Elsevier Editorial System(tm) for Biological Psychology
Manuscript Draft

Manuscript Number: BIOPSY-D-10-00384R2

Title: Acute ingestion of different macronutrients differentially enhances aspects of memory and attention in healthy young adults

Article Type: Research Paper

Keywords: macronutrients; cognitive function; memory; attention; glucose; protein; fat

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Abstract: The role of carbohydrates on mood and cognition is fairly well established, however research examining the behavioural effects of the other macronutrients is limited. The current study compared the effects of a 25g glucose drink to energetically-matched protein and fat drinks and an inert placebo. Following a blind, placebo-controlled, randomized crossover design, 18 healthy young adults consumed drinks containing fat, glucose, protein and placebo. Cognitive performance was examined at baseline and again 15- and 60-minutes post drink. Mood was assessed at baseline and then 10-, 35- and 80-minutes post drink. Attention and speed were enhanced 15-minutes following fat or glucose ingestion and working memory was enhanced15-minutes following protein ingestion. Sixty minutes post drink memory enhancements were observed after protein and memory impairment was observed following glucose. All drinks increased ratings of alertness. The findings suggest that macronutrients: i) have different windows of opportunity for effects ii) target different cognitive domains.

Dear Professor Ring,

Thank you for inviting us to respond to the final reviewer comment and resubmit our paper to Biological Psychology. Below we have addressed and outlined in detail our response to the comment made by reviewer 2, which we had not sufficiently addressed previously. We hope you find our comments and amendments acceptable and our paper now worthy of publication.

Best wishes

Emma Jones

Response to Review:

When selecting the current design we acknowledged that both between-subjects and repeated measures have their own problems and it is necessary to identify and attempt to reduce theses as much as possible. We selected a crossover design, the particular strength of which is that the interventions under investigation are evaluated within the same participant and so eliminates between-subject variability (Maclure, 1991). However, we do agree that the design has certain weaknesses, including carry-over of effect of treatments across study periods, which could potentially distort the results (Cleophas, 1990; Wallenstein & Fisher, 1977) and observed treatment effects will depend upon the order in which they were received.

Some have argued against consistent testing for carryover effects of interventions across periods as carry-over effects are rare and statistical manipulation after the fact cannot address the impact of a carry-over effect (Senn, D'Angelo & Potvin, 2004). It has been argued that tests for carry-over are generally underpowered even with an appreciable carry-over effect (Senn, 1988). Another complicating matter when assessing order effects is that effects may interact with participant, and this is difficult to assess. Consequently any effect of order or interaction effects may not detect the order effect.

However what is of particular relevance in the current study is that statistical analysis was carried out on change from baseline levels, which in itself controls for potential carry over effects. The treatment we used is not an endogenous entity nor has administration of fat, protein and glucose any long lasting effects. Moreover, we employed a 5-7 day washout between study days and treatment order was randomised. In addition, to reduce potential effect of familiarity with tests, the current study employed four practice sessions prior to the start of the study.

We have however followed the recommendation of reviewer 2 and incorporated order in the ANOVA model and the results showed no significant effects of drink order on any of the measures and only one significant three-way interaction (drink*time*drink order) which was on quality of working memory [$F(3,36)=3.44$, $p=0.013$]. Given the number of “orders” it is unclear what this means. The drink*time interaction is also significant (as already reported) and this has been explored further in the paper. We have highlighted this issue in the discussion section of the paper (first para. Pg 20).

Consequently, although we do appreciate the concerns of reviewer 2, we are confident that the design of the study (practice session, randomisation, pre-and post treatment assessment) minimised order effects and is overall preferable to the potential confounding variables that present with between participant designs.

References

Maclure M: The case-crossover design: a method for studying transient effects on the risk of acute events. Am J Epidemiol. 1991, 133(2):144-153.

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Senn SJ, D'Angelo G, Potvin D: Carry-over in cross-over trials in bioequivalence: theoretical concerns and empirical evidence. Pharmaceutical Statistics 2004, 3:13-142.

Senn SJ: Cross-over trials, carry-over effects and the art of self-delusion. Stat Med 1988, 7:1099-101

Dear Reviewer,

Thank you for your helpful comments and suggestions. I apologise that we failed to fully address your concerns in our previous response and hope we have dealt with the issue to your satisfaction below. We hope that our paper is now worthy of publication. Best wishes

Emma Jones

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***Highlights**

- Attention and speed of processing were enhanced 15-minutes following fat or glucose ingestion
- Working memory was enhanced 15-minutes following protein ingestion
- Sixty minutes post drink memory enhancements were observed after protein and memory impairment was observed following glucose
- All drinks (including placebo) increased ratings of alertness immediately post drink

Acute ingestion of different macronutrients differentially enhances aspects of memory and attention in healthy young adults

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Abstract

The role of carbohydrates on mood and cognition is fairly well established, however research examining the behavioural effects of the other macronutrients is limited. The current study compared the effects of a 25g glucose drink to energetically-matched protein and fat drinks and an inert placebo. Following a blind, placebo-controlled, randomized crossover design, 18 healthy young adults consumed drinks containing fat, glucose, protein and placebo. Cognitive performance was examined at baseline and again 15- and 60-minutes post drink. Mood was assessed at baseline and then 10-, 35- and 80-minutes post drink. Attention and speed were enhanced 15-minutes following fat or glucose ingestion and working memory was enhanced15-minutes following protein ingestion. Sixty minutes post drink memory enhancements were observed after protein and memory impairment was observed following glucose. All drinks increased ratings of alertness. The findings suggest that macronutrients: i) have different windows of opportunity for effects ii) target different cognitive domains.

