During the 6 weeks, food intake was recorded 3 days p/w to ensure ppts had the same diet over the 6 week period. On test days (baseline) and endpoint), ppts consumed a standardised breakfast. Acute stress was induced	- omission (target letters not crossed out) and confusion (non-target letters crossed out). Outcomes: Total number of letters worked on calculated as concentration performance (CP), omissions (O) and false answers (F).			

Reference	Ingredient	Sample characteristics	Design and intervention	Cognitive measures (battery and sub tests)	Reported findings	Subgroup/Further analyses	Effect summary
			using delayed auditory feedback (DAF) and cognitive measures administered (stroop then D2 test) following this on both test occasions, with cognitive measures being interrupted by EEG measurement (2 minutes) immediately after each.				
Parker et al. (2011)	PS from soy	Healthy, physically active* males (n=18). Age: 22.5 ± 2.2 years. *participated in lower body resistance	Single-centre RDBPC cross-over study. Ppts took 2 x serving's per day for 2 weeks:- (1) IQPLUS Foods LLC containing 200 mg soy-based PS per serving (400	(a) Serial subtraction task: Two-minute timed test where ppts subtracted 7 from a random 4-digit number. Outcomes: RT and accuracy - average time per correct calculation, number of correct	Paired samples t-test analysed performance at pre-exercise bout only:- average time per correct calculation (seconds) reduced and number of correct calculations increased following PS vs. placebo (p = .001, .07, respectively).	None	+

Reference	Ingredient	Sample characteristics	Design and intervention	Cognitive measures (battery and sub tests)	Reported findings	Subgroup/Further analyses	Effect summary
		exercise at least once p/w for the prior 3 months.	mg per day); (2) Matching placebo (rice flour). No washout period. Cognitive measure administered during a familiarisation visit, then post 14 and 28 days (at the end of each 14 day supplementation period). On test days, ppts administered cognitive measure pre-acute exercise bout, then 5 and 60 minutes post-acute exercise bout.	calculations, number of errors (mistakes).	2 x 3 repeated measures ANOVA: No significant main effect of condition. Significant main effect for time (p = .02) where there was a significant decrease in time per correct calculation across both conditions between pre- and 60 minutes post-acute exercise bout. There was also a significant time x condition interaction (p = .007) with the PS condition demonstrating quicker average time per correct calculation vs. placebo at pre-acute exercise bout only. No significant differences were found for serum cortisol, total testosterone, or cortisol to testosterone ratio by condition or condition and time.		

Reference	Ingredient	Sample characteristics	Design and intervention	Cognitive measures (battery and sub tests)	Reported findings	Subgroup/Further analyses	Effect summary
Boyle et al. (2019)	PL-20 bovine milk concentrate	High stress vulnerable (high perfectionist*) males (n=54**). PL condition (n=26): Age 22.04 ± 0.76. Control condition (n=27): Age 20.81 ± 0.34. *Score of ≥ 13 on Perfectionism: organisation subscale. **n=1 removed owing to non-compliance	Single-centre RDBPC parallel group study. Ppts took 1 x milk drink per day for 6 weeks (each in plain white TetraBrik carton):- (1) 250 ml milk drink containing 2.7g of PLs per day (including 300 mg PS); (2) 250 ml placebo (0% PLs). Fat content of placebo matched to PL drink by adding butter oil containing only triglycerides. Upon arrival at study laboratory, ppts consumed a standardised meal	(a) N-back task (memory load of 2). Outcome: Accuracy and RT; (b) Attention switch task. Outcome: Accuracy and RT. Switch costs (within colour, trial 1 (switch) - trial 2 (nested) and repeat costs (within colour, trial 3 (pre-switch) - trial 2 (nested).	(a) No significant difference. (b) Significant main effect of drink on RT repeat cost where PL condition incurred significantly lower performance costs on repeat trials vs. placebo condition (p= 0.01). PL drink did not significantly attenuate the cortisol response but marginal significance was found for reduced anticipatory subjective stress ratings post-intervention, PL milk drink vs. the placebo (p = .06). PL drink condition also reported significantly increased subjective energy and arousal during peak stress exposure following 6 week intervention (p = .03).	None	+

Reference	Ingredient	Sample characteristics	Design and intervention	Cognitive measures (battery and sub tests)	Reported findings	Subgroup/Further analyses	Effect summary
		with study drink intake.	to standardise baseline nutritional state. Cognitive measure delivered post-acute psychosocial stressor (TSST and SECPT) on visit 1 (baseline) and visit 2 (endpoint). Ppts consumed drinks each morning for 6 weeks.				
Schubert et al. (2011)	PL-20 bovine milk concentrate	Chronically stressed men* (n=75)**. Age: 30-51. 0.5% PL condition (n=25). Age: 39.76 ± 1.29. 1% PL condition	Single-centre RDBPC parallel group study. Ppts took 1 x milk drink per day for 6 weeks:- (1) 250 ml bovine milk enriched with 0.5% PL per carton (150 mg; SM	(a) VISGED Visual Memory Test (electronic): Recall (immediate) of the position of symbols on a city map. Outcome: person parameter representing the ppts level of skill for the task.	Age accounted for in the analyses of memory data - established 2 groups of similar age using a median split at 41 years. Ppts assigned to either middle-aged group (30-40 years) or older group (41-51 years). Data of n=71 were available for analysis (30-40 years, n=33; 41-51	None	+

Reference	Ingredient	Sample characteristics	Design and intervention	Cognitive measures (battery and sub tests)	Reported findings	Subgroup/Further analyses	Effect summary
		(n=24). Age: 39.04 ± 1.28. Placebo condition (n=24). Age: 40.35 ± 1.35. *As assessed by the Trier Inventory of Chronic Stress. **Data not obtained for n=2 – n=1 placebo, n=1 1.0% PL	0.12%, PC 0.14%, PE 0.14%, PS 0.06%, PI 0.04%); (2) 250 ml bovine milk enriched with 1.0% PL per carton (300 mg; SM 0.23%, PC 0.28%, PE 0.28%, PS 0.12%, PI 0.08%); (3) 250 ml placebo bovine milk with 0% PL per carton. Cognitive measure delivered after 42 day supplementation period before and post-acute stress exposure (TSST). Ppts consumed drinks at breakfast.		years, n=8). Older group: 0.5% PL n=11, 1.0% PL n=16, placebo n=11. Prior to acute stress exposure: placebo and 1.0% PL condition had higher scores in memory performance vs. 0.5% PL condition, but this was not significant. Post-acute stress exposure: No main effect of age, time, condition. Time x age x condition interaction: visual memory performance significantly better in the 1.0% PL older group vs. placebo and 0.05% PL older groups (p = .042). Performance by placebo older group was lower post-acute stress exposure, but this was not significant. 0.5% PL older group maintained similar		

Reference	Ingredient	Sample characteristics	Design and intervention	Cognitive measures (battery and sub tests)	Reported findings	Subgroup/Further analyses	Effect summary
					memory performance. 1.0% PL milk drink condition demonstrated higher cortisol levels, whereas the placebo has the lowest levels before and after acute stress exposure, but this was not significant.		
Hellhammer et al. (2010)	PL-20 bovine milk concentrate	Healthy male adults (n=45)*. Age: 30-55. PL condition (n=23): Age 41.61 ± 7.18. Control condition (n=22): Age 41.43 ± 6.82. *Further analysis: sample split based on group	Single-centre RDBPC study parallel group study. Ppts took 1 x milk drink per day for 3 weeks:- (1) 0.325L milk drinks containing 13.5g of PLs per day (5% SM, 5% PC, 4% PE, 2.3% PS, 1.5% PI); (2) 0.325L placebo compound. Cog measure	(a) Item recognition test: Twenty trials. Presentation of 3-4 uppercase letters, followed by recognition display of 2-4 uppercase letters. Ppts responded either yes/no - whether or not one of the targets was identical to one of the stimuli in the recognition display. Processing load manipulated by the number of comparisons that had to be made in	Trend (p = 0.09) with the PL condition demonstrating quicker RTs vs. placebo. After controlling for inter-individual variation in cortisol concentration, this effect became marginally significant (p = 0.06). Further analysis determined that the PL composite milk drink also dampened endocrine and psychological stress	High vs. low stress analyses not conducted on cognitive measures.	+

Reference	Ingredient	Sample characteristics	Design and intervention	Cognitive measures (battery and sub tests)	Reported findings	Subgroup/Further analyses	Effect summary
		median into high vs. low stress groups based on Trier Inventory of Chronic Stress scores. No demographics reported for these groups	delivered after 3 week supplementation period 20 minutes before and 10 minutes post-acute stress exposure (TSST). Ppts consumed drinks 30 minutes before breakfast.	order to respond. Outcomes: Error and RT	response but only in highly stressed individuals.		
Crook et al. (1991)	PS derived from bovine cortex (BC-PS).	Adults with age-associated memory impairment (AAMI) 50-75 years (n=149). Mean age of 64 years. BC-PS condition (n=74; Male=45.9%): Age 62.61 ± 6.31.	Multicentre RDBPC parallel group study. 300mg of BC-PS per day with a matched placebo (capsules) for 12 weeks. Paper neuropsychological test administered at baseline & endpoint. Computerised	(a) Paper neuropsychological tests: Benton Visual Retention Test; WMS Logical Memory Subtest (Form A); WMS Associative Learning Subtest (Form A). (b) Computerised psychometric battery: normative data showing age associated performance decline	(a) No significant difference was found between the experimental and control conditions. (b) Significantly better performance was demonstrated by the BC-PS condition on Name Face Association: Acquisition at weeks 3 and 6 ($p < .001$); on Name Face Association: Delayed recall at weeks 3 ($p = .04$) and 6 ($p = .03$); and on Facial Recognition	Cluster analysis of baseline data identified two subgroups. First subgroup (n=92) had relatively good memory function. Second subgroup (n=57) had relatively poor memory function. Subgroup entered into the model as a main effect and subgroup x treatment as an	+

Reference	Ingredient	Sample characteristics	Design and intervention	Cognitive measures (battery and sub tests)	Reported findings	Subgroup/Further analyses	Effect summary
		Control condition (n=75; Male=37.3%): Age 64.88 ± 6.81. Subgroup analysis: First subgroup (n=92) Age 61.6 years. Second subgroup (n=57) Age 64.3. Ppts scored at least 27 on MMSE.	psychometric battery undertaken at baseline then at weeks 3, 6, 9, 12 and 16 (last visit +4 weeks after treatment ended). Clinical global rating scale done at week 12 and 16 (ratings of improvement).	was used to select primary and secondary subtests: Primary: Name Face Association: Acquisition; Name Face Association: Delayed recall; Facial Recognition (delayed non-matching to sample paradigm); Telephone number recall (with interference); Misplaced objects recall. Secondary: Selective reminding: Acquisition; Selective reminding: Delayed recall; First-Last Names: Acquisition; Divided attention. (c) Clinical global rating scale undertaken by a study psychologist/registered	at week 12 (p = .01), whereas this was marginally significant at week 3 (p = .05). (c) No significant difference was found between the experimental and control conditions.	interaction term. Significant differences were identified on all primary measures and 3/4 secondary measures. This difference in performance was consistently seen in subgroup 2 - those in the control condition performed less well vs. BC-PS condition. (a) Ppts in BC-PS condition scored significantly higher on the Logical Memory Subtest of the WMS (Form A; paragraph recall; p < .03) at week 12. (b) Significantly better performance was demonstrated by the BC-PS condition on	

Reference	Ingredient	Sample characteristics	Design and intervention	Cognitive measures (battery and sub tests)	Reported findings	Subgroup/Further analyses	Effect summary
				nurse during interview with ppt. 12 items: 10/12 on specific cognitive symptoms and 2/12 on overall cognitive status.		Name Face Association: Acquisition at weeks 3 (p= .01), 6 (*p* = .04) and 12 (*p* = .01); on Name Face Association: Delayed recall at week 12 (*p* = .04) but marginally significant at week 3 (p = .05); on Facial Recognition at weeks 3 (*p* = .03), 6 (*p* = .02), 12 (*p* = .02) and 16 (*p* = .01); on Telephone number recall at week 16 (*p* < .001) and on Misplaced objects recall at week 6 (*p* < .001) and week 16 (*p* = .03). (c) Ppts in BC-PS condition scored significantly higher on 2 items at week 12: memory for names of	

Reference	Ingredient	Sample characteristics	Design and intervention	Cognitive measures (battery and sub tests)	Reported findings	Subgroup/Further analyses	Effect summary
						persons after introductions (p = .02); and ability to maintain concentration when reading, conversing or performing tasks (p = .02). Marginal significance was seen at week 12 for overall global change in cognitive status (p = .05); and visual analogue scale of global improvement (p = .05). These perceived improvements were lost at week 16, when a follow-up found no significant differences.	
Vakhapova et al. (2010)	Vayacog: PS conjugated to omega-3 LC PUFA - attached to	Adults with memory complaints between 50-90 years (n=131)*	Single-centre RDBPC parallel group study. Ppts took 3 x capsules per day for 15	(a) Rey Auditory Verbal Learning Test (RAVLT). Sub scores - immediate memory recall (trial 1), verbal total learning	(a) Significant improvement in number of words recalled in immediate recall trial for the PS-DHA/EPA condition	Ppts with relatively good cognitive performance at baseline - fulfilled 2/3: MMSE score >	+