Introduction

The effects of nutrition on brain and behaviour and more specifically the cognitive effects of foods, food components and nutritional interventions are very much on the public agenda. More specifically, it has been demonstrated that acute administration of glucose (a simple carbohydrate), can facilitate verbal declarative memory in healthy young adults and adolescents (e.g. Foster, Lidder & Sünram, 1998; Smith & Foster, 2008; Smith Hii, Foster & van Eekelen, 2009; Sünram-Lea, Foster, Durlach & Perez, 2001; 2002a; 2002b) and older populations (e.g. Craft, Murphy & Wemstrom, 1994; Kaplan, Greenwood, Winocur & Wolever, 2001; Messier, Gagnon & Knott, 1997; Riby, Meikle & Glover, 2004). In addition, administration of a glucose drink has been shown to improve working memory performance in healthy, young adults (e.g. Scholey, Harper & Kennedy, 2001); to ameliorate impairment of a secondary, psychomotor task during a divided attention (encoding plus psychomotor) paradigm (Scholey, Sünram-Lea, Greer, Elliott, & Kennedy, 2009) and to enhance prospective memory (Riby, Laws, McLaughlin & Murray, 2011). Hoyland, Lawton and Dye (2008) conducted a comprehensive review of the literature and concluded that the most robust glucose-mediated enhancement has been demonstrated on memory although there are numerous examples of glucose facilitation of other cognitive tasks (see Hoyland et al., 2008; Messier, 2004; Riby, 2004 for reviews).

Glucose index (GI) is the rate at which an ingested substance increases and maintains blood glucose levels. Pure glucose has a high GI as it increases levels quickly with a fast return to baseline, whereas foodstuff with lower GI ratings tend to elicit a slower rise, smaller peak and are slower to return to baseline. Glycaemic Load (GL) is another way of describing response to a carbohydrate and takes into account both GI and the quantity of food. It has been found that foods with different GIs and

GLs can differentially influence cognitive function. For example, some authors report that cognitive performance benefits can be more readily observed following consumption of low GI foods compared to high GI foods (e.g. Benton et al., 2003). More specifically, researchers have demonstrated beneficial effects of low GI breakfasts compared to high GI breakfasts for children (e.g. Ingwersen, Defeyter, Kennedy, Wesnes & Scholey, 2007; Mahoney, Taylor, Kanarek & Samuel, 2005; Wesnes, Pincock, Richardson, Helm & Hails, 2003). Ingwersen et al. (2007) found that following a low GI breakfast cognitive benefits were observed for 2-hours post ingestion whereas performance following the low GI breakfast declined over this time period. A review by Gilsenan and colleagues (Gilsenan, Bruin & Dye, 2009) concluded that evidence for effects of different GLs over relatively short periods of time (from between 100-390 minutes) is inconsistent. However, in general it appears that high GI or GL foods (including glucose drinks) appear to have short term cognitive benefit, whereas over a longer time frame foods which allow a more sustained energy supply are more beneficial (also see e.g. Kaplan, Greenwood, Winocur & Wolver, 2000).

To date, a large body of literature reports on the influence of glucose ingestion on cognitive function whereas considerably fewer studies have examined the influence of protein or fat ingestion and/or compared the effects of different macronutrients on cognitive performance. Kaplan et al., (2001) compared the effects of protein, glucose and fat ingestion in a sample of older participants (61–79 years) and found that immediately after ingestion all three macronutrients improved memory performance compared to placebo, whereas 60-minutes post-ingestion memory improvements were only observed following a glucose drink. In addition, fat administration improved attention 60 minutes post ingestion and protein led to a reduced rate of forgetting when assessed 15 min after consumption. Fischer, Colombani, Langhans and Wenk (2001) administered carbohydrate-, protein- and fat-containing meals to healthy, young adults and tested cognitive performance at various time-points over the course of 180-minutes. Their data indicated that different macronutrients influence cognitive performance in a different manner, with the best performance usually observed after fat ingestion. More specifically, faster reaction times, improved short-term memory and improved attention were observed at all time points (60-, 120- and 180 minutes) following fat ingestion. In a placebo-controlled study Jones, Sünram-Lea and Threadgold (2005) compared the effects of glucose and protein administration and observed glucose-mediated enhancement of reaction times following glucose administration. However, this study failed to replicate the immediate beneficial effects of macronutrients observed by Kaplan et al. or the well-documented glucose facilitation of memory. Overall, findings to date tentatively suggest that macronutrients may differentially influence cognition and mood and that the effects are time-dependent (e.g. Fischer et al., 2001; Kaplan et al., 2001).

In addition to cognitive facilitation, previous research has also demonstrated complex macronutrient-specific effects on mood. For example, Fischer et al. (2001) observed a reduction in depression scores on the Profile of Mood Scale (POMS) following carbohydrate ingestion compared to protein. Conversely, Gibson et al. (1999) found that 120-minutes post ingestion, a meal high in protein (and low in carbohydrate) led to increased positive affect on the PANAS mood scale compared to a meal low in protein (and high in carbohydrate). In our own laboratory we have previously observed an increase in negative affect following protein ingestion (Jones et al., 2005). These findings appear to be contradictory but methodological differences between studies may account for these discrepancies. For example, Fischer et al.

(2001) administered pure macronutrients whereas Gibson et al. (1999) administered macronutrient combinations, which preclude a clear conclusion as to whether the mood effects are due to specific macronutrients or interaction effects of multiple macronutrients. In addition, the use of different mood rating scales further impedes direct comparison.

It is evident from this brief review of the literature that the effects of glucose on cognitive functioning and mood are relatively well described whereas those of protein and fat still remain to be explored in detail. It is also important to note that although investigating the effects of combined administration of different macronutrients (for example Benton & Sargent, 1992) is important to for our understanding of macronutrient interaction they do not inform us about the contributions of individual macronutrients and their potential underlying mechanisms. It is important to establish whether different macronutrients influence cognitive performance via a specific mechanism or whether they exert their influence via a shared, generalised mechanism. Kaplan et al. (2001) found evidence to indicate a generalised effect of macronutrients on cognitive function in older adults (e.g. effects 15 and 60 minutes post ingestion - earlier than would be expected if metabolite-mediated) suggesting a pre-digestive influence possibly due to the release of gut hormones such as cholecystokinin (CCK), gastrin-releasing peptide or amylin which have all been demonstrated to influence memory in animals (Flood and Morley, 1989; 1992; Flood, Smith & Morley, 1987; Morley, Flood, Silver & Kaiser, 1994). Whether such effects have nutrient and/ or domain specificity is yet to be examined. There is now growing interest in the potential of specific foods to influence mood and cognitive performance. The main components of our diet that can be readily manipulated are the macronutrients, glucose (carbohydrates), protein and fat.

Therefore, the aim of the current research was to further explore the effects of acute macronutrient ingestion on cognitive performance and mood in healthy young adults. Specifically, the effects of acute fat, protein, glucose and placebo ingestion on a range of cognitive tests and mood scales were examined.

Methods and Materials

Power calculation

A medium overall effect size was previously found in a meta-analytic review of the glucose facilitation effect (d = 0.56; Riby, 2004). An a priori power calculation using G-power (Erdfelder, Faul, & Buchner, 1996) revealed that for a medium effect size, with alpha set to 0.05 (two-tailed) a sample size of 16 would be required for 95% power. The effect size of fat and protein is as yet unclear due to the relatively few numbers of studies carried out in this area, however Kaplan et al. (2001) observed effects using 22 participants.

Participants

Eighteen healthy young male and female participants (5 males, 13 females) with a mean BMI of 21.1 kg/m^2 took part in this study. Ages ranged from 18-37 years (mean age =19 years). Participants were excluded from the study on the basis of several criteria. Information regarding these criteria was gathered using a confidential medical questionnaire which was completed before signing the consent form. Exclusion criteria included i) history of neurological and/or psychiatric illness, ii) Diabetes Mellitus, iii) BMI $\geq$ 25, iv) intolerance or allergic reaction to substances that contain phenylalanine. Participants were recruited from the University of Lancaster 1st year cohort and received 30 pounds sterling and 4 course credits for taking part in the experiment. The study was approved by the Ethics Committee of the Department of Psychology, Lancaster University, and carried out in accordance to national and local ethics guidelines. Written informed consent was obtained from each participant prior to participation.

Design

Following a blind, placebo controlled, balanced, cross-over, repeated measures design, participants were administered 40g protein in solution, 16g fat emulsion, 40g glucose solution and an inert placebo (matched for volume, sweetness and flavour) over four study days, with a 5-7 day washout period between treatments. Treatment order was randomly assigned. Treatment order was randomised and counterbalanced using a Latin Square.

Treatments

Three isoenergetic (145 Kcal) and isovolumic (300ml) drinks and an inert placebo were administered. All drinks were matched for volume, sweetness and flavour and administered in opaque cups, covered by lids and ingested through a straw. Drinks were flavoured with lemon juice in order to improve palatability and participants' compliance. Drinks were prepared in the laboratory and refrigerated prior to testing. The composition of test drinks is shown in Table 1.

<Table 1. here>

Blood Glucose Measurement

Blood glucose readings were obtained using the ExacTech blood glucose monitoring equipment (supplied by MediSense Britain Ltd, 16/17 The Courtyard, Gorsey Lane, Coleshill, Birmingham B46 1JA), following the manufacturers recommended procedure.

Measuring Cognition and Mood

A tailored version of the CDR System (www.unitedbiosource.com) was used to assess participants' cognitive performance and mood. The battery was administered on PCs and responses were made via a two-button (yes and no) response box. Responses for the Visual Analogue Scales (VAS) were made by mouse click. Completion of the whole battery took around 20-minutes and tasks were presented in following order:

Word presentation. – A list of 15 words matched for frequency, concreteness and imagery was presented on the monitor at the rate of one every two seconds for participants to remember. During encoding, participants were required to perform two complex hand-movement sequences (Sünram-Lea et al., 2001). Each sequence was performed using both hands and contained three movements: fist – chop - slap and back-slap – chop – fist. Participants were told to alternate the sequence every fifth word and they were not informed when to change, only that they had to keep track of this themselves. Hand-movements were performed continually during word presentation.

Immediate word recall. - Immediately after the words had been presented participants were given 60-seconds to write down as many words as they could from the list they had just seen. Participants' responses were marked according to total number of errors, intrusions and percentage of words recalled correctly (accuracy).

Picture presentation. – Twenty photographs of objects were individually, displayed in the centre of the screen at a rate of one every three seconds. Each picture was displayed for one second. Participants were required to remember the pictures.

Simple reaction time. – The word 'yes' was presented repeatedly in the centre of the screen with inter trial intervals varying randomly between 1 and 3.5 seconds.

Participants were required to respond by pressing the ‘yes’ button on their response box as quickly as possible, whenever the word appeared. Reaction times were recorded in milliseconds.

Digit vigilance. – A single target digit was randomly selected and continuously displayed on the right side of the screen. In the centre a series of rapidly changing digits was displayed at the rate of 150 digits per minute. Participants were required to press the ‘yes’ button as quickly as possible, whenever the digit in the centre matched the target digit. The task lasted for three minutes Reaction times (milliseconds), percentage accuracy and number of false alarms were recorded.

Choice reaction time. - The target words ‘yes’ and ‘no’ were repeatedly, randomly displayed individually in the centre of the screen. The inter-trial intervals varied randomly between 1 second and 3.5 seconds. Participants were instructed to respond by pressing the appropriate button on their response box as quickly and accurately as possible. Reaction times (milliseconds) and percentage accuracy were recorded.

Spatial working memory. - A picture of a house was displayed on the screen with nine evenly distributed windows. Four of the windows were lit up in the original picture and participants were asked to remember the position of these windows. Following this, the house was presented again, repeatedly but each time only one window was lit up. Participants were required to answer whether the window was lit up or not in the original house by pressing the appropriate button on their answer box. Percentage accuracy of identifying novel stimuli (distractors) and target stimuli were recorded in addition to reaction times (milliseconds) to distractors and targets and overall reaction times. Sensitivity Index (SI) was calculated by combining an individual’s ability to discriminate targets and their ability to discriminate distractors.

SI ranges between +1 and -1 whereby +1 indicates perfect performance, zero indicates chance performance, and a negative score indicates performance which is worse than chance.

Numeric working memory. - A series of five digits were displayed individually on the screen for participants to remember. These were followed by 30 probe digits to which participants were required to respond using their answer box, indicating whether or not each probe digit had been in the original sequence. Percentage accuracy and reaction times for both distractors and targets were recorded in addition to overall reaction times and SI.

Delayed word recall. - Participants were given 60-seconds to write as many words as they could from the list they had seen at the beginning of the battery. Participant's responses were marked according to total number of errors, intrusions and percentage of words recalled correctly (accuracy).

Delayed word recognition. – The 15 original words and 15 distractor words were presented individually in a randomised order. Participants were asked to indicate whether each word had been in the original list or not by responding 'yes' or 'no' on their response box. Percentage accuracy and reaction times for both distractors and targets were recorded in addition to overall reaction times and SI.