Reference	Ingredient	Sample characteristics	Design and intervention	Cognitive measures (battery and sub tests)	Reported findings	Subgroup/Further analyses	Effect summary
	the glycerol backbone	PP analysis PS-DHA/EPA condition (n=60; Male=48%): Age 72.9 ± 8.20. Control condition (n=62; Male=53%): Age 73.01 ± 8.28. Subgroup analysis: PS-DHA/EPA condition (n=40; Male=37.5%): Age 71.8 ± 8.14. Control condition (n=38; Male=44.7%):	weeks:- (1) PS-DHA/EPA (300 mg PS, 79 mg DHA+EPA, DHA/EPA ratio 3:1) per day. (2) Placebo (cellulose - matched, identically looking). Cog measures delivered at baseline and endpoint. Clinical Global Impression of Change (CGI-C) given at week 7 and endpoint.	(sum of scores of trial 1-5), delayed recall (trial 8), recognition (hits, trial 9); (b) Rey Complex Figure Test (RCFT) - visuospatial perception/construction and memory. Outcome = task duration (copy time, secs) and accuracy (copy quality), immediate recall, delayed recall; (c) CGI-C. Scores range from 1-7, lower meaning improvement, higher meaning deterioration, 4 meaning no change. Interviewer independent of testing - no knowledge of how the ppt performed on cognitive measures. Ppts who scored	vs. control (p = .041). No significant differences between conditions for the rest of the RAVLT tasks; however, improvement occurred more commonly in the PS-DHA/EPA condition than in the placebo condition in 2 of 3 items. ITT analysis of immediate recall scores from 131 ppts revealed marginal significance (p = .069) for the PS-DHA/EPA condition vs. controls. (b)Trend in copy time in favour of PS-DHA/EPA condition (p = .079) vs. control. Overall, in all trials for this measure except for the delayed recall trial (similar improvement in both conditions), more improvement shown by PS-DHA/EPA condition.	26*; baseline performance in RAVLT delayed recall trial > PP group mean (7 words); academic education > 12 years. Included 78 ppts (n=40 PS-DHA/EPA / n=38 control condition). *inclusion criteria = MMSE score ≥ 26 for ppts without a college education. (a) Significant improvements demonstrated by PS-DHA/EPA condition for immediate recall (p = .006), total learning (p = .002) and delayed recall (p = .045). There was no significant difference	

Reference	Ingredient	Sample characteristics	Design and intervention	Cognitive measures (battery and sub tests)	Reported findings	Subgroup/Further analyses	Effect summary
		Age 72.2 ± 8.34. *n=9 were excluded from the PP analysis (n=6 from placebo group, n=3 from PS-DHA/EPA condition): 1 due to short interval between visits and 8 failed to meet post-specified compliance criteria (≥65%). All ppts scored at least ≥ 26 on MMSE and ≤ 0.5 on the Clinical		themselves as improved from last visit (scores of either 1, 2, 3), in at least 1/2 visits (weeks 7/15), classified as improved; unless ppts rated improved at week 7 and deterioration at week 15. Otherwise, classified as unchanged (score 4) or worse (scores of either 5, 6, 7). (d) NexAde computerised cognitive tool. Subtasks - symbol spotting, pattern identification, pattern recall, digit-symbol substitution, digit span forward, digit span backward and delayed pattern recall. Outcome = 8 composite scores relating to focused attention, sustained	(c) No significant difference found. However, more ppts in PS-DHA/EPA condition were classified as clinically improved (37%/n=22) vs. control condition (27%/n=17). (d) No significant differences. Responder analysis Ppts classified as responders when improvement in RAVLT immediate recall was at least 2 words & when they had an overall improvement in the CGI-C. Following intervention, a significant correlation between responder status and treatment was found, p = .034 in PP analysis: responders in the PS-DHA/EPA condition = 22%,	for the recognition trial. (b) Marginal significance was found for copy duration where the PS-DHA/EPA condition showed the largest improvement (p = .055). Accuracy remained stable in both conditions - indicating the improvement observed in the time to complete the copy task was not at the expense of the quality of the copied figure (c) No significant difference found. However, more ppts in PS-DHA/EPA condition were classified as clinically	

Reference	Ingredient	Sample characteristics	Design and intervention	Cognitive measures (battery and sub tests)	Reported findings	Subgroup/Further analyses	Effect summary
		Dementia Rating Scale.		attention, memory recognition and recall, visuospatial learning, spatial short-term memory, executive functions and mental flexibility.	control condition = 8% (n=13 and n=5, respectively). Also, significant association found for ITT analysis *p* = .035: PS-DHA/EPA condition = 23%, control condition = 9%.	improved (40%/n=16) vs. control condition (32%/n=12). (d) NexAde No significant differences. Responder analysis Significant correlation found between responder status and treatment (*p* = .016) in the PP analysis: PS-DHA/EPA condition = 25%/n=10, control condition = 5%/n=2.	

Key: + positive treatment effect; x no treatment effect; AAMI: Age-associated memory impairment; BC-PS: bovine cortex phosphatidylserine; CGI-C: Clinical Global Impression of Change; DHA: Docosahexaenoic acid; EEG: Electroencephalogram; EPA: Eicosapentaenoic acid; ITT: Intention-to-treat; LC PUFA: Long chain polyunsaturated fatty acid; MMSE: Mini-Mental State Examination; PC: Phosphatidylcholine; PE: Phosphatidylethanolamine; PI: Phosphatidylinositol; PL: Phospholipid; PP: Per protocol; Ppts: Participants; PS: Phosphatidylserine, PS-DHA/EPA: Phosphatidylserine-Docosahexaenoic acid/Eicosapentaenoic acid; RAVLT: Rey Auditory Verbal Learning Test; RCFT: Rey Complex Figure Test; RDBPC: Randomised, double-blind, placebo-controlled; SECPT: Socially evaluated cold-pressor test; SM: Sphingomyelin; TSST: Trier Social Stress Test; WAIS: Wechsler Adult Intelligence Scale; WMS: Wechsler Memory Scale.

Appendix 4. Study 1 opt-out permission letters

UNIVERSITY OF LEEDS

Institute of Psychological Sciences

Leeds LS2 9JT

Dear Parent/Carer,

An intervention study of the effects of enriched milk compared with non-enriched milk on cognitive performance and subjective state in 6-8 year old school children

My name is Claire Champ and I am a Research Officer from the University of Leeds. I am leading a team who are conducting a study in your pupils' school. This work is a research collaboration between the University of Leeds and a food (milk) manufacturing company. Some results from the study will be used towards an educational qualification by members of the research team. We are supervised by Professor Louise Dye and Dr Clare Lawton at the Institute of Psychological Sciences.

We would like to invite your child to take part in a study examining how two different milk drinks affect children's performance over a period of 6 weeks. The study milk drinks are A) Cow's milk and B) Cow's milk enriched with a milk ingredient already present in cow's milk. This ingredient is a type of fat that is thought to be beneficial for cognitive performance.

This letter is designed to provide you with enough information about the study in order for you to make an informed decision about your child's participation.

The tests are administered using touch screen laptops and most children find them good fun. We would like children aged 6-8 years to take part and would test your child on 3 occasions within the 6 week period. The tasks should take about 30 minutes and will take place during the school day on the school premises. The tasks will include tests of different abilities such as reaction time and memory. These tasks are not intended to be stressful and are appropriate for your child's age group. Half way through the tasks your child will be given a 5 minute refresher break. They will also be asked to complete some simple questionnaires asking them about their hunger and mood at each session. Your child will have to option to stop the tests and leave the study at any time.

During the 6 weeks of testing each child will be randomly assigned to consuming either Milk A or Milk B on 5 days per week (i.e. on Mondays-Fridays). Allocation to each milk type will be decided by chance – rather like tossing a coin. There is an equal chance that

your child will receive Milk A or Milk B. Neither you nor the researchers will be able to choose which milk your child receives. Children will, however, be able to choose between banana, strawberry or raspberry flavoured milk. University staff will be responsible for delivering the study milk to school, for giving this out to the children and for supervising milk consumption during school hours.

All the children who take part will undergo a colour blindness test to make sure that they can see the colours used in the touch screen computerised tests and complete the Wechsler Abbreviated Scale of Intelligence (WASI). If your child is found to be colour-blind we will pass this information on to you via the school.

This study has received ethical approval from the Institute of Psychological Sciences Research Ethics Committee (Ref 14-0101; date of approval 17/05/2014). All of the information collected from your child during the study will be kept strictly confidential and will only be used for the purposes of this research. Participation in the study is completely voluntary. If you decide to allow your child to take part they are free to withdraw at any time without providing a reason. All results from the study will be kept strictly anonymous and at no point will any identifiable personal information be linked with the results. At the end of the study both teachers and parents will be invited to a post study dissemination event where top line results will be presented by us and our supervisors.

This study will take place in school during the period of Wednesday, 3rd September until Wednesday, 22nd October.

If you are you happy for your child to participate in this study then you do not need to do anything further. We will assume that you are happy for your child to take part unless you inform the school otherwise using the slip below.

Please note: School staff will ensure that children who have an allergy or who are intolerant to cow's milk do not take part in this study.

If you have any questions about the study please contact:

Claire Champ or Fiona Croden on 0113 343 5753 or milkstudy@leeds.ac.uk (study email)

Alternatively you can email or phone our project supervisors Professor Louise Dye and Dr Clare Lawton at the Institute of Psychological Sciences:

Prof Louise Dye: 0113 3435707 or l.dye@leeds.ac.uk
Dr Clare Lawton: 0113 3435741 or c.l.lawton@leeds.ac.uk

An intervention study of the effects of enriched milk compared with non-enriched milk on cognitive performance and subjective state in 6-8 year old school children

I **DO NOT** wish my son/daughter .....(Please insert child's name)

to take part in the above research study.

Signed.....(parent/carer)

Date.....

Please only return this slip if you DO NOT wish your child to take part in this study.

Appendix 5. Expert panel approved Lacprodan®PL-20 as part of nutrition bars and milk-based nutritional beverages as safe, suitable and GRAS in July 2014

EXPERT PANEL OPINION THE GENERALLY RECOGNIZED AS SAFE (GRAS) STATUS OF THE PROPOSED USES OF LACPRODAN PL-20

Introduction

The undersigned, an independent panel of experts, qualified by their scientific training and national and international experience to evaluate the safety of food and food ingredients (the "Expert Panel"), was specially convened on behalf of Arla Foods Ingredients Group P/S, and asked to evaluate the safety and "generally recognized as safe" ("GRAS") status of the proposed use of Lacprodan PL-20 as an ingredient in certain specified foods.

Lacprodan PL-20 is a milk protein concentrate rich in phospholipids. Lacprodan PL-20 is proposed for use in nutrition bars (e.g., high protein bars, meal replacement bars) at a maximum level of 6 g/serving, in ready-to-drink milk-based nutritional beverages (e.g., RTD meal replacements/supplements such as Boost) at a maximum level of 16 g/serving, in protein/high protein milk-based nutritional drinks at a maximum level of 6 g/serving , and in other milk-based nutritional beverages (e.g., instant breakfast beverages, powder mixes) at a maximum of 8 g/serving.

Exponent Inc. ("Exponent") performed a comprehensive search of the scientific literature in November 2013 relating to the safety of the milk-derived ingredient and its specific components for human consumption. The specific milk-derived components that were the subject of the safety review included milk fat globular membrane (MFGM), phospholipids, and sphingolipids. Exponent summarized the results of the literature search and prepared a safety dossier, "GRAS Determination for the Use of Lacprodan PL-20 in Select Foods," for consideration by the Expert Panel.

The Expert Panel critically evaluated Exponent's safety documentation (the dossier), and other available data and information that the members of the Expert Panel believed to be pertinent to the safety of Lacprodan PL-20 under the conditions of intended use. In addition, the Expert Panel critically evaluated the method of manufacture and specifications for Lacprodan PL-20, analytical data confirming compliance with appropriate food-grade specifications and consistency of production, the conditions of its intended use as an ingredient in foods, and the estimated dietary exposure to Lacprodan PL-20. After independent review, the Expert Panel convened via telephone conference call on April2, 2014, and independently, jointly, and unanimously concluded that the intended use of Lacprodan PL-20 as a food ingredient

in select foods, produced consistent with current good manufacturing practice (cGMP) and meeting appropriate food-grade specifications, is safe and suitable. The Expert Panel further concluded that such intended use is GRAS based on scientific procedures. It is also the opinion of this Expert Panel that other qualified experts would concur with these conclusions.

Summarized below is the Expert Panel's scientific analysis supporting our conclusions.

Description

Lacprodan PL-20 is a milk protein concentrate rich in phospholipids. Protein is the primary constituent of Lacprodan PL-20, accounting for 49-55% of the product. Approximately 55% of the proteins in Lacprodan PL-20 are comprised of casein or whey, and the remaining 45% of proteins are MFGM proteins. Lipid accounts for 24-30% of Lacprodan PL-20 by weight, with the majority of lipid present as phospholipids and a small fraction present as triglycerides. Lacprodan PL-20 contains a minimum of 16% phospholipids. The phospholipid profile in Lacprodan PL-20 is comparable to the phospholipid profile found in both bovine and human milk, as the predominant phospholipids are phosphatidylcholine, sphingomyelin, and phosphatidylethanolamine, with each accounting for approximately 27%, 27%, and 22% of total phospholipids, respectively. The remaining constituents of Lacprodan PL-20 include lactose (up to 10%); ash (up to 6%), predominantly as phosphorus and calcium; and moisture (up to 5%).

Manufacturing Process

Lacprodan PL-20 is made from standard cream with a lipid content of approximately 40% using standard separation techniques commonly applied in the dairy industry. In the production of Lacprodan PL-20, lipid in the form of triglycerides is removed from cream, leaving the serum phase (or aqueous phase) which contains proteins and phospholipids from the MFGM along with milk proteins, carbohydrates, and minerals. Lacprodan PL-20 is made under cGMP from food grade materials using only mechanical separation steps (homogenization, centrifugation and ultrafiltration). No processing aids are used in the production process; consequently, Lacprodan PL-20 contains only components intrinsic to cream, or the milk from which cream is derived. Ascorbyl palmitate (21 CFR§182.3149) and tocopherol (21 CFR§184.1890) are added to prevent oxidation during storage; the concentration of each of these additives is approximately 100 ppm as calculated on the powder.

Intended Use and Estimated Intake

Lacprodan PL-20 is rich in phospholipids naturally occurring in milk fat, including sphingomyelin, phosphatidylcholine, phosphatidylethanolamine, phosphatidylserine and phosphatidylinositol. The intended effect of using Lacprodan PL-20 as an ingredient in foods is to provide a dietary source of the milk-derived components in the ingredient.