Picture recognition. - The 20 original pictures and 20 distractor pictures were presented, individually in a randomised order. Participants were asked to indicate whether each word had been in the original list or not by responding 'yes' or 'no' on their response box. Percentage accuracy and reaction times for both distractors and targets were recorded in addition to overall reaction times and SI.

Subjective Mood

The Bond and Lader visual analogue scales (VAS) were used to assess subjective mood (Bond & Lader, 1974). Sixteen VAS were presented on the screen immediately after the cognitive tests. Participants used the mouse to position an arrow at the point on the scale that represented their feelings at that moment. The 16 scales were combined as recommended by Bond and Lader (1974) to form three mood factors: ‘alertness’, ‘calmness’ and ‘contentment’.

Cognitive Outcome Measures

Scores from individual measures were combined to form seven secondary outcome measures (‘power of attention’, continuity of attention’, ‘quality of working memory’, ‘quality of episodic secondary memory’, ‘quality of memory’, ‘speed of memory’ and ‘combined speed’) derived from factor analysis of the Cognitive Drug Research computerised test battery (Wesnes, Ward, Ayre, & Pincock, 1999; Wesnes, Ward, McGinty & Petrini, 2000), and previously used (e.g. Wesnes et al., 1997; 1999; 2000; Kennedy, Scholey, & Wesnes, 2001; 2002; Kennedy, Scholey, Tildsley, Perry & Wesnes, 2002; Sünram-Lea, Birchall, Wesnes & Petrini, 2004). See Figure 1.

Power of attention factor (also referred to as ‘speed of attention’): Derived by combining reaction times of three attention tasks: simple reaction time, choice reaction time, and digit vigilance (units are summed milliseconds for the three tasks).

Continuity of attention factor (also referred to as ‘accuracy of attention’): Derived by calculating the combined percentage accuracy across choice reaction time and digit vigilance tasks (with adjustment for false alarms on the latter test). 100 percent accuracy across the two tasks would result in a maximum score of 95.

Quality of working memory: Derived by combining SI scores from the two working memory tests: spatial working memory and numeric working memory. Range from -2 to +2. Perfect performance on both tasks result in a maximum score of +2.

Quality of episodic secondary memory: Derived by calculating the combined percentage accuracy scores (adjusted for proportion of novel and new stimuli where appropriate) from all secondary memory tests: word recognition, picture recognition, immediate word recall, delayed word recall (with adjustment to the total percentage correct for errors and intrusions on the latter two tasks). One hundred percent accuracy across the four tasks would result in a maximum score of 400.

Quality of memory factor: Derived by calculating the combined percentage accuracy scores (adjusted for proportion of novel and new stimuli where appropriate) of all working memory tests and secondary memory tests: spatial working memory, numeric working memory, word recognition, picture recognition, immediate word recall, delayed word recall (with adjustment to the total percentage correct for errors and intrusions on the latter two tasks). One hundred percent accuracy across the six tasks would generate a maximum score of 600.

Speed of memory factor: Derived by combining reaction times of the numeric working memory task, spatial memory task, delayed word recognition and delayed picture recognition task (units are summed milliseconds for the four tasks).

Combined speed: Derived by combining the two speed outcome factors: ‘speed of memory’ and ‘power of attention’.

<Figure 1 goes here>

Procedure

Each participant attended four 20-minute practice sessions in order to familiarise them with the cognitive test battery. Upon arrival at the laboratory for the first practice session, participants gave informed consent and demographic information. They were given complete instructions for each task including the secondary hand movement task. No treatments were administered during the practice sessions and performance data from these sessions was not included in the analysis.

Once the practice sessions had been successfully completed there were four experimental sessions. All followed the same procedure. On arrival at their first session participants were randomly allocated to a treatment regime which counterbalanced the order of drinks across the study days. Sessions were separated by a 5-7 day wash-out period and they were conducted in the mornings following a 12-hour, over-night fast. In addition, participants were instructed to refrain from nicotine, alcohol and stimulants for 12-hours prior to each session. Sessions were 1 hour and 45 minutes long and started at either 9am or 11am.

On entering the laboratory participants were asked to report if they had complied to the 12-hour fast. The first blood glucose measure (T0) was taken and the cognitive test battery was administered (pre-treatment assessment) followed by drink administration. 10-minutes were allowed for drink consumption and immediately following consumption the visual analogue scales were administered to assess any immediate effects of drink ingestion on mood. The second blood glucose measure was taken 12-minutes following drink ingestion (T12). 15-minutes following drink administration the cognitive test battery and mood scales were administered again.

Upon completion of the test battery and mood scales the third blood glucose reading was taken (T37). Participants were then allowed to engage in silent reading of their own choice for 20-25 minutes, after which the fourth blood glucose reading was taken (T55). Sixty minutes post-drink, the cognitive battery was, again, administered, followed by the last blood glucose measure (T82). This time-scale was employed in this study as glucose facilitation appears to be optimal when testing starts 15 to 20 minutes post ingestion (e.g. Foster et al., 1998; Owens & Benton, 1994; Sünram-Lea et al., 2001; 2002a; 2002b; 2004), whereas beneficial effects of fat and protein ingestion have been observed following longer delays (Kaplan et al., 2001; Fischer et al., 2001)

Statistical Analysis

Blood Glucose Levels

Blood glucose levels (mmol/litre) were analysed using a two-way (4*5) repeated measures ANOVA (drink: fat, protein, glucose and placebo and time: T0, T12, T37, T55 and T82). Significant main effects and interactions were analysed using the Bonferroni post hoc test.

Cognitive Data and Visual Analogue Scales

Scores on the cognitive outcome measures and the three factors derived from the visual analogue scales were analysed as ‘change from baseline’. Comparisons of all drinks were made using repeated measures ANOVAs. To further explore main effects and interactions, planned comparisons of each treatment drink with placebo were made using t-tests with MSE from omnibus ANOVA as an error term. Post hoc comparisons were made using Bonferroni tests. For all cognitive outcome factors the ANOVAs were 4*2 (drink: fat, protein, glucose and aspartame and assessment: 15-

minutes post and 60-minutes post). For the visual analogue scales the levels of assessment were: 10-minutes post, 35-minutes post and 80-minutes post. In order to minimise the risk of type 1 errors planned comparisons were only conducted when significant main effects or interactions (or trends, p<0.1) were observed.

Results

Blood Glucose Levels

For mean blood glucose levels (±SE) see Figure 2. There was a main effect of drink on blood glucose levels [F(3,39)=59.42, p<0.01]. Post hoc analyses indicated that the glucose drink lead to significantly higher blood glucose levels than the other drinks (all p-values <0.01). A main effect of time [F(4,52)=37.43, p<0.001] was due to increasing blood glucose levels over the course of the experimental session(all p-values <0.01) and a significant time*drink interaction [F(12,156)=15.74, p<0.001] showed that blood glucose levels were significantly higher at each post dose time point following a glucose drink compared to any of the other treatments.

Cognitive Outcome Measures

Mean (±SD) baseline and post-drink change from baseline scores on the cognitive outcome factors and VAS, on which significant drink effects were observed, are displayed in Figures 3 and 4.

Power of Attention

The main effect of drink just failed to reach significance [F(3,48)=2.30, p=0.09]. Planned comparisons between treatment drink and placebo demonstrated a significant performance enhancement following glucose compared to placebo (t(48)=2.34, p<0.05) particularly 15-minutes post ingestion (t(48)=-3.71, p<0.01). See Figure 4(b).

Continuity of Attention

There was a significant main effect of time [F(3,48)=7.37, p<0.05] whereby regardless of drink, performance was significantly worse 60-minutes post-drink than 15-minutes after drink ingestion (p<0.05).

Quality of Working Memory

A significant time*drink interaction was observed [F(3,48)=3.34, p<0.05]. Post hoc analyses revealed performance improvements 60-minutes after a fat drink compared to 15-minutes after a fat drink (p<0.01). Planned comparisons revealed that 15-minutes post drink, protein was associated with enhanced performance compared to placebo (t(48)=2.15, p<0.05) and 60-minutes post drink glucose was associated with impaired working memory compared to placebo (t(48)=2.45, p<0.05). See Figure 3(b).

Quality of Episodic, Secondary Memory

There was a significant main effect of drink [F(3,48)=3.90, p<0.05] with significantly better performance following a protein drink compared to a glucose drink (p<0.05). In addition, there was a significant time*drink interaction [F(2.01, 32.18)=8.81, p<0.01] which was due to the fact that performance was significantly better 60-minutes after a protein drink than after a fat or a glucose drink (both p-values <0.01). In addition, performance was enhanced 60-minutes following protein ingestion compared to placebo ingestion (t(48)=4.42, p<0.001). Furthermore, following a protein drink, performance improved significantly over time (p<0.01) whereas performance following a glucose drink deteriorated over time (p<0.05). See Figure 3(a).

Quality of Memory

There was a significant main effect of drink [F(3,48)=4.969, p<0.01]. Post hoc comparisons showed that performance was significantly better following a protein drink than a glucose drink ($p<0.05$). There was also a significant time*drink interaction [$F(3,48)=8.14$, $p<0.01$] with significantly enhanced performance 60-minutes after a protein drink compared to performance following a fat drink ($p<0.01$) or a glucose drink ($p<0.01$). Planned comparisons revealed that protein ingestion significantly enhanced memory compared to placebo ($t(48)=2.49$, $p<0.05$), particularly at the 60-minutes post time point ($t(48)=4.72$, $p<0.001$). In contrast, at 60-minutes post ingestion glucose led to significantly impaired memory compared to placebo ($t(48)=2.52$, $p<0.05$). See Figure 3(c).

Speed of Memory

The time*drink interaction just missed significance [F(3,48=2.54, p=0.07]. This may be a result of one of the following observations: the slowest memory processing was observed following the placebo drink, the glucose drink produced consistently fast performance and speed following protein ingestion improved over time. Planned comparisons revealed faster responses 15-minutes following glucose ingestion ($t(48)=3.27$, $p<0.05$) and following fat-ingestion ($t(48)=2.62$, $p<0.05$) compared to placebo. See Figure 3(d).

Combined Speed

The interaction of time*drink almost reached significance [$F(3,48)=2.70$, $p=0.06$]. Planned comparisons revealed that 15-minutes post drink, fat and glucose were associated with faster responses compared to placebo ($t(48)=2.77$, $p<0.01$ and $t(48)=3.85$, $p<0.001$ respectively). See Figure 4(a).

Subjective Mood Measures

There was a main effect of time on alertness [$F(1,16)=7.23$, $p<0.01$] with participants reporting significantly higher alertness levels 10-minutes post drink compared to either 15-minutes or 60-minutes post ingestion ($p<0.05$ and $p<0.01$, respectively). See Figure 4(c).

<Figures 3 and 4 here>

Discussion

The present study further demonstrated the ability of macronutrients to affect cognition and mood and that the effects are time-dependant and vary between macronutrients. Protein facilitated working memory performance 15-minutes post ingestion, and enhanced episodic memory 60-minutes post ingestion. Glucose ingestion enhanced attentional processes (power of attention), speed of processing (combined speed) and speed of memory 15-minutes post ingestion. Fat ingestion was associated with enhanced speed of processing 15-minutes post drink. However, glucose was also associated with impaired working memory (quality of working memory) 60-minutes post ingestion.

Beneficial effects of protein (compared to placebo) were observed 15 and 60-minutes post drink and were specifically targeting memory processes with enhanced working memory 15-minutes post drink and enhanced episodic memory 60-minutes post drink. Given the significant effects of protein on these memory factors, it is not surprising that protein ingestion was also associated with enhanced memory accuracy (quality of memory) as this factor is a combination of accuracy scores from all of the memory tasks.

Beneficial effects following fat and glucose ingestion were also observed. Fifteen minutes after fat ingestion cognitive processing speed (combined speed) and speed of memory were faster than following placebo. Cognitive processing speed, speed of memory and the ability to allocate attentional processes (‘power of attention’ factor) were also faster 15-minutes following a glucose drink compared to placebo.