Lacprodan PL-20 is intended for use in nutrition bars (e.g., high protein bars, meal replacement bars) at a maximum level of 6 g/serving, in ready-to-drink milk-based

nutritional beverages (e.g., RTD meal replacements/supplements such as Boost) at a maximum level of 16 g/serving, in protein/high protein milk-based nutritional drinks at a maximum level of 6 g/serving, and in other milk-based nutritional beverages (e.g., instant breakfast beverages, powder mixes) at a maximum of 8 g/serving.
For the population aged 2 years and older, the per user mean and 90th percentile estimated daily intakes (EDIs) of Lacprodan PL-20 are 7.3 and 15.3 g/day, respectively. Adults 19 years of age and older are estimated to have the highest intake of Lacprodan PL-20 from the proposed uses; the per user mean and 90th percentile EDIs of Lacprodan PL-20 for adults are 7.5 and 15.9 g/day, respectively. At the 90th percentile of intake among adults, Lacprodan PL-20 will deliver approximately 3.8g MFGM proteins and 2.7g phospholipids.

Safety

Lacprodan PL-20 contains no novel dietary components in that the MFGM components in Lacprodan PL-20, i.e. phospholipids and proteins, are present in milk and milk-derived products. Milk and milk-derived ingredients have a long history of use and are widely consumed in the U.S. diet. Phospholipids account for no more than approximately 1% of total lipids in milk, and approximately 1 to 4% of total proteins in milk are MFGM proteins. The concentration of MFGM phospholipids and proteins in standard milk and milk products therefore is lower than the concentration in Lacprodan PL-20. The remaining components in Lacprodan PL-20, namely casein and whey, triglycerides, and minerals including calcium, phosphorus, potassium, and magnesium, are widely consumed in relatively large quantities in dairy products and other dietary sources. As such, the exposure from the additional intake of these components resulting from the intended use of Lacprodan PL-20 is not considered to present a safety concern.

The safety of the intended use of Lacprodan PL-20 therefore was evaluated by a critical evaluation of information pertinent to the safety of the key constituents in the ingredient, namely the phospholipids and MFGM proteins that account for the distinct compositional and nutritional profile of Lacprodan PL-20, and also the safety of the consumption of Lacprodan PL-20 and similar milk-derived substances. All the information critically evaluated that formed the basis for this GRAS determination is available in the publicly available literature.

Polar lipids present in the highest quantity in bovine milk are glycerophospholipids and sphingolipids (primarily sphingomyelin) and their composition also reflects the phospholipid profile in human milk. It is reasonable to assume that the absorption of glycerophospholipids from this milk-derived ingredient (Lacprodan PL-20) is similar to that of other dietary glycerophospholipids, which is known to be rapid and nearly complete. Following absorption, the fatty acids contained in dietary glycerophospholipids are incorporated into cellular membranes or enter the pool of absorbed fatty acids and share their metabolic fate.

Phospholipids are ubiquitously found in the human diet as components of animal- and plant- derived foods, and as approved food additives, including the widely used lecithin

(i.e., a naturally occurring mixture of phosphatides of choline, ethanolamine and inositol; 21 CFR §184.1400) and serine-containing phosphatides that have also been determined to be GRAS. The estimated daily intake of phospholipids from the diet (background) is in the range of0.9 to 8 g per day. Adverse effects are not associated with these levels of phospholipid intake. In addition to dietary sources, the biliary pathway delivers approximately 10 to 20 g of phospholipids to the intestinal lumen per day. International regulations allow for the addition of up to 2 g phospholipids per Litre infant formula (Codex Stan 72-1981, rev 2007; Directive 2006/141/EC); in the USA, egg-phospholipids may be added to infant formula at a level up to 2 g per L. Assuming the maximum allowable level of phospholipids in infant formula and the typical formula intake of 1 L per day, the daily intake of phospholipids by infants from this source could be approximately 2 g with no anticipated adverse effects.

Sphingolipids represent a structurally diverse category of lipids. The sphingolipids of mammalian tissues include ceramides, sphingomyelins, cerebrosides, gangliosides, and sulfatides, with sphingosine as the principal sphingoid base. Sphingomyelin is synthesized mainly via *de novo* pathways, but it is also obtained from the diet. The intake of sphingolipids (as sphingomyelin) is estimated at 300 to 400 mg/day. While the major dietary glycerophospholipids are rapidly digested and absorbed by pancreatic enzymes, dietary sphingolipids are more slowly digested by mucosal enzymes. The available data indicate that humans digest and absorb most (approximately 81%) of the sphingomyelin in normal diets. Adverse effects from sphingomyelin intake have not been observed or reported in animal or human studies.

The MFGM proteins belong to a variety of functional classes, with the most frequently observed classes comprised of immunity and defence, signal transduction, protein transport, and lipid metabolism. While there is limited published evidence regarding the specific safety or of safety evaluations of key MFGM proteins, no information was identified to suggest untoward or adverse effects from their consumption in the diet. MFGM proteins account for approximately 1 to 4% of total proteins in bovine milk; therefore, each one-cup serving of whole milk provides in the range of 80 to 310 mg MFGM. MFGM proteins account for approximately 2 to 4% of total protein in human milk. Assuming an intake of 1 L human milk per day with a total protein content of 9 g per L, the daily MFGM protein intake by breastfeeding infants is in the range of 180 to 360 mg.

Several published repeat-dose feeding studies of Lacprodan PL-20 and its primary components conducted in humans and animals were identified. Although the human studies were not designed specifically to assess the safety of the MFGM-derived ingredients, the studies present indicators of safety including reports of tolerance of the test articles, blood lipid responses, and body weight; animal studies typically include measures of feed intake, body weight and growth.

Findings from the clinical studies support the safe intake of Lacprodan PL-20 at the tested doses. The highest intake of Lacprodan PL-20 was 16.6 g daily for a period of 4 weeks in a sample of 48 adults; this level of Lacprodan PL-20 delivered 8.4 g protein (approximately 3.8 g MFGM protein) and 2.8 g phospholipids. No untoward effects were observed in the study or in additional studies in which adults consumed

approximately 13.5 or 14.7 g Lacprodan PL-20 daily. In a small study of short duration (10 days), women consumed up to 16.8 g protein and 6 g phospholipid from 32.4 g of a butter serum-derived ingredient compositionally similar to Lacprodan PL-20. Children were also exposed to daily intakes of approximately 4 g protein (0.4 g MFGM protein) and 0.4 g phospholipids in a 6 month intervention with a milk-derived ingredient, and children consumed 0.5 g supplemental phospholipids in another study for a period of 6 months.

Studies conducted in rats and mice examining the supplementation of their diets with formulations prepared using isolated milk fat globule membrane components that range in length from 3 weeks to 13 weeks did not reveal any untoward effects. These studies demonstrated that the formulations were well-tolerated and supported growth and appropriate body weight gain and composition when provided concurrently with a suitable animal diet. Feeding isolates of milk fat globule membranes also did not produce any untoward effects in pregnant dams supplemented during gestation, nor in pups exposed *in utero,* in terms of physical characteristics, growth, body weight, weight gain, and body composition. Litter size and survival were not explicitly repmied, however, the authors stated that litter size was adjusted to 8, indicating typically sized litters. Findings from a 13-week rat study feeding of a milk fat globule isolate formulation (MFGM) support an acceptable daily intake of at least 85 mg/kg bw/d proteins and 6.25 mg/kg bw/d MFGM phospholipids, the highest doses tested in the study. This level of protein intake is equivalent to 5.1 g/day for a 60 kg adult, or approximately 0.6 g MFGM proteins assuming that MFGM-specific proteins account for 12.5% of total protein. The safety of intake of the MFGM isolate demonstrated in this animal study is consistent both with the lack of adverse effects noted in the clinical trials and the available information regarding the safety of phospholipids, including sphingomyelin, and the safety of MFGM specific proteins.

Summary and Conclusion

The 90th percentile per user EDI of Lacprodan PL-20 from the maximum proposed use levels in nutrition bars and milk-based nutritional beverages is 15.9 g/day among adults, which is below the level determined to be an acceptable daily intake. Therefore, it is reasonable to conclude that the proposed use of Lacprodan PL-20 in nutrition bars (e.g., high protein bars, meal replacement bars) at a maximum level of 6 g/serving, in ready-to-drink milk-based nutritional beverages (e.g., RTD meal replacements/supplements such as Boost) at a maximum level of 16 g/serving, in protein/high protein milk-based nutritional drinks at a maximum level of 6 g/serving, and in other milk-based nutritional beverages (e.g., instant breakfast beverages, powder mixes) at a maximum of 8 g/serving is safe and suitable, and GRAS.

We, the undersigned expert panel members, have individually and collectively critically evaluated published and unpublished data and information pertinent to the safety of the proposed use of Lacprodan PL-20, a milk protein concentrate rich in phospholipids, in nutrition bars (e.g., high protein bars, meal replacement bars) at a maximum level of 6 g/serving, in ready-to-drink milk-based nutritional beverages (e.g., RTD meal replacements/supplements such as Boost) at a maximum level of 16 g/serving, in protein/high protein milk-based nutritional drinks at a maximum level of6 g/serving,

and in other milk-based nutritional beverages (e.g., instant breakfast beverages, powder mixes) at a maximum of 8 g/serving, produced consistent with cGMP and meeting appropriate food-grade specifications, and unanimously conclude that it is "generally recognized as safe" (GRAS) based on scientific procedures.

It is our opinion that other qualified experts would concur with our conclusions. By:

Stanley M. Tarka Jr. 08 July 2014

Stanley M. Tarka, Jr., Ph.D.
Chair of the Expert Panel
President, The Tarka Group, Inc.
United States

Date

P Jones JULY 9, 2014

Peter Jones, Ph.D.
Director, Richardson Centre for Functional Foods
University of Manitoba, Canada

Date

7/9/2014

Robert E. Ward, Ph.D.
Associate Professor
Department of Nutrition, Dietetics, and Food Sciences
Utah State University, United States

Date

Appendix 6. Pre-cognitive measures 10-point Likert scale to measure subjective evaluation of appetite, mood, motivation and mental alertness

Participant Number: .....

Date: .....

Visual Analogue Scale items for measurement of subjective state

Pre Cognitive Test VAS

1. How **hungry** do you feel right now?

Not at all hungry	1 2 3 4 5 6 7 8 9 10 	Very hungry

2. How **cheerful** do you feel right now?

Not at all cheerful	1 2 3 4 5 6 7 8 9 10 	Very cheerful

3. How much **energy** do you have right now**?**

No energy at	1 2 3 4 5 6 7 8 9 10 	A lot of energy

4. How keen are you to **try hard** right now?

Not at all keen	1 2 3 4 5 6 7 8 9 10 	Very keen _____

5. Are you **easily distracted** right now?

Not at all	1 2 3 4 5 6 7 8 9 10 	Very easily

6. How easy are you finding it to **concentrate** right now?

Not at all easy	1 2 3 4 5 6 7 8 9 10 	Very easy

7. How **awake** do you feel right now?

Not at all awake	1 2 3 4 5 6 7 8 9 10 	Very awake

8. How **bad tempered** do you feel right now?

Not at all bad tempered	1 2 3 4 5 6 7 8 9 10 	Very bad tempered

Appendix 7. Post-cognitive measures 10-point Likert scale for cognitive measure evaluation ratings

Participant Number: .....

Date: .....

Visual Analogue Scale items for measurement of subjective state

<u>Post Cognitive Test VAS</u>

1. How **hard** did you find these tests you just completed?

Not at all hard	1 2 3 4 5 6 7 8 9 10 	Extremely hard

2. How much did you **concentrate** in the tests that you just completed?

A small amount	1 2 3 4 5 6 7 8 9 10 	A large amount

3. How **well** do you think you did in the tests that you just completed?

Not at all well	1 2 3 4 5 6 7 8 9 10 	Extremely well

4. How **frustrating** did you find the tests that you just completed?

Not at all frustrating	1 2 3 4 5 6 7 8 9 10 	Extremely frustrating

Appendix 8. SAS PROC mixed models for the Choice Reaction Time (CRT)

	Number of correct trials	Reaction time of correct trials	Movement time of correct trials
Main effect terms			
Condition	$F(1,67) = 0.61$, $p = .437$	$F(1,65) = 7.25$, $p = .009$	$F(1,65) = 0.54$, $p = .467$
Week	$F(1,63) = 2.41$, $p = .126$	$F(1,65) = 0.25$, $p = .617$	$F(1,65) = 0.05$, $p = .818$
Covariates			
Baseline	$F(1,67) = 8.05$, $p = .006$	$F(1,65) = 89.32$, $p < .001$	$F(1,65) = 82.72$, $p = <.001$
Age			
IQ		$F(1,65) = 0.30$, $p = .584$	$F(1,65) = 2.40$, $p = .126$
Gender			$F(1,65) = 4.00$, $p = .050$
Trial	*Not used in the model*	$F(14,923) = 6.44$, $p < .001$	$F(14,922) = 1.70$, $p = .050$
Interaction terms			
Baseline*condition		$F(1,65) = 5.94$, $p = .018$	
Baseline*week	$F(1,63) = 1.79$, $p = .185$		
Condition*week	$F(1,63) = 0.98$, $p = .325$	$F(2,64) = 4.42$, $p = .016$	$F(1,65) = 9.67$, $p = .003$
Trial*condition*week	*Not used in the model*		$F(42,788) = 1.07$, $p = .361$
Baseline*condition*week			$F(3,1768) = 2.79$, $p = .039$
Gender*condition*week	$F(4,63) = 3.16$, $p = .020$	$F(4,64) = 7.08$, $p < .001$	$F(3,65) = 8.55$, $p < .001$
Age*condition*week			$F(4,1768) = 1.46$, $p = .213$
IQ*condition*week		$F(3,1830) = 4.12$, $p = .006$	

Note. Where no *F* value is presented, this main effect, covariate or interaction term was not retained in the final model.

Appendix 9. SAS PROC mixed models for the Simple Reaction Time (SRT)

	Number of correct trials	Reaction time of correct trials	Movement time of correct trials
Main effect terms			
Condition	$F(1,65) = 2.37$, $p = .129$	$F(1,65) = 2.20$, $p = .143$	$F(1,64) = 2.45$, $p = .122$
Week	$F(1,59) = 0.01$, $p = .919$	$F(1,65) = 5.25$, $p = .025$	$F(1,64) = 3.54$, $p = .064$
Covariates			
Baseline	$F(1,65) = 9.61$, $p = .003$	$F(1,65) = 108.03$, $p < .001$	$F(1,64) = 54.69$, $p < .001$
Age			$F(1,64) = 3.74$, $p = .058$
IQ	$F(1,65) = 0.23$, $p = .632$	$F(1,65) = 0.95$, $p = .334$	$F(1,64) = 0.31$, $p = .582$
Gender	$F(1,65) = 3.09$, $p = .084$	$F(1,65) = 4.87$, $p = .031$	
Trial	*Not used in the model*	$F(14,900) = 2.49$, $p = .002$	
Interaction terms			
Baseline*condition			$F(1,64) = 4.83$, $p = .032$
Baseline*week		$F(1,1775) = 5.32$, $p = .021$	
Condition*week	$F(1,59) = 5.81$, $p = .019$	$F(1,65) = 1.28$, $p = .262$	$F(1,64) = 8.37$, $p = .005$
Trial*condition*week	*Not used in the model*		$F(56,1673) = 1.37$, $p = .038$
Baseline*condition*week			
Gender*condition*week	$F(3,59) = 5.95$, $p = .001$	$F(3,65) = 5.67$, $p = .002$	$F(4,64) = 2.98$, $p = .025$
Age*condition*week			$F(3,1731) = 3.80$, $p = .010$
IQ*condition*week	$F(3,59) = 2.57$, $p = .063$		$F(3,1731) = 5.19$, $p = .001$

Note. Where no *F* value is presented, this main effect, covariate or interaction term was not retained in the final model.

Appendix 10. SAS PROC mixed models for the Rivermead Behavioural Memory Test for Children (RBMT-C) Task

	Immediate recall	Delayed recall
Main effect terms		
Condition	$F(1,65) = 2.06$, $p = .156$	$F(1,64) = 3.82$, $p = .055$
Week	$F(1,61) = 0.18$, $p = .675$	$F(1,61) = 0.07$, $p = .786$
Covariates		
Baseline	$F(1,65) = 47.77$, $p < .001$	
Age	$F(1,65) = 3.31$, $p = .073$	$F(1,64) = 3.52$, $p = .065$
IQ		$F(1,64) = 3.23$, $p = .077$
Gender	$F(1,65) = 4.10$, $p = .047$	$F(1,64) = 0.03$, $p = .859$
Interaction terms		
Baseline*condition		$F(1,64) = 2.32$, $p = .132$
Baseline*week		$F(1,61) = 0.02$, $p = .882$
Condition*week	$F(1,61) = 3.54$, $p = .065$	$F(1,61) = 1.25$, $p = .267$
Baseline*condition*week		$F(1,61) = 1.50$, $p = .225$
Gender*condition*week	$F(3,61) = 0.51$, $p = .678$	$F(3,61) = 2.17$, $p = .101$
Age*condition*week	$F(3,61) = 1.76$, $p = .164$	
IQ*condition*week		

Note. Where no *F* value is presented, this main effect, covariate or interaction term was not retained in the final model.

Appendix 11. SAS PROC mixed models for the Spatial Recognition Memory (SRM)

	Reaction time of correct trials	Number of correct trials
Main effect terms		
Condition	$F(1,64) = 4.10$, $p = .047$	$F(1,66) = 0.11$, $p = .739$
Week	$F(1,63) = 3.37$, $p = .071$	$F(1,60) = 4.12$, $p = .047$
Covariates		
Baseline	$F(1,64) = 77.07$, $p < .001$	$F(1,66) = 3.31$, $p = .074$
Age		
IQ	$F(1,64) = 3.96$, $p = .051$	
Gender		
Trial	$F(16,851) = 3.63$, $p < .001$	*Not used in the model*
Interaction terms		
Baseline*condition	$F(1,64) = 4.17$, $p = .045$	
Baseline*week	$F(1,1570) = 3.49$, $p = .062$	
Condition*week	$F(1,63) = 0.53$, $p = .468$	$F(1,60) = 0.15$, $p = .703$
Trial*condition*week		*Not used in the model*
Baseline*condition*week	$F(1,1570) = 4.74$, $p = .030$	$F(3,60) = 0.93$, $p = .433$
Gender*condition*week	$F(4,63) = 0.80$, $p = .532$	
Age*condition*week	$F(4,1570) = 0.30$, $p = .876$	
IQ*condition*week	$F(3,1570) = 1.41$, $p = .238$	$F(4,60) = 1.48$, $p = .220$

Note. Where no *F* value is presented, this main effect, covariate or interaction term was not retained in the final model.

Appendix 12. SAS PROC mixed models for the Motor Screening Task (MOT)

	Reaction time	Distance
Main effect terms		
Condition	$F(1,66) = 0.91$, $p = .344$	$F(1,68) = 0.00$, $p = .995$
Week	$F(1,68) = 0.30$, $p = .583$	$F(1,69) = 5.57$, $p = .021$
Covariates		
Baseline	$F(1,1260) = 6.28$, $p = .012$	$F(1,1282) = 4.52$, $p = .034$
Age	$F(1,66) = 0.16$, $p = .692$	
IQ	$F(1,66) = 0.11$, $p = .739$	
Gender		$F(1,68) = 9.09$, $p = .004$
Trial	$F(9,621) = 7.32$, $p < .001$	$F(9,621) = 1.67$, $p = .094$
Interaction terms		
Baseline*condition		$F(1,1282) = 2.03$, $p = .155$
Baseline*week	$F(1,1260) = 4.21$, $p = .041$	$F(1,1282) = 0.93$, $p = .335$
Condition*week	$F(1,68) = 7.01$, $p = .010$	
Trial*condition*week	$F(27,581) = 1.08$, $p = .362$	$F(29,599) = 0.75$, $p = .828$
Baseline*condition*week	$F(2,1260) = 9.64$, $p < .001$	
Gender*condition*week		
Age*condition*week	$F(3,1260) = 2.47$, $p = .060$	$F(4,1282) = 1.82$, $p = .122$
IQ*condition*week	$F(3,1260) = 3.70$, $p = .011$	

Note. Where no *F* value is presented, this main effect, covariate or interaction term was not retained in the final model.

Appendix 13. SAS PROC mixed models for the Spatial Span (SSP)

	Reaction time for correct trials	Number of correct trials	Highest span
Main effect terms			
Condition	*F*(1,62) = 2.39, *p* = .127	*F*(1,61) = 3.04, *p* = .086	*F*(1,63) = 3.33, *p* = .073
Week	*F*(1,62) = 0.00, *p* = .963	*F*(1,45) = 0.02, *p* = .895	*F*(1,51) = 0.69, *p* = .410
Covariates			
Baseline	*F*(1,62) = 21.82, *p* <.001	*F*(1,61) = 1.85, *p* = .179	*F*(1,63) = 21.98, *p* <.001
Age		*F*(1,61) = 6.42, *p* = .014	*F*(1,63) = 2.78, *p* = .100
IQ	*F*(1,62) = 0.20, *p* = .653	*F*(1,61) = 7.95, *p* = .007	
Gender	*F*(1,62) = 0.75, *p* = .391	*F*(1,61) = 7.71, *p* = .007	
Trial	*F*(26,983) = 240.90, *p* <.001	*Not used in the model*	*Not used in the model*
Sumattempts	*F*(10,41) = 3.31, *p* = .003	*F*(10,38) = 84.85, *p* <.001	*F*(10,41) = 36.41, *p* <.001
Hspan	*Not used in the model*	*F*(1,45) = 14646.3, *p* <.001	*Not used in the model*
Interaction terms			
Baseline*condition			
Baseline*week			
Condition*week	*F*(1,62) = 5.87, *p* = .018	*F*(1,45) = 0.89, *p* = .351	*F*(1,51) = 0.49, *p* =.487
Baseline*condition*week	*F*(3,1727) = 4.86, *p* = .002		
Trial*condition*week		*Not used in the model*	*Not used in the model*
Gender*condition*week	*F*(3,62) = 6.14, *p* = .001	*F*(3,45) = 1.32, *p* = .279	
Age*condition*week	*F*(4,1727) = 4.59, *p* = .001		
IQ*condition*week	*F*(3,1727) = 4.37, *p* = .005	*F*(3,45) = 1.40, *p* = .254	*F*(4,51) = 1.09, *p* = .372

Note. Where no *F* value is presented, this main effect, covariate or interaction term was not retained in the final model. Sumattempts = total number of attempts at each span to the highest span achieved (inclusive) on each test occasion. HSpan = Highest span achieved on each test occasion.

Appendix 14. SAS PROC mixed models for the Subjective Evaluation of Appetite, Mood, Motivation and Mental Alertness

	Hunger	Cheerfulness	Bad temper	Energy levels
Main effect terms				
Condition	$F(1,67) = 0.36$, $p = .550$	$F(1,66) = 2.73$, $p = .103$	$F(1,67) = 0.51$, $p = .478$	$F(1,67) = 1.89$, $p = .174$
Week	$F(1,67) = 1.10$, $p = .297$	$F(1,67) = 1.06$, $p = .306$	$F(1,60) = 0.07$, $p = .794$	$F(1,67) = 0.29$, $p = .594$
Covariates				
Baseline	$F(1,67) = 25.95$, $p < .001$	$F(1,66) = 9.69$, $p = .003$	$F(1,67) = 30.41$, $p < .001$	$F(1,67) = 35.78$, $p < .001$
Interaction terms				
Baseline*condition		$F(1,66) = 1.48$, $p = .228$		
Baseline*week			$F(1,60) = 3.01$, $p = .088$	
Condition*week	$F(1,67) = 0.04$, $p = .843$	$F(1,67) = 0.35$, $p = .555$	$F(1,60) = 0.05$, $p = .818$	$F(1,67) = 0.05$, $p = .823$
Baseline*condition*week			$F(2,60) = 9.61$, $p < .001$	

Appendix 14 continued. SAS PROC mixed models for the Subjective Evaluation of Appetite, Mood, Motivation and Mental Alertness

	Keenness to try hard	Ease of distraction	Ease of focussing	Wakefulness
Main effect terms				
Condition	$F(1,67) = 0.16$, $p = .692$	$F(1,66) = 6.27$, $p = .015$	$F(1,66) = 0.72$, $p = .399$	$F(1,67) = 0.13$, $p = .722$
Week	$F(1,63) = 3.96$, $p = .051$	$F(1,67) = 0.00$, $p = .966$	$F(1,65) = 0.11$, $p = .742$	$F(1,66) = 4.20$, $p = .045$
Covariates				
Baseline	$F(1,67) = 22.37$, $p < .001$	$F(1,66) = 19.43$, $p < .001$	$F(1,66) = 17.13$, $p < .001$	$F(1,67) = 41.73$, $p < .001$
Interaction terms				
Baseline*condition		$F(1,66) = 4.99$, $p = .029$	$F(1,66) = 1.94$, $p = .168$	
Baseline*week			$F(1,65) = 0.06$, $p = .812$	$F(1,66) = 2.05$, $p = .157$
Condition*week	$F(1,63) = 8.99$, $p = .004$	$F(1,67) = 0.17$, $p = .682$	$F(1,65) = 4.66$, $p = .035$	$F(1,66) = 1.12$, $p = .294$
Baseline*condition*week	$F(3,63) = 4.10$, $p = .010$		$F(1,65) = 4.80$, $p = .032$	

Note. Where no *F* value is presented, this main effect, covariate or interaction term was not retained in the final model.

Appendix 15. SAS PROC mixed models for the Cognitive Test Evaluation Ratings

	Test battery difficulty	Perceived concentration	Perceived performance	Frustration
Main effect terms				
Condition	$F(1,67) = 0.32$, $p = .574$	$F(1,67) = 1.37$, $p = .247$	$F(1,67) = 1.73$, $p = .193$	$F(1,67) = 0.30$, $p = .585$
Week	$F(1,68) = 2.67$, $p = .107$	$F(1,66) = 0.05$, $p = .832$	$F(1,68) = 0.26$, $p = .611$	$F(1,68) = 0.08$, $p = .783$
Covariates				
Baseline	$F(1,67) = 25.61$, $p < .001$	$F(1,67) = 37.29$, $p < .001$	$F(1,67) = 20.09$, $p < .001$	$F(1,67) = 17.72$, $p < .001$
Interaction terms				
Baseline*condition				
Baseline*week				
Condition*week	$F(1,68) = 0.04$, $p = .840$	$F(1,66) = 0.32$, $p = .575$	$F(1,68) = 0.64$, $p = .427$	$F(1,68) = 2.86$, $p = .096$
Baseline*condition*week				

Note. Where no *F* value is presented, this main effect, covariate or interaction term was not retained in the final model.