Furthermore, 60-minutes after glucose ingestion working memory was significantly impaired compared to placebo. This latter finding is not surprising since glucose is metabolised quickly and enhancement is observed up to 20-minutes post ingestion (e.g. Foster et al., 1998; Owens & Benton, 1994; Sünram-Lea et al., 2001; 2002a; 2002b; 2004) so enhancement at a later time point would not be expected. And indeed impairments might be explained by a drop in blood glucose levels subsequent to ingestion of a glucose load due to increased insulin output.

In general, the findings show beneficial effects on cognition soon after glucose and fat ingestion (15-minutes post ingestion), whereas protein enhanced cognition at later time points. There are some notable exceptions e.g. protein also enhanced working memory 15-minutes post drink. These findings suggest that i) different macronutrients have different windows of opportunity for performance improvements and ii) different macronutrients target different cognitive domains. More specifically it appears that protein has a beneficial effect on general memory processes, whereas fat and glucose apparently target attentional processes and speed of processing.

In terms of glycaemic response the observed trajectories were nutrient-dependent. As expected glucose ingestion led to significantly increased blood glucose levels compared to fat, protein or placebo ingestion. This further supports the notion that ingestion of energy, regardless of source, appears to improve certain aspects of cognition and that at least some of the effects are independent of increases in blood glucose levels (Kaplan et al., 2001).

The current findings are consistent with those of Kaplan et al. (2001) who demonstrated macronutrient-mediated cognitive enhancement 15-minutes following protein, glucose and fat. However, in contrast to the current findings, they also observed maintained performance improvements following glucose ingestion 60-

minutes post ingestion. Moreover, the current study employed a repeated measures design in order to reduce inter-participant variability. Steps were taken to minimise the effects of drink order. Despite this a drink*time*drink order interaction was observed on working memory. There were no main effects of drink order on any of the measures. Future work should consider potential order effects perhaps studies could employ between-participant designs.

The glucose facilitation effect of cognition has been widely reported in the literature and has previously been reliably demonstrated with dosages of 25g glucose in healthy, young adults (e.g. Foster et al., 1998; Kennedy & Scholey, 2000; Sünram-Lea et al., 2001; 2002a; 2002b, 2004) and dosages of 25g and 50g in elderly adults (e.g. Craft et al., 1994; Messier et al., 1997; Riby et al., 2004). The most robust facilitation appears to be on memory (see reviews by Riby, 2004; Hoyland et al., 2008); however glucose-mediated enhancement of other tasks including attention and speed of processing (those that were enhanced in the current study) has also been reported in previous research (e.g. reaction times: Owens & Benton, 1994; information processing: Benton, Owens & Parker, 1994; Donohoe & Benton, 1999). Taken together these findings suggest that glucose administration may not specifically target memory processes and the effects of glucose may be more widespread.

Some previous papers have reported fat-mediated cognitive impairments (e.g. Cunliffe, Obeid & Powell-Tuck, 1997; Kaplan et al., 2001; and Wells & Read, 1995). However, Fischer et al. (2001) observed a beneficial effect of fat on a variety of cognitive tasks which was not replicated in the current study. This could be due to the optimal time-frame of fat metabolism which may have been missed as a result of the relatively short experimental sessions employed in the current study (final testing was 60-minutes post ingestion). However, Fischer et al. (2001) observed faster reaction times, improved short-term memory and improved attention at all time points (60-, 120- and 180 minutes) following fat ingestion. In the current study we observed faster processing 15-minutes following a fat drink. The discrepancies between findings may be a result of different dosages, time-frames and tasks that were employed by different research groups. Further research is required to establish the effects of different fat dosages on a variety of cognitive domains.

The current experiment attempted to separate nutritionally-mediated effects on cognition and the effects of food perception on mood by assessing mood prior to nutrient metabolism (10-minutes post drink) and 35-minutes and 80-minutes post-drink. However, it is important to note that although the 10-minutes post drink time point would precede absorption of both fat and protein, glucose would be metabolically active so any observed effects could be nutritionally-mediated. Macht, Gerer, & Ellgring (2003) found that increasing energy content of food was associated with increased negative emotions and increased negative perceptions of the food (e.g. more unhealthy and dangerous etc). However, in the current study no significant main effects of drink type on self-rated alertness, contentment or calmness were observed suggesting that energy content does not influence mood in healthy adults. However, increased alertness was observed immediately post-drink, regardless of drink-type. The fact that all drinks increased alertness suggests a general mechanism, possibly the impact of hydration from all drinks. For example, Neave, Scholey & Emmett et al. (2001) and Rogers, Kainth & Smit (2001) have shown that, compared to no drink, water ingestion increases subjective alertness in both fasted and non-fasted, healthy, young adults.

Alternatively, the experimental situation may have been responsible for the immediate post-drink increase in alertness observed. The 10-minutes post-drink mood scale was administered in isolation whereas the other mood scales were all administered immediately after the cognitive test battery. It may be the case that participants felt significantly less alert at these times because they had just been engaged, for 20-minutes, in cognitive tasks.

Macronutrient-specific mood effects demonstrated in previous research (e.g. Fischer et al., 2001; Gibson et al., 1999) have not been replicated in the current study. Fischer et al. (2001) found that carbohydrate ingestion led to an overall reduction in depression scores on the POMS, which was not related to time of testing. Gibson et al. found improved positive affect 120-minutes after a meal high in protein compared to a meal low in protein. The observation of protein-mediated mood effects may depend on a longer post-dose time-frame than that employed in the current study. Alternatively, it may be that the mood scales used in the current study lacked sensitivity to these effects, for example, previous research appears to demonstrate specific improvement of subjective depression ratings associated with carbohydrate ingestion (e.g. Fischer et al., 2001; Sayegh, Schiff, Wurtman, Spiers, McDermott & Wurtman, 1995; Wurtman, Brzezinski, Wurtman, & Laferrere, 1989) but depression was not measured in the current study. Moreover, Gibson administered a more general mood scale and measured negative and positive affect. This was shown to be sensitive to protein ingestion. Furthermore, Gibson did not administer a cognitive test battery which in itself might affect mood.

In terms of mechanisms, we can only speculate as biomarkers were not measured in this study. There are, however, a number of potential mechanisms that could be responsible for the effects of macronutrient ingestion on cognitive performance and mood. Kaplan et al. (2001) suggest that carbohydrates, fat and protein may improve performance via distinct mechanisms that are mediated by different brain regions. Moreover, they argue that facilitation of certain aspects of cognitive performance after administration of macronutrients which do not significantly raise blood glucose levels suggest that facilitation of cognitive performance might be due to more generic aspects of energy supply to the brain (Kaplan et al., 2001). There is substantial evidence suggesting possible hippocampal mediation for the glucose facilitation effect in both cognitive and physiological terms. However, the fact that macronutrients which do not raise blood glucose levels also improve certain aspects of cognitive functioning suggests that the enhancement effect of certain foodstuffs on cognitive function may be nutrient-specific whereby the action of glucose is on specific central mechanisms and other macronutrients have their effects on more generalised peripheral mechanisms.

It has been suggested that food-related memory enhancement may occur through release of gastrointestinal peptides in response to ingestion of fat, protein, and glucose (Flood et al., 1987; Flood & Morley, 1989). This notion is supported by animal studies which have demonstrated that gastrointestinal peptides, such as cholecystokinin (CCK; Flood & Morley, 1989; Flood et al., 1987), gastrin-releasing peptide (Morley et al., 1994), and amylin (Flood & Morley, 1992) enhance memory performance through vagus nerve stimulation. Moreover, enterostatin, an intestinal peptide produced after food ingestion, has been shown to attenuate scopolamine-induced amnesia (Takenaka, Nakamura, Jinsmaa, Lipowski, & Yoshikawa, 2001).

Furthermore, Flood et al. (1987) and Morley et al. (1994) demonstrated that CCK memory enhancement is abolished by vagotomy, suggesting that the central effects of this peptide are mediated by activation of the ascending pathways of the vagus nerve. In terms of mood effects, Cunliffe et al (1997) argue that the previously observed fat mediated reduction in alertness and flicker fusion frequency could be due to unspecified hormonal changes. Wells & Read (1996) attributed impaired Bakkan performance to concomitant changes in mood and reduced alertness following fat ingestion to increased CCK release stimulated by the presence of lipid in the duodenum. CCK has been shown to increase sleepiness (e.g. Kapas et al., 1991; Stacher, Bauer, & Steinringer, 1979). Thus CCK is attributed to both performance enhancements and impairments. However, the findings reported in the current study did not reveal fat-mediated performance impairments or mood effects. Alternatively, cognitive enhancement could be related to circulating insulin. Previous research has shown that insulin administration can enhance cognitive performance (e.g. Benedict et al., 2004; Craft et al., 1999; Moosavi, Naghdi, Maghsoudi, & Zahedi, 2007). The ingestion of protein and glucose is associated with increased levels of circulating insulin (e.g. Nuttall, Mooradian, Gannon, Billington, & Krezowski, 1984) that could potentially act directly on the CNS. However, this would not explain why fat-mediated enhancements have been previously observed (Fischer et al., 2001) as fat does not stimulate insulin secretion. Moreover, if insulin were responsible for glucose- and protein-mediated cognitive enhancement the same or at least similar effects would be observed following these two macronutrients.

The nutrient-specific profiles of peptide and hormone release are yet to be elucidated and the effects of different peptides and peptide combinations on the CNS and behaviour are not fully understood. However, it is possible that macronutrient-specific peptide profiles may target different brain regions or neurotransmitter systems.

In conclusion the current experiment has provided additional support for nutritionally-mediated cognitive enhancement following the ingestion of macronutrients, particularly 15-minutes post ingestion with sustained memory enhancement 60-minutes after protein ingestion. Furthermore, the current findings have revealed different temporal patterns of effects which suggest that the action of different macronutrients on cognition may be related to nutrient-specific mechanisms. However, a number of crucial questions remain to be answered before the beneficial effects of macronutrient administration can be fully understood. For example, this study has failed to clarify the basis of post-prandial mood effects and further investigation is required. Moreover, there are other limitations which future studies should aim to address. For example, we only investigated one dosage per macronutrient and further research needs to be carried out to elucidate the effects of different dosages of macronutrients on different cognitive domains. Moreover, controlling for the potential confounding effects of cognitive testing on subjective mood measures might help to elucidate macronutrient effects on mood. Finally, future research should employ a longer post-dose period in order to further clarify the window of opportunity of effects. In Western countries, the high incidence of obesity, Type 2 diabetes and AD are associated with diet and increased fat intake (Martins et al., 2006). The data suggests that modifiable lifestyle factors including diet may contribute significantly to the risk of cognitive decline, including dementia. Understanding the way nutrients affect behaviour will provide scientific evidence for nutritional interventions aimed to increase health including optimal cognition and psychological ‘wellbeing’.

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Table(s)

Table 1. *Composition of treatment drinks*

Glucose	Fat	Protein	Placebo
40g glucose dextrose powder (Thornton and Ross Ltd, Huddersfield, UK)	16g Pura Vegetable oil (supplied by Sainsbury's Plc, UK)	40g Casilan 90% protein powder (supplied by Boots Plc., UK)[a]	
	2g aspartame (Candarel, Merisant UK Ltd)	2g aspartame (Candarel, Merisant UK Ltd)	2g aspartame (Candarel, Merisant UK Ltd)
10ml Lemon juice	10ml Lemon juice	10ml Lemon juice	10ml Lemon juice
260 ml water	249 ml water	260ml water	290ml water

[a] The other 10% is made up of small amounts of carbohydrate, fat, fibre, sodium and calcium.

Table(s)

Table 2. *Mean (± SD) Cognitive Performance Scores at Baseline and the Post-drink Change from Baseline*