Appendix 16. SAS PROC mixed models for the Attention Switching Task (acute supplementation)

	Target accuracy	Target reaction time	Switch cost accuracy	Switch cost reaction time
Main effect terms				
Condition	$F(1,142) = 0.96$, $p = .328$	$F(1,141) = 4.24$, $p = .041$	$F(1,92) = 0.02$, $p = .898$	$F(1,91) = 7.46$, $p = .008$
Trial	$F(2,142) = 1.11$, $p = .334$	$F(2,141) = 5.90$, $p = .004$	$F(1,92) = 5.55$, $p = .021$	$F(1,91) = 6.38$, $p = .013$
Covariates				
Baseline	$F(1,142) = 168.68$, $p < .001$	$F(1,141) = 0.92$, $p = .339$	$F(1,92) = 11.63$, $p = .001$	$F(1,91) = 0.03$, $p = .863$
Age			$F(1,92) = 4.44$, $p = .038$	$F(1,91) = 1.27$, $p = .262$
IQ			$F(1,92) = 0.58$, $p = .447$	
Gender			$F(1,92) = 1.62$, $p = .206$	$F(1,91) = 2.89$, $p = .093$
Accuracy	*Not used in the model, as this was the outcome*	$F(1,141) = 605.26$, $p < .001$	*Not used in the model, as this was the outcome*	$F(1,91) = 324.43$, $p < .001$
Interaction term				
Baseline*condition		$F(1,141) = 6.20$, $p = .014$		$F(1,91) = 3.86$, $p = .052$
Trial*condition	$F(2,142) = 0.06$, $p = .939$	$F(2,141) = 1.41$, $p = .248$	$F(1,92) = 0.12$, $p = .727$	$F(1,91) = 0.00$, $p = .989$

Notes. Where no *F* value is presented, this main effect, covariate or interaction term was not retained in the final model. Accuracy: target accuracy used in target reaction time analysis / switch cost accuracy used in switch cost reaction time analysis.

Appendix 17. SAS PROC mixed models for the Rapid Visual Information Processing Task (acute supplementation)

	Total hits	**Total false alarms**	**Reaction time for hits**
Main effect terms			
Condition	$F(1,46) = 6.80$, $p = .012$	$F(1,42) = 0.44$, $p = .508$	$F(1,864) = 8.41$, $p = .004$
Covariates			
Baseline	$F(1,46) = 158.97$, $p < .001$	$F(1,42) = 91.37$, $p < .001$	$F(1,864) = 83.16$, $p < .001$
Age			$F(1,864) = 12.54$, $p < .001$
IQ		$F(1,42) = 10.44$, $p = .002$	
Gender		$F(1,42) = 0.55$, $p = .461$	$F(1,864) = 8.02$, $p = .005$
Trial	*Not used in the model*	*Not used in the model*	
Total correct	*Not used in the model*	*Not used in the model*	$F(1,864) = 25.49$, $p < .001$
Interaction term			
Baseline*condition		$F(1,42) = 1.19$, $p = .281$	$F(1,864) = 10.86$, $p = .001$

Notes. Where no *F* value is presented, this main effect, covariate or interaction term was not retained in the final model. Total correct: Number of correct trials obtained.

Appendix 18. SAS PROC mixed models for the N-back Task (acute supplementation)

	Target accuracy	Total accuracy	Reaction time for targets	Reaction time for nontargets
Main effect terms				
Condition	$F(1,44) = 7.55$, $p = .009$	$F(1,44) = 3.57$, $p = .066$	$F(1,1891) = 1.98$, $p = .160$	$F(1,4971) = 147.23$, $p < .001$
Covariates				
Baseline	$F(1,44) = 84.60$, $p < .001$	$F(1,44) = 65.56$, $p < .001$	$F(1,1891) = 252.76$, $p < .001$	$F(1,4971) = 228.61$, $p < .001$
Age				$F(1,4971) = 3.87$, $p = .049$
IQ	$F(1,44) = 7.30$, $p = .010$	$F(1,44) = 7.21$, $p = .010$	$F(1,1891) = 1.30$, $p = .255$	$F(1,4971) = 172.29$, $p < .001$
Gender	$F(1,44) = 2.89$, $p = .096$	$F(1,44) = 2.12$, $p = .152$	$F(1,1891) = 2.79$, $p = .095$	$F(1,4971) = 19.18$, $p < .001$
Trial	*Not used in the model*	*Not used in the model*	$F(1,1891) = 5.25$, $p = .022$	$F(1,4971) = 4.92$, $p = .027$
Total correct	*Not used in the model*	*Not used in the model*	$F(1,1891) = 6.90$, $p = .009$	$F(1,4971) = 335.01$, $p < .001$
Interaction term				
Baseline*condition	$F(1,44) = 6.90$, $p = .012$	$F(1,44) = 3.82$, $p = .057$	$F(1,1891) = 1.56$, $p = .211$	$F(1,4971) = 138.37$, $p < .001$

Notes. Where no *F* value is presented, this main effect, covariate or interaction term was not retained in the final model. Total correct: Number of correct trials obtained.

Appendix 19. SAS PROC mixed models for the Pattern Separation Task (acute supplementation)

	Pattern separation score	Recognition score
Main effect terms		
Condition	$F(1,44) = 1.04$, $p = .312$	$F(1,45) = 4.60$, $p = .038$
Covariates		
Baseline	$F(1,44) = 30.71$, $p < .001$	$F(1,45) = 36.05$, $p < .001$
Age		
IQ		
Gender	$F(1,44) = 3.05$, $p = .088$	$F(1,45) = 0.37$, $p = .547$
Interaction term		
Baseline*condition		$F(1,45) = 8.25$, $p = .006$

Note. Where no *F* value is presented, this main effect, covariate or interaction term was not retained in the final model.

Appendix 20. SAS PROC mixed models for the Face-Name Associative Memory Exam (acute supplementation)

	Immediate recall	Delayed recall
Main effect terms		
Condition	$F(1,46) = 0.00$, $p = .978$	$F(1,46) = 3.60$, $p = .064$
Covariates		
Baseline	$F(1,46) = 10.60$, $p = .002$	$F(1,46) = 45.09$, $p < .001$
Age		
IQ		$F(1,46) = 2.03$, $p = .161$
Gender	$F(1,46) = 1.97$, $p = .167$	
Interaction term		
Baseline*condition		

Note. Where no *F* value is presented, this main effect, covariate or interaction term was not retained in the final model.

Appendix 21. SAS PROC mixed models for the Visual Verbal Learning Test (VVLT) (acute supplementation)

	Rate of learning	New learning	Retroactive interference	Proactive interference
Main effect terms				
Condition	$F(1,140) = 0.11$, $p = .737$	$F(1,44) = 0.16$, $p = .689$	$F(1,45) = 1.46$, $p = .233$	$F(1,45) = 0.09$, $p = .770$
Trial	$F(2,140) = 20.29$, $p < .001$	*Not used in the model*	*Not used in the model*	*Not used in the model*
Covariates				
Baseline	$F(1,140) = 25.36$, $p < .001$	$F(1,44) = 15.69$, $p < .001$	$F(1,45) = 2.43$, $p = .126$	$F(1,45) = 1.38$, $p = .247$
Age	$F(1,140) = 7.21$, $p = .008$	$F(1,44) = 4.30$, $p = .044$		$F(1,45) = 4.46$, $p = .040$
IQ	$F(1,140) = 17.22$, $p < .001$			
Gender		$F(1,44) = 0.92$, $p = .343$	$F(1,45) = 0.54$, $p = .468$	$F(1,45) = 0.79$, $p = .379$
Interaction term				
Baseline*condition		$F(1,44) = 0.16$, $p = .690$	$F(1,45) = 0.26$, $p = .609$	
Trial*condition	$F(2,140) = 0.06$, $p = .941$	*Not used in the model*	*Not used in the model*	*Not used in the model*

Note. Where no *F* value is presented, this main effect, covariate or interaction term was not retained in the final model.

Appendix 21 continued. SAS PROC mixed models for the Visual Verbal Learning Test (VVLT) (acute supplementation)

	Delayed recall
Main effect terms	
Condition	*F*(1,44) = 3.94, *p* = .053
Trial	*Not used in the model*
Covariates	
Baseline	*F*(1,44) = 36.21, *p* <.001
Age	*F*(1,44) = 6.05, *p* = .018
IQ	
Gender	*F*(1,44) = 0.13, *p* = .722
Interaction term	
Baseline*condition	*F*(1,44) = 2.01, *p* = .164
Trial*condition	*Not used in the model*

Note. Where no *F* value is presented, this main effect, covariate or interaction term was not retained in the final model.

Appendix 22. SAS PROC mixed models for the Attention Switching Task (chronic supplementation)

	Target accuracy	Target reaction time	Switch cost accuracy	Switch cost reaction time
Main effect terms				
Condition	$F(1,45) = 0.65$, $p = .426$	$F(1,46) = 0.65$, $p = .424$	$F(1,45) = 2.85$, $p = .098$	$F(1,45) = 0.05$, $p = .833$
Week	$F(1,41) = 5.37$, $p = .026$	$F(1,41) = 5.83$, $p = .020$	$F(1,41) = 0.00$, $p = .982$	$F(1,42) = 2.00$, $p = .165$
Trial	$F(2,92) = 16.20$, $p < .001$	$F(2,94) = 12.99$, $p < .001$	$F(1,47) = 28.65$, $p < .001$	$F(1,47) = 11.66$, $p = .001$
Covariates				
Baseline	$F(1,218) = 85.67$, $p < .001$		$F(1,127) = 26.81$, $p < .001$	$F(1,127) = 1.25$, $p = .266$
Age	$F(1,45) = 5.70$, $p = .021$			
IQ			$F(1,45) = 3.88$, $p = .055$	
Gender				$F(1,45) = 1.11$, $p = .297$
Accuracy	*Not used in the model, as this was the outcome*	$F(1,220) = 1075.44$, $p < .001$	*Not used in the model, as this was the outcome*	$F(1,127) = 487.83$, $p < .001$
Interaction terms				
Baseline*condition		$F(1,220) = 5.02$, $p = .026$	$F(1,127) = 5.28$, $p = .023$	
Baseline*week		$F(1,220) = 4.73$, $p = .031$	$F(1,127) = 2.57$, $p = .111$	$F(1,127) = 4.07$, $p = .046$
Condition*week	$F(1,41) = 5.15$, $p = .029$	$F(1,41) = 0.22$, $p = .643$	$F(1,41) = 2.61$, $p = .114$	$F(1,42) = 5.36$, $p = .026$
Trial*condition	$F(2,92) = 1.27$, $p = .285$			
Trial*condition*week			$F(3,43) = 0.92$, $p = .439$	
Baseline*condition*week		$F(1,220) = 4.09$, $p = .045$	$F(1,127) = 3.97$, $p = .049$	$F(2,127) = 0.51$, $p = .602$
Gender*condition*week	$F(4,41) = 1.53$, $p = .212$	$F(4,41) = 1.59$, $p = .196$	$F(4,41) = 3.17$, $p = .023$	$F(3,42) = 1.12$, $p = .354$
Age*condition*week	$F(3,218) = 1.92$, $p = .128$			
IQ*condition*week	$F(4,218) = 3.52$, $p = .008$	$F(4,220) = 1.16$, $p = .330$		$F(4,127) = 1.95$, $p = .106$

Notes. Where no *F* value is presented, this main effect, covariate or interaction term was not retained in the final model. Accuracy: target accuracy used in target reaction time analysis / switch cost accuracy used in switch cost reaction time analysis.

Appendix 23. SAS PROC mixed models for the Rapid Visual Information Processing Task (chronic supplementation)

	Total hits	Total false alarms	Reaction time for hits
Main effect terms			
Condition	$F(1,43) = 0.46$, $p = .502$	$F(1,41) = 7.22$, $p = .010$	$F(1,42) = 1.88$, $p = .178$
Week	$F(1,35) = 0.91$, $p = .346$	$F(1,38) = 2.18$, $p = .148$	$F(1,41) = 4.66$, $p = .037$
Covariates			
Baseline	$F(1,43) = 137.44$, $p < .001$	$F(1,41) = 61.25$, $p < .001$	$F(1,42) = 29.43$, $p < .001$
Age	$F(1,43) = 4.94$, $p = .032$	$F(1,41) = 6.30$, $p = .016$	
IQ	$F(1,43) = 7.82$, $p = .008$		$F(1,42) = 4.52$, $p = .039$
Gender			$F(1,42) = 3.80$, $p = .058$
Trial	*Not used in the model*	*Not used in the model*	$F(1,1628) = 5.34$, $p = .021$
Total correct	*Not used in the model*	*Not used in the model*	$F(1,1628) = 10.07$, $p = .002$
Interaction term			
Baseline*condition		$F(1,41) = 7.46$, $p = .009$	$F(1,42) = 2.16$, $p = .149$
Baseline*week		$F(1,38) = 3.08$, $p = .087$	$F(1,1628) = 5.29$, $p = .022$
Condition*week	$F(1,35) = 0.31$, $p = .581$	$F(1,38) = 0.03$, $p = .863$	$F(1,41) = 0.12$, $p = .728$
Baseline*condition*week			
Gender*condition*week	$F(4,35) = 0.81$, $p = .527$	$F(4,38) = 0.21$, $p = .929$	$F(3,41) = 0.87$, $p = .463$
Age*condition*week	$F(3,35) = 2.21$, $p = .104$		
IQ*condition*week	$F(3,35) = 2.45$, $p = .080$		

Notes. Where no *F* value is presented, this main effect, covariate or interaction term was not retained in the final model. Total correct: Number of correct trials obtained.

Appendix 24. SAS PROC mixed models for the N-back Task (chronic supplementation)

	Target accuracy	Total accuracy	Reaction time for targets	Reaction time for nontargets
Main effect terms				
Condition	$F(1,45) = 3.36$, $p = .073$	$F(1,45) = 4.66$, $p = .036$	$F(1,43) = 3.60$, $p = .064$	$F(1,42) = 0.02$, $p = .878$
Week	$F(1,33) = 0.13$, $p = .724$	$F(1,31) = 0.46$, $p = .502$	$F(1,44) = 0.41$, $p = .527$	$F(1,45) = 11.38$, $p = .002$
Covariates				
Baseline	$F(1,45) = 96.29$, $p < .001$	$F(1,45) = 89.13$, $p < .001$	$F(1,43) = 111.90$, $p < .001$	$F(1,42) = 18.63$, $p < .001$
Age				
IQ				$F(1,42) = 4.61$, $p = .038$
Gender				$F(1,42) = 2.28$, $p = .139$
Trial	*Not used in the model*	*Not used in the model*		$F(1,9423) = 15.42$, $p < .001$
Total correct	*Not used in the model*	*Not used in the model*		$F(1,9423) = 108.22$, $p < .001$
Interaction term				
Baseline*condition			$F(1,43) = 1.50$, $p = .228$	$F(1,42) = 14.35$, $p = .001$
Baseline*week	$F(1,33) = 3.68$, $p = .064$	$F(1,31) = 4.94$, $p = .034$		
Condition*week	$F(1,33) = 1.20$, $p = .282$	$F(1,31) = 0.51$, $p = .479$	$F(1,44) = 1.73$, $p = .195$	$F(1,45) = 4.82$, $p = .033$
Baseline*condition*week	$F(2,33) = 2.36$, $p = .111$	$F(2,31) = 2.02$, $p = .150$		
Gender*condition*week	$F(4,33) = 0.92$, $p = .465$	$F(4,31) = 0.55$, $p = .702$		
Age*condition*week	$F(4,33) = 1.69$, $p = .175$	$F(4,31) = 1.83$, $p = .148$	$F(4,3539) = 1.13$, $p = .342$	$F(4,9423) = 5.04$, $p = .001$
IQ*condition*week			$F(4,3539) = 1.53$, $p = .191$	$F(3,9423) = 2.88$, $p = .034$

Notes. Where no *F* value is presented, this main effect, covariate or interaction term was not retained in the final model. Total correct: Number of correct trials obtained.

Appendix 25. SAS PROC mixed models for the Pattern Separation Task (chronic supplementation)

	Pattern separation score	Recognition score
Main effect terms		
Condition	$F(1,45) = 5.08$, $p = .029$	$F(1,44) = 0.17$, $p = .685$
Week	$F(1,37) = 0.41$, $p = .525$	$F(1,38) = 0.08$, $p = .778$
Covariates		
Baseline	$F(1,45) = 47.15$, $p < .001$	$F(1,44) = 21.91$, $p < .001$
Age		
IQ		
Gender		
Interaction term		
Baseline*condition		$F(1,44) = 0.69$, $p = .410$
Baseline*week		$F(1,38) = 0.26$, $p = .612$
Condition*week	$F(1,37) = 0.43$, $p = .518$	$F(1,38) = 2.31$, $p = .137$
Baseline*condition*week		$F(1,38) = 5.73$, $p = .022$
Gender*condition*week	$F(4,37) = 0.65$, $p = .629$	$F(4,38) = 4.99$, $p = .003$
Age*condition*week	$F(4,37) = 2.06$, $p = .106$	
IQ*condition*week		

Note. Where no *F* value is presented, this main effect, covariate or interaction term was not retained in the final model.

Appendix 26. SAS PROC mixed models for the Face-Name Associative Memory Exam (chronic supplementation)

	Immediate recall	Delayed recall
Main effect terms		
Condition	$F(1,43) = 1.76$, $p = .192$	$F(1,42) = 0.04$, $p = .837$
Week	$F(1,39) = 1.70$, $p = .199$	$F(1,40) = 6.72$, $p = .013$
Covariates		
Baseline	$F(1,43) = 53.27$, $p < .001$	$F(1,42) = 70.94$, $p < .001$
Age	$F(1,43) = 5.37$, $p = .025$	$F(1,42) = 4.67$, $p = .036$
IQ		$F(1,42) = 0.00$, $p = .957$
Gender		
Interaction term		
Baseline*condition	$F(1,43) = 0.68$, $p = .414$	$F(1,42) = 0.11$, $p = .736$
Baseline*week	$F(1,39) = 0.57$, $p = .453$	
Condition*week	$F(1,39) = 4.83$, $p = .034$	$F(1,40) = 0.24$, $p = .628$
Baseline*condition*week	$F(1,39) = 5.39$, $p = .026$	$F(2,40) = 6.23$, $p = .004$
Gender*condition*week	$F(4,39) = 1.24$, $p = .309$	
Age*condition*week		
IQ*condition*week		$F(3,40) = 2.69$, $p = .059$

Note. Where no *F* value is presented, this main effect, covariate or interaction term was not retained in the final model.

Appendix 27. SAS PROC mixed models for the Visual Verbal Learning Test (VVLT) (chronic supplementation)

	Rate of learning	New learning	Retroactive interference	Proactive interference
Main effect terms				
Condition	$F(1,43) = 2.25$, $p = .141$	$F(1,44) = 0.44$, $p = .512$	$F(1,40) = 2.86$, $p = .098$	$F(1,42) = 0.60$, $p = .442$
Week	$F(1,42) = 2.32$, $p = .136$	$F(1,42) = 0.44$, $p = .511$	$F(1,41) = 3.89$, $p = .055$	$F(1,38) = 0.13$, $p = .726$
Trial	$F(2,94) = 81.73$, $p < .001$	*Not used in the model*	*Not used in the model*	*Not used in the model*
Covariates				
Baseline	$F(1,225) = 14.07$, $p < .001$	$F(1,44) = 33.11$, $p < .001$		$F(1,42) = 1.15$, $p = .290$
Age	$F(1,43) = 6.13$, $p = .017$		$F(1,40) = 7.55$, $p = .009$	$F(1,42) = 0.32$, $p = .577$
IQ	$F(1,43) = 11.73$, $p = .001$		$F(1,40) = 0.31$, $p = .582$	$F(1,42) = 0.43$, $p = .516$
Gender	$F(1,43) = 5.77$, $p = .021$	$F(1,44) = 0.45$, $p = .505$	$F(1,40) = 7.48$, $p = .009$	
Interaction term				
Baseline*condition			$F(2,40) = 6.60$, $p = .003$	$F(1,42) = 3.56$, $p = .066$
Baseline*week				$F(1,38) = 4.15$, $p = .049$
Condition*week	$F(1,42) = 13.31$, $p < .001$	$F(1,42) = 5.09$, $p = .029$	$F(1,41) = 5.53$, $p = .024$	$F(1,38) = 0.61$, $p = .441$
Trial*condition		*Not used in the model*	*Not used in the model*	*Not used in the model*
Trial*condition*week		*Not used in the model*	*Not used in the model*	*Not used in the model*
Baseline*condition*week				
Gender*condition*week	$F(3,42) = 0.66$, $p = .580$	$F(3,42) = 1.83$, $p = .156$	$F(3,41) = 1.42$, $p = .251$	$F(4,38) = 2.56$, $p = .054$
Age*condition*week	$F(3,225) = 5.10$, $p = .002$			
IQ*condition*week				

Note. Where no *F* value is presented, this main effect, covariate or interaction term was not retained in the final model.

Appendix 27 continued. SAS PROC mixed models for the Visual Verbal Learning Test (VVLT) (chronic supplementation)

	Delayed recall
Main effect terms	
Condition	$F(1,43) = 6.27$, $p = .016$
Week	$F(1,38) = 2.17$, $p = .149$
Trial	*Not used in the model*
Covariates	
Baseline	$F(1,43) = 39.85$, $p < .001$
Age	
IQ	
Gender	$F(1,43) = 2.38$, $p = .130$
Interaction term	
Baseline*condition	$F(1,43) = 3.68$, $p = .062$
Baseline*week	
Condition*week	$F(1,38) = 0.25$, $p = .621$
Trial*condition	*Not used in the model*
Trial*condition*week	*Not used in the model*
Baseline*condition*week	
Gender*condition*week	$F(3,38) = 0.89$, $p = .454$
Age*condition*week	
IQ*condition*week	$F(4,38) = 3.37$, $p = .019$

Note. Where no *F* value is presented, this main effect, covariate or interaction term was not retained in the final model.

Appendix 28. SAS PROC mixed models for the Cognitive Failures Questionnaire

	Cognitive Failures Questionnaire
Main effect terms	
Condition	$F(1,44) = 1.21$, $p = .277$
Week	$F(1,41) = 2.29$, $p = .138$
Covariates	
Baseline	$F(1,44) = 77.74$, $p < .001$
Age	
IQ	
Gender	
Interaction term	
Baseline*condition	$F(1,44) = 0.53$, $p = .473$
Baseline*week	$F(1,41) = 4.89$, $p = .033$
Condition*week	$F(1,41) = 4.89$, $p = .033$
Baseline*condition*week	$F(1,41) = 5.16$, $p = .028$
Gender*condition*week	
Age*condition*week	
IQ*condition*week	

Note. Where no *F* value is presented, this main effect, covariate or interaction term was not retained in the final model.

Appendix 29. Study 2 study advert

IS YOUR MEMORY AS GOOD AS IT USED TO BE?

Nutrition & Cognition

If not, how about volunteering for a research study exploring the potential beneficial effects of a nutritional supplement (all ingredients from everyday food items) on memory and other cognitive functions.

You will receive up to £40.00 to cover travel costs and to compensate for time lost.

Arla

UNIVERSITY OF LEEDS

This research is subject to ethical guidelines set out by the British Psychological Society and has received ethical approval (ref no: PSC-289; date approved: 15/02/2018). The primary supervisor of this research is Professor Louise Dye (l.dye@leeds.ac.uk).

Eligibility:

- Aged 50 or over;
- No serious medical condition;
- Not lactose intolerant.

What you will do:

- Take a flavoured (strawberry or chocolate) drink based supplement once a day for 12 weeks;
- Be seen on 4 occasions. Following the screening visit, study visits, lasting 1 hour, are spaced 6 weeks apart and can take place either at home or at the University;
- Undertake cognitive exercises on a laptop and paper based questionnaires during study visits.

Contact:

For more information, contact Claire Champ:

07480 518 960

memory-study@leeds.ac.uk

Appendix 30. Study 2 participant information sheet

Investigating the benefits of a 12 week phospholipid intervention on cognitive performance in adults with a subjective memory complaint.

You are being invited to take part in a research project. Before you decide, it is important for you to understand why the research is being done and what it will involve. Please take time to read the following information carefully and discuss it with others if you wish. Ask me if there is anything that is not clear or if you would like more information. Take time to decide whether or not you wish to take part.

Purpose of the research

We wish to find out whether consuming a nutritional intervention, the details of which will follow, for 12 weeks will improve performance on a range of cognitive measures that assess memory and processing speed, and reduce the number of cognitive lapses e.g. entering a room and forgetting why you wanted to go in there, experienced day to day. Cognitive failures include behaviours such as clumsiness and episodes of forgetfulness.

What exactly are the supplements?

The active supplement contains multiple phospholipids, which are thought to help cell function and brain activity. Phospholipids are available from a range of foods consumed as part of a regular diet and therefore, feature part of people's every day nutritional intake. The phospholipids in the active supplement have been extracted from cow's milk. The non-active or placebo supplement does not contain phospholipids but instead contains maltodextrin, a food additive used in many food products. The additional ingredients that make up the rest of both supplements include items typically found in a regular diet. Both supplements are available in strawberry and chocolate flavour and are provided as a powder, which is added to water to form a nutritional drink. If you decide to attend a screening appointment, you will have the opportunity to sample the supplement in both flavours. Before doing so, you will be shown a full list of the ingredients in case of a pre-existing food intolerance/allergy. This can be supplied prior to screening if necessary.

Why have I been chosen?

There is a need to develop the research area concerning phospholipid supplementation. An increasing aged population means that greater emphasis is placed on ageing well. This study will add to the findings of similar studies and explore whether phospholipids can promote healthy ageing. To see whether you may be eligible to take part, please refer to the study inclusion and exclusion criteria in Appendix 1 at the end of this document.

What will I have to do?

A study appointment break down and study timeline both available in Appendix 1 of this document accompany this section.

All appointments can take place at the University or at home.

Screening appointment:
You will learn more about the study, sample the supplement in both flavours, complete a version of the cognitive measures and be shown the self-report measures to be completed over the course of the study. Further, conditional upon you signing the study consent form, you will undertake screening measures led by the researcher, provide sociodemographic and some health details and undertake a measure of anthropometry (waist-to-hip measurement). At the end of this appointment, provided that you have chosen to enter into the study *and* you meet the study inclusion and exclusion criteria (based on the details you have provided), you will be given six short forms to takeaway and complete in your own time before the next appointment. All future appointments - you will be seen on three further occasions across 12 weeks - will also be scheduled at this time. The screening appointment is expected to take ~120 minutes.

Intervention study:
You will complete a number of self-report questionnaires and computerised cognitive measures (that you will be shown during the screening appointment). In order to explore whether there are any benefits of the active supplement after a single dose, at the first appointment following screening, the self-report and computerised cognitive measures will be conducted twice: once before the consumption of the supplement and again 90 minutes post-ingestion of the supplement. This appointment will take approx. 2 hours, with a break of 90 minutes in between. Please note: there is no requirement for the researcher to remain with you during the 90 minute break. All subsequent appointments will last up to 1 hour. Fifty sachets (packaged together) containing the supplement that you have been randomly allocated to receive (so there is an equal chance of getting either the active or the placebo supplement) will be provided at the first appointment following screening. You will be required to take the contents of 1 sachet per day with 120ml of water. You will also be given a consumption diary, which you will be asked to complete each day. Using this, you can say whether or not you took the supplement on each day of the 12 week intervention period. Please note: all participants are strongly encouraged to take the supplement each day for the whole intervention period. All empty and part-empty supplement sachets should be kept with the remaining sachets to be collected at the next appointment. Half way through the 12 week period, you will receive the next 50 sachets to be consumed over the rest of the 12 weeks, again having 1 per day.

What are the possible disadvantages and risks of taking part?

There are no known risks/disadvantages to taking part in this study. The composite of phospholipids found in the active supplement has been approved for use by an expert panel as safe, suitable, and 'Generally Recognized As Safe' (GRAS). Given that the other ingredients in both supplements are available from / used in regular food products, it is not anticipated that there is any risk associated with consuming the supplements.

You will receive £10.00 per appointment to cover travel costs and compensate for time lost.

What are the possible benefits of taking part?

For those allocated to the active supplement, the increased intake of phospholipids may facilitate improvements in your cognitive function. However, bear in mind that there is mixed

evidence regarding the efficacy of phospholipid supplementation. By participating in this study, you will play a role in extending the current knowledge base.

Do I have to take part?

It is up to you to decide whether or not to take part. If you do decide to take part, you will be given this information sheet to keep (and be asked to sign a consent form at screening). You will be able to withdraw at any time up to 2 weeks (10 working days) following your participation. You do not have to give a reason should you wish to withdraw and will not be disadvantaged if you choose to do so. We may ask for your permission to keep the data collected so far to use in the final analysis.

Will my taking part in this project be kept confidential?

All procedures for handling, processing, storing, sharing and destroying participant data meet with Data Protection Act 1998. All email communication will be stored on the University secure network and will be password protected. The consent form along with all paper records and measures will be stored in a lockable cabinet on the University campus, which can be accessed by staff and project students only. The consent form will be the only form that records both your full name and your unique participant ID. This will be stored separately to all other paperwork. The latter will be identified using participant IDs only.

All data obtained from the cognitive measures will be anonymised, so will not contain any of your personal information, stored on the University secure network, and will be password protected. Following the completion of the study, a copy of the final composite data set (containing the data for all participants) will be deposited onto the Research Data Leeds repository in an anonymised format. This will be referenced in any subsequent publication, where readers will be able to access the data on the repository. We may share this anonymised data with Arla Food Ingredients Group P/S based in Denmark, who have prepared the supplements, should they request this.

Withdrawing

Participation is voluntary and refusal to participate / withdrawal from the study up to 10 working days following participation over the 12 weeks will not result in any consequences.

What will happen to the results of the research project?

The results will further the current knowledge concerning the use of phospholipids as a nutritional supplement. They will also be used towards an educational qualification by the researcher (Claire Champ). There may also be scope to use the results to support Level 3 undergraduate dissertation projects. Whether the data gets used for this purpose depends upon whether such a project is selected by year three undergraduate students. The results may further form the basis for a publication; however, participants will not be identifiable from any details in reports, presentations or scientific publications. All data, apart from the anonymised composite data set available on the Research Data Leeds repository, obtained from participants whom completed the study and did not withdraw within the 10 working day period following participation will be retained for a maximum period of 3 years before being destroyed.

What if there is a problem?

If you have a concern about the study, please contact the researcher (Claire Champ) using the details below in the first instance. If you have a complaint about the researcher or would prefer to speak to someone other than Claire, please contact the main supervisor – details given below.

Who is funding the research?

This study is funded by the Economic and Social Research Council (ESRC) and the supplements were manufactured by Arla Food Ingredients Group P/S.

Contact information

If you would like any more information on the study, please contact:

Claire Champ	memory-study@leeds.ac.uk	07480 518 960

The main supervisor of this research is:

Professor Louise Dye	l.dye@leeds.ac.uk	0113 343 5707

This research is subject to ethical guidelines set out by the British Psychological Society and has received ethical approval (ref no:PSC-289; date approved:14/02/2018).

Study inclusion and exclusion criteria

Inclusion criteria	Exclusion criteria
- Aged 50 years of age and over.	- Any psychiatric or medical disorder that could interfere with cognitive function.
- Experience reduced memory function where you feel your memory is not as good as it was.	- Use of any drugs that affect the brain, including prescription, herbal and diet supplements.
- Do not have any cognitive impairment as diagnosed by a health professional.	- Evidence of delirium, confusion or other disturbance of consciousness.
- Able to consent to participate in the study and willing to consume the supplement and complete a consumption diary daily for 12 weeks.	- Any neurological disorder including dementia, Parkinson's disease, stroke, focal brain lesions, multiple sclerosis, and epilepsy.
- Willing and able to participate in screening and testing measures on four occasions (screening x 1, testing session x 4).	- Current diagnosis or history of alcoholism or drug dependence.
- Able to follow verbal and simple written instructions in English.	- Have a history of any of infective or inflammatory brain disease.
- Has normal vision and hearing, with appropriate corrective aids if required.	- Have a history of head injury.
- Able to understand cognitive testing instructions and responding requirements.	- Have colour vision deficiency or dyslexia.
- Comfortable with a researcher conducting screening and testing measures.	- Lactose intolerant and/or suspects or knows they have an allergy to any ingredient in the active and placebo supplements.

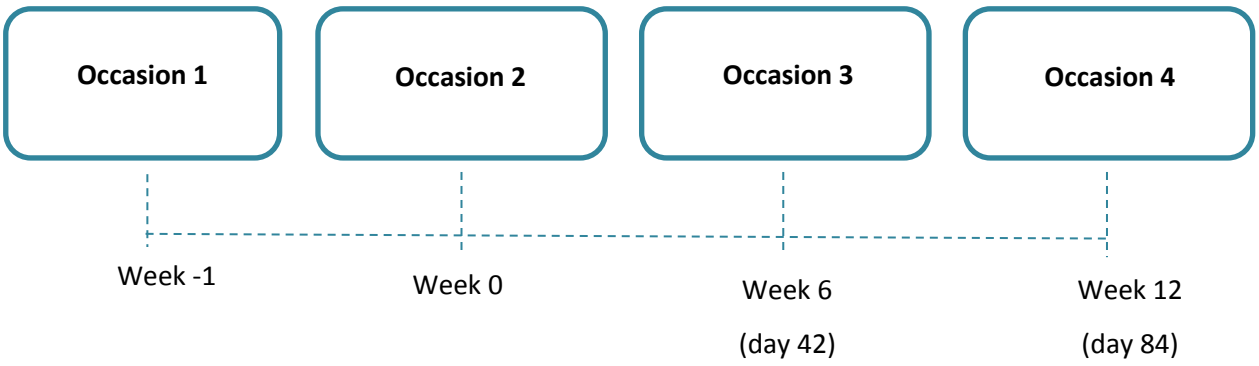
Should you have any questions about any of the above criteria, please contact Claire Champ using the contact details on the last page of this form.

Study appointment break down

First occasion: screening appointment	1	Study briefing: Discussion focused on what the study is and what you will be required to do	**Appointment length**
	2	Supplement tasting	
	3	Completion of a practise version of the cognitive measures and familiarisation with the self-report measures.	**Up to 120 minutes**
	4	Opportunity to ask any questions followed by signing of the study consent form	
	5	Completion of screening measures, sociodemographic and health form, and anthropometric measurement.	
Second occasion	1	Cognitive measures x 1	**Up to 1 hour either side of a 90 minute break***
	2	First supplement - demonstration and consumption	
	3	90 minutes after taking supplement, cognitive measures x 1	
Third occasion	1	Cognitive measures x 1	**Up to 1 hour**
Fourth occasion	1	Cognitive measures x 1	**Up to 1 hour**

*The first set of cognitive measures on the second occasion will act as baseline measures. After taking the supplement, the second set of cognitive measures will be ran 90 minutes later to explore the effects of a single dose of the supplement.

Study timeline



Appendix 31. Study 2 consumption diary

Supplement consumption diary

Day 1

Date: ...../...../.....

Mix the supplement with 120ml of water. Have you taken the supplement today?

Yes, I have taken it ☐

No, I have not taken it ☐

Not taken the supplement today? Please give your reason(s)below:

Have you experienced an adverse event? Please give details below:

Day 2

Date: ...../...../.....

Mix the supplement with 120ml of water. Have you taken the supplement today?

Yes, I have taken it ☐

No, I have not taken it ☐

Not taken the supplement today? Please give your reason(s)below:

Have you experienced an adverse event? Please give details below:

UNIVERSITY OF LEEDS Arla

Supplement consumption diary

Day 3

Date: ...../...../.....

Mix the supplement with 120ml of water. Please tick to confirm you have consumed the supplement today:

Yes, I have taken it ☐

No, I have not taken it ☐

Not taken the supplement today? Please give your reason(s)below:

Have you experienced an adverse event? Please give details below:

Day 4

Date: ...../...../.....

Mix the supplement with 120ml of water. Have you taken the supplement today?

Yes, I have taken it ☐

No, I have not taken it ☐

Not taken the supplement today? Please give your reason(s)below:

Have you experienced an adverse event? Please give details below:

UNIVERSITY OF LEEDS Arla

Appendix 32. Adverse event form

Adverse Events/Serious Adverse Events

Participant ID:

Date of report: **Name of reporter:**

Source of information:

Description of event:

Dates of event: Start: **End:** **Still ongoing: ? Yes ? No**

Any medication taken for this AE? **Yes** **No**

If YES, please specify:

Appendix 33. Study 2 consent form

Study title: Investigating the benefits of a 12 week phospholipid intervention on cognitive performance in adults with a subjective memory complaint.

	Please initial
1. I confirm that I have read and understand the participant information sheet version 3, dated 13th February 2018, explaining the above research project, and I have had the opportunity to ask questions about the project. If I have asked any questions, I have received satisfactory answers.	_____
2. I agree for the data collected as a result of my participation to be stored on the University campus and systems, and deposited onto the Research Data Leeds repository. I am happy for the anonymised data to be used in relevant future research. I am also happy for the anonymised data to be shared with Arla Food Ingredients Group P/S should they request it. I understand that all data, apart from the anonymised composite data set available on the Research Data Leeds repository, will be retained for a maximum period of 3 years before being destroyed.	_____
3. I understand that relevant sections of the data collected during the study may be looked at by auditors from the University of Leeds or from regulatory authorities, where it is relevant to my taking part in this research. I give permission for these individuals to have access to my records.	_____
4. I confirm that I do not have any food allergies/intolerances which would exclude me from taking part in this study and understand that I will be required to consume the study supplement for 12 weeks, 7 days per week, and be seen on three occasions across this period. I agree to complete the consumption diary and keep all used and part-used supplement sachets. I understand that I may be contacted in between appointments from time to time.	_____

Continued …

5. I agree to take part in the above study. _____

Participant's name……Date ____ / ____ /_____

Signature
…….....

*Researcher's name…….Date ____ / ____ /_____

*Signature
…….....

*To be signed and dated in the presence of the participant.

Once this has been signed by all parties, the participant should receive a copy of the signed and dated participant consent form, the information sheet and any other written information provided to the participants. A copy of the signed and dated consent form should be kept with the project's main documents which must be kept in a secure location.

This research is subject to ethical guidelines set out by the British Psychological Society and has received ethical approval (ref no:PSC-289; date approved:14/02/2018). The primary supervisor of this research is Professor Louise Dye (l.dye@leeds.ac.uk)

Appendix 34. Waist:hip ratio standard operating procedures

Standard Operating Procedure: Measuring the Waist to Hip Ratio (WHR)

The purpose of this procedure is to determine the ratio of waist circumference to the hip circumference.

Procedure

The researcher will perform the following in order of appearance:

1. Explain the procedure to the participant.
2. Ask the participant to remove any outdoor clothing, which may restrict accurate measurement.
3. Use a flexible plastic tape measure to perform the measurements.
4. Ensure both measurements are taken with the participant standing.

Waist measurement:

5. Ensure the waist measurement is taken at the narrowest waist level, or if this is not apparent, at the mid-point between the lowest rib and the top of the hip bone (iliac crest).
6. Ensure the tape is not too tight or too loose, is lying flat on the skin, and is horizontal.
7. Ensure the measurement is recorded on the participant's sociodemographic and health form.

Hip measurement:

8. Ensure that the hip girth measurement is taken over minimal clothing.
9. Ensure that the participant stands erect with their weight evenly distributed on both feet and legs. Request that participants do not tense the gluteal (buttock) muscles – these should be relaxed.
10. Ensure that the hip girth measurement is taken at the level of the greatest protrusion of the gluteal (buttock) muscles.
11. Ensure the tape is not too tight or too loose, is lying flat, and is horizontal.
12. Ensure the measurement is recorded on the participant's sociodemographic and health form.

Post procedure

Calculate the WHR as follows:

Waist to Hip Ratio (WHR) = Gw / Gh, where Gw = waist girth, Gh = hip girth.

Appendix 35. Geriatric Depression Scale 15-item questionnaire

Geriatric Depression Scale: Short Form

DIRECTIONS: Choose the best answer for how you have felt over the past week:

1. Are you basically satisfied with your life?
   YES / NO

2. Have you dropped many of your activities and interests?
   YES / NO

3. Do you feel that your life is empty?
   YES / NO

4. Do you often get bored?
   YES / NO

5. Are you in good spirits most of the time?
   YES / NO

6. Are you afraid that something bad is going to happen to you?
   YES / NO

7. Do you feel happy most of the time?
   YES / NO

8. Do you often feel helpless?
   YES / NO

9. Do you prefer to stay at home, rather than going out and doing new things?
   YES / NO

10. Do you feel you have more problems with memory than most?
    YES / NO

11. Do you think it is wonderful to be alive now?
    YES / NO

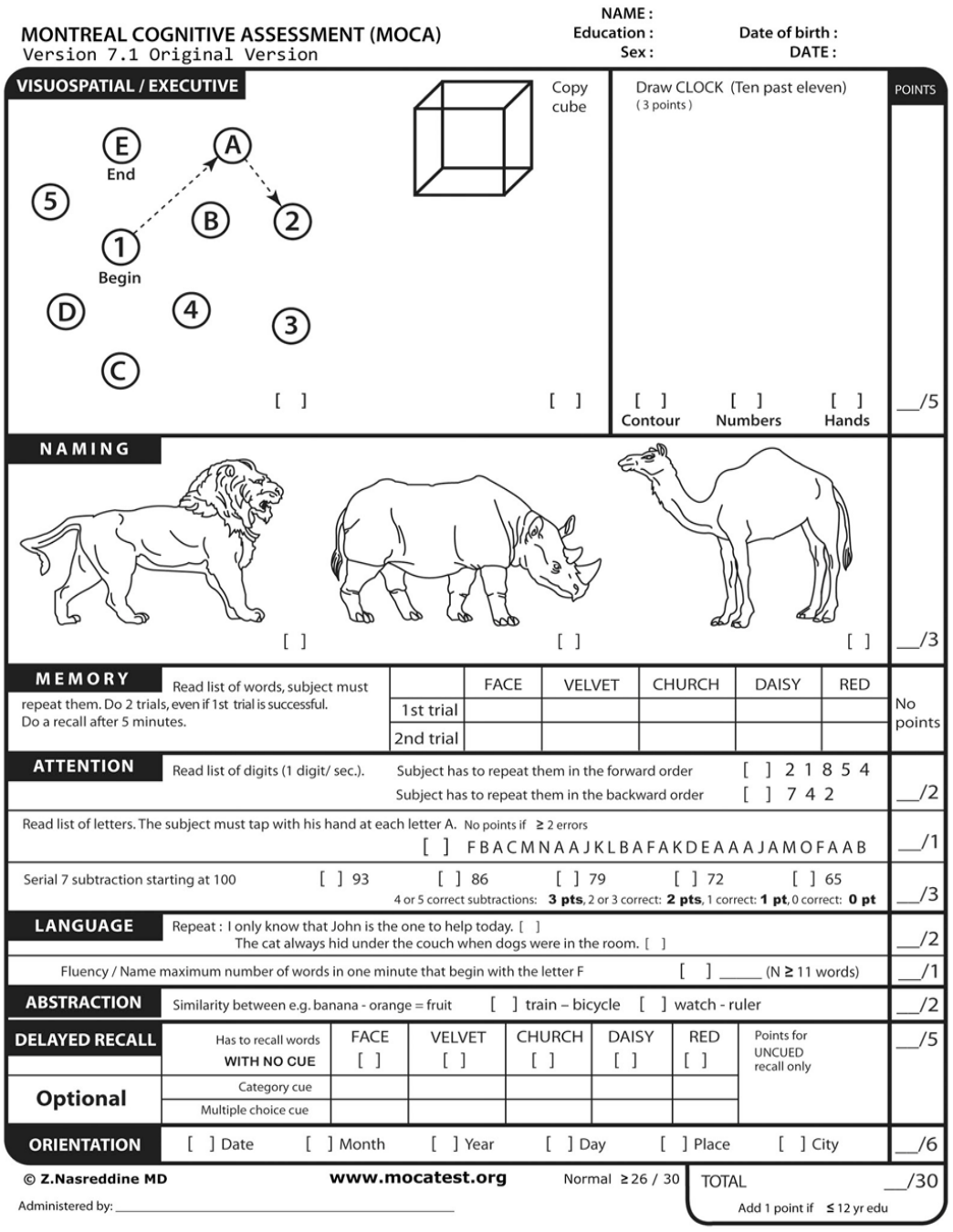
12. Do you feel pretty worthless the way you are now?
    YES / NO

13. Do you feel full of energy?
    YES / NO

14. Do you feel that your situation is hopeless?
    YES / NO

15. Do you think that most people are better off than you are?
    YES / NO

Appendix 36. Montreal Cognitive Assessment



MONTREAL COGNITIVE ASSESSMENT (MOCA)
Version 7.1 Original Version

NAME :
Education : Date of birth :
Sex : DATE :

VISUOSPATIAL / EXECUTIVE — POINTS

Copy cube [ ]

Draw CLOCK (Ten past eleven) (3 points) [ ] Contour [ ] Numbers [ ] Hands — /5

NAMING — /3

MEMORY Read list of words, subject must repeat them. Do 2 trials, even if 1st trial is successful. Do a recall after 5 minutes.

	FACE	VELVET	CHURCH	DAISY	RED
1st trial					
2nd trial					

No points

ATTENTION Read list of digits (1 digit/ sec.). Subject has to repeat them in the forward order [ ] 2 1 8 5 4
Subject has to repeat them in the backward order [ ] 7 4 2 — /2

Read list of letters. The subject must tap with his hand at each letter A. No points if ≥ 2 errors
[ ] F B A C M N A A J K L B A F A K D E A A A J A M O F A A B — /1

Serial 7 subtraction starting at 100 [ ] 93 [ ] 86 [ ] 79 [ ] 72 [ ] 65
4 or 5 correct subtractions: **3 pts**, 2 or 3 correct: **2 pts**, 1 correct: **1 pt**, 0 correct: **0 pt** — /3

LANGUAGE Repeat : I only know that John is the one to help today. [ ]
The cat always hid under the couch when dogs were in the room. [ ] — /2

Fluency / Name maximum number of words in one minute that begin with the letter F [ ] _____ (N ≥ 11 words) — /1

ABSTRACTION Similarity between e.g. banana - orange = fruit [ ] train – bicycle [ ] watch - ruler — /2

DELAYED RECALL — /5

	Has to recall words WITH NO CUE	FACE [ ]	VELVET [ ]	CHURCH [ ]	DAISY [ ]	RED [ ]	Points for UNCUED recall only
Optional	Category cue						
	Multiple choice cue						

ORIENTATION [ ] Date [ ] Month [ ] Year [ ] Day [ ] Place [ ] City — /6

© Z.Nasreddine MD **www.mocatest.org** Normal ≥ 26 / 30

TOTAL — /30
Add 1 point if ≤ 12 yr edu

Administered by: _____

Appendix 37. Study 2 sociodemographic details

Sociodemographic and health form

Participant ID……..

Date ……..

Thank you for agreeing to take part in this study. This questionnaire is designed to collect sociodemographic and health details. If you have any questions as you go through the questionnaire, feel free to ask.

Section one

Full name:

……
…

Address:

……..……
…

……..……
…

……..……
…

Post code: ……..

Contact telephone number(s): …….

Email: ……

Section Two

Date of birth:
……../……../……..

Age:
……

Gender:
M F

Highest qualification achieved. Please tick:

A Level or equivalent ☐

O Level or equivalent ☐

Trade (commercial or clerical) ☐

None ☐

Language spoken at home: ……..

Language spoken in country of birth: ……..

Are you currently employed? Please circle: Yes No

Are you married? Please circle: Yes No

Do you live alone? Please circle: Yes No

Section Three

Anthropometric measurements

Waist measure: ……

Hip measure: ……

Body impedance:

……

……

Please list all **medications** that you currently take:

……
……
……
……

Please list all supplements that you currently take:

……
……
……
……
……

Do you have a hearing impairment? Please circle: Yes No

If you answered yes, do you use a corrective aid? Please circle Yes No

Appendix 38. Study 2 Socioeconomic indicator

Deprivation Indicator

Participant ID:

Date:

Can you look at the things listed below and tell me which you have and which you do not have by marking the appropriate column?

For all items you have said you *don't have*, please can you tell me whether this is because you do not want them or because you can't afford them. To indicate that you can't afford them, please place a tick in the third column.

		Yes	No	Can't afford
1	A cooked meal every day..……..			
2	Meat or fish every other day ……..……..			
3	A roast meat joint or equivalent once a week …..			
4	A warm winter coat ……..			
5	Two pairs of all-weather shoes ……..			
6	New, not second hand clothes when you need them ..			
7	Presents for friends or family once a year ……			
8	Celebrations on special occasions such as Christmas ..			
9	A holiday away from home each year ……			
10	A holiday abroad every year or so …….			

Appendix 39. State Anxiety Scale (S-Anxiety)

State-Trait Anxiety Inventory: State

Participant ID:

Date:

DIRECTIONS: A number of statements which people have used to describe themselves are given below. Read each statement and then circle the response option to the right to indicate ***how you feel right now***, that is, at this moment. There are no right or wrong answers. Do not spend too much time on any one statement but give the answer which seems to describe. our present feelings best.

		NOT AT ALL	SOMEWHAT	MODERATELY	VERY MUCH SO
1	I feel calm ……..	1	2	3	4
2	I feel secure ……	1	2	3	4
3	I am tense ……..	1	2	3	4
4	I am regretful ……..	1	2	3	4
5	I feel at ease ……..	1	2	3	4
6	I feel upset ……	1	2	3	4
7	I am presently worrying about possible misfortunes ……..	1	2	3	4
8	I feel rested ……	1	2	3	4
9	I feel anxious ……	1	2	3	4
10	I feel comfortable ……..	1	2	3	4
11	I feel self-confident ……	1	2	3	4
12	I feel nervous ……	1	2	3	4
13	I am jittery ……..	1	2	3	4
14	I feel "high strung" ……..	1	2	3	4
15	I am relaxed ……	1	2	3	4
16	I feel content ……..	1	2	3	4
17	I am worried ……	1	2	3	4
18	I feel over-excited and rattled ……..	1	2	3	4
19	I feel joyful ……..	1	2	3	4
20	I feel pleasant ……	1	2	3	4

Appendix 40. The Stress Arousal Checklist (SACL)

The Stress Arousal Checklist

Participant ID:

Date:

The adjectives shown below describe different feelings and moods. Please use this list to describe your feelings at this moment in time.

If the adjective definitely describes your feelings circle the: (++) + ? -

If the adjective more or less describes your feelings circle the: ++ (+) ? -

If you do not understand the adjective, or you cannot decide whether it describes how you feel circle the: ++ + (?) -

If the adjective does not describe the way you feel circle the: ++ + ? (-)

Your first reactions will be the most reliable; therefore, do not spend too long thinking about each adjective. Please be as honest and accurate as possible.

Tense	++	+	?	-	Tired	++	+	?	-
Relaxed	++	+	?	-	Idle	++	+	?	-
Restful	++	+	?	-	Up tight	++	+	?	-
Active	++	+	?	-	Alert	++	+	?	-
Apprehensive	++	+	?	-	Lively	++	+	?	-
Worried	++	+	?	-	Cheerful	++	+	?	-
Energetic	++	+	?	-	Contented	++	+	?	-
Drowsy	++	+	?	-	Jittery	++	+	?	-
Bothered	++	+	?	-	Sluggish	++	+	?	-
Uneasy	++	+	?	-	Pleasant	++	+	?	-
Dejected	++	+	?	-	Sleepy	++	+	?	-
Nervous	++	+	?	-	Comfortable	++	+	?	-
Distressed	++	+	?	-	Calm	++	+	?	-
Vigorous	++	+	?	-	Stimulated	++	+	?	-
Peaceful	++	+	?	-	Activated	++	+	?	-

Appendix 41. The Profile of Mood States – Short form (POMS-SF)

The Profile of Mood States – Short form

Participant ID:

Date:

Instructions

Please circle the number which best describes how you are feeling at this moment in time. The numbers correspond to the descriptions at the top of each column (0 = ”Not at all“, 4 = "Extremely”)

		Not at all	A Little	Moderately	Quite a bit	Extremely
1	Tense	0	1	2	3	4
2	Peeved	0	1	2	3	4
3	Sad	0	1	2	3	4
4	Hopeless	0	1	2	3	4
5	Restless	0	1	2	3	4
6	Active	0	1	2	3	4
7	Bewildered	0	1	2	3	4
8	Discouraged	0	1	2	3	4
9	Fatigued	0	1	2	3	4
10	Anxious	0	1	2	3	4
11	Cheerful	0	1	2	3	4
12	Uncertain about things	0	1	2	3	4
13	Exhausted	0	1	2	3	4
14	Blue	0	1	2	3	4
15	Miserable	0	1	2	3	4
16	Angry	0	1	2	3	4
17	Worthless	0	1	2	3	4
18	Annoyed	0	1	2	3	4

		Not at all	A Little	Moderately	Quite a bit	Extremely
19	Vigorous	0	1	2	3	4
20	Grouchy	0	1	2	3	4
21	Resentful	0	1	2	3	4
22	On edge	0	1	2	3	4
23	Bitter	0	1	2	3	4
24	Unable to concentrate	0	1	2	3	4
25	Furious	0	1	2	3	4
26	Full of pep	0	1	2	3	4
27	Uneasy	0	1	2	3	4
28	Lively	0	1	2	3	4
29	Nervous	0	1	2	3	4
30	Bushed	0	1	2	3	4
31	Helpless	0	1	2	3	4
32	Confused	0	1	2	3	4
33	Unhappy	0	1	2	3	4
34	Energetic	0	1	2	3	4
35	Forgetful	0	1	2	3	4
36	Worn out	0	1	2	3	4
37	Weary	0	1	2	3	4

Appendix 42. Cognitive Failures Questionnaire (CFQ)

Cognitive Failures Questionnaire

Participant ID:

Date:

The following questions are about minor mistakes which everyone makes from time to time, but some of which happen more often than others.

We want to know how often these things have happened to you in the past ***6 weeks***.

Please use the following scale:

0	1	2	3	4
Never	Very Rarely	Occasionally	Quite often	Very often

1	Do you read something and find you haven't been thinking about it and must read it again?	Rating
2	Do you find you forget why you went from one part of the house to the other?	
3	Do you fail to notice signposts on the road?	
4	Do you find you confuse right and left when giving directions?	
5	Do you bump into people?	
6	Do you find you forget whether you've turned off a light or a fire or locked the door?	
7	Do you fail to listen to people's names when you are meeting them?	
8	Do you say something and realize afterwards that it might be taken as insulting?	
9	Do you fail to hear people speaking to you when you are doing something else?	
10	Do you lose your temper and regret it?	
11	Do you leave important letters unanswered for days?	

12	Do you find you forget which way to turn on a road you know well but rarely use?	
13	Do you fail to see what you want in a supermarket (although it's there)?	
14	Do you find yourself suddenly wondering whether you've used a word correctly?	
15	Do you have trouble making up your mind?	
16	Do you find you forget appointments?	
17	Do you forget where you put something like a newspaper or a book?	
18	Do you find you accidentally throw away the thing you want and keep what you meant to throw away -- as in the example of throwing away the matchbox and putting the used match in your pocket?	
19	Do you daydream when you ought to be listening to something?	
20	Do you find you forget people's names?	
21	Do you start doing one thing at home and get distracted into doing something else (unintentionally)?	
22	Do you find you can't quite remember something although it's "on the tip of your tongue"?	
23	Do you find you forget what you came to the shops to buy?	
24	Do you drop things?	
25	Do you find you can't think of anything to say?	