Measure	Baseline score	Post-drink change from baseline	
		15-minutes	60-minutes
Immediate word recall (% accuracy)			
Placebo	35.93 (11.35)	0.00 (13.54)	-4.12 (16.65
Fat	37.78 (14.99)	-1.57 (13.29)	-6.67 (13.69)
Protein	42.78 (13.00)	-5.10 (15.42)	-4.31 (13.63)
Glucose	43.14 (10.24)	-7.26 (10.82)*	-11.57 (12.91)*
Total	**39.91 (12.40)**	**-3.48 (13.27)**	**-6.67 (5.90)**
Delayed word recall (% accuracy)			
Placebo	24.63 (11.15)	-10.39 (13.64)	-7.65 (16.06)
Fat	25.74 (14.09)	-10.78 (10.37)	-16.08 (14.96)
Protein	28.70 (16.01)	-13.53 (14.12)	-14.31 (14.56)
Glucose	25.29 (9.79)	-10.98 (10.72)	-14.12 (8.86)
Total	**26.09 (12.76)**	**-11.42 (12.13)**	**-13.04 (13.61)**
Simple reaction time (ms)			
Placebo	265.99 (23.92)	16.54 (25.31)	18.35 (23.86)
Fat	266.89 (33.99)	11.29 (25.05)	16.05 (22.90)
Protein	267.71 (39.18)	16.23 (23.72)	17.78 (18.97)
Glucose	265.30 (28.74)	5.56 (18.73)	10.78 (22.62)
Total	**266.47 (31.46)**	**12.41 (23.20)**	**15.74 (22.09)**
Choice reaction time accuracy (%)			
Placebo	94.56 (4.94)	0.00 (3.46)	-1.41 (3.80)
Fat	95.11 (4.66)	-2.24 (6.08)	-0.71 (4.36)
Protein	94.78 (5.49)	0.59 (3.52)	-0.12 (2.78)
Glucose	93.41 (6.96)	0.59 (4.35)	2.00 (4.53)
Total	**94.47 (5.51)**	**-0.265 (4.35)**	**-0.06 (3.87)**
Choice reaction time (ms)			
Placebo	398.29 (41.25)	4.56 (36.37)	-2.94 (33.32)
Fat	397.22 (49.16)	-5.46 (23.63)	-1.83 (30.19)
Protein	391.08 (42.22)	1.15 (26.69)	12.80 (31.26)
Glucose	391.81 (45.94)	-7.70 (25.63)	9.11 (27.39)
Total	**394.60 (44.64)**	**-1.86 (28.08)**	**4.29 (30.54)**
Digit vigilance accuracy (%)			
Placebo	94.82 (8.14)	0.91 (6.76)	-2.48 (5.26)
Fat	95.19 (5.62)	0.39 (4.98)	-3.66 (5.33)
Protein	95.31 (4.81)	0.52 (4.75)	-0.52 (4.87)
Glucose	95.95 (4.66)	-0.65 (5.32)	-2.75 (6.16)
Total	**95.32 (5.81)**	**0.29 (5.45)**	**-2.35 (5.41)**
Digit vigilance reaction time (ms)			
Placebo	423.18 (47.70)	17.43 (38.98)	11.23 (26.17)
Fat	412.15 (36.04)	11.09 (27.93)	15.07 (31.08)
Protein	417.99 (38.75)	14.86 (33.57)	13.37 (29.16)
Glucose	433.48 (41.02)	-8.81 (26.08)	-7.31 (34.13)
Total	**421.70 (40.88)**	**8.643 (31.64)**	**8.09 (30.14)**
Spatial memory original item accuracy (%)			
Placebo	93.40 (6.60)	-2.57 (13.08)	1.10 (10.65)
Fat	94.10 (6.24)	-2.21 (11.04)	-0.37 (7.48)
Protein	88.89 (13.14)	6.25 (15.93)	4.78 (12.01)
Glucose	94.12 (5.62)	-1.84 (7.25)	-5.15 (15.51)
Total	**92.63 (7.90)**	**-0.09 (11.83)**	**0.09 (11.41)**
Spatial memory new item accuracy (%)			
Placebo	97.22 (4.28)	-1.76 (8.09)	-3.24 (9.99)
Fat	98.89 (2.14)	-3.82 (5.74)	-2.35 (4.72)
Protein	92.78 (11.14)	2.06 (12.00)	3.53 (13.32)
Glucose	95.88 (5.93)	2.35 (4.37)	-3.82 (10.83)
Total	**96.19 (5.87)**	**-0.29 (7.55)**	**-1.47 (9.72)**
Spatial memory sensitivity index (SI)			
Placebo	0.91 (0.09)	-0.04 (0.19)	-0.02 (0.19)
Fat	0.94 (0.06)	-0.06 (0.13)	-0.03 (0.05)
Protein	0.82 (0.23)	0.08 (0.28)	0.08 (0.24)
Glucose	0.90 (0.88)	0.01 (0.07)	-0.09 (0.23)
Total	**0.89 (0.32)**	**-0.0025 (0.17)**	**-0.015 (0.18)**
Spatial memory reaction time (ms)			
Placebo	564.36 (127.08)	-22.62 (60.40)	-37.32 (53.37)
Fat	586.87 (151.34)	-62.16 (93.23)	-54.58 (107.43)
Protein	599.05 (157.44)	-41.68 (167.28)	-76.87 (152.22)
Glucose	616.11 (191.16)	-73.34 (153.69)	-86.22 (93.94)
Total	**591.60 (156.76)**	**-49.95 (118.65)**	**-63.748 (101.74)**

Measure	Baseline score	Post-drink change from baseline	
		15-minutes	60-minutes
Numeric working memory original item accuracy (%)			
Placebo	87.28 (13.55)	-1.18 (8.32)	-0.78 (7.20)
Fat	88.27 (14.09)	-2.22 (5.27)	0.13 (7.18)
Protein	87.78 (11.79)	-2.09 (6.00)	-1.31 (5.21)
Glucose	88.76 (14.18)	-3.40 (7.08)	-2.88 (6.61)
Total	**88.02 (13.40)**	**-2.22 (6.67)**	**-1.21 (6.55)**
Numeric working memory new item accuracy (%)			
Placebo	94.08 (9.88)	1.57 (5.14)	1.31 (4.58)
Fat	92.96 (9.50)	-0.13 (5.00)	2.61 (4.86)
Protein	94.57 (11.36)	0.00 (5.21)	-0.65 (6.61)
Glucose	93.60 (8.16)	1.18 (3.69)	-1.05 (4.85)*
Total	**93.80 (9.73)**	**0.66 (4.76)**	**0.56 (5.23)**
Numeric working memory sensitivity index (SI)			
Placebo	0.82 (0.22)	0.01 (0.10)	0.01 (0.09)
Fat	0.82 (0.23)	-0.02 (0.08)	0.03 (0.10)
Protein	0.83 (0.22)	-0.16 (0.09)	-0.02 (0.11)
Glucose	0.83 (0.21)	-0.02 (0.72)	-0.04 (0.08)
Total	**0.83 (0.22)**	**-0.05 (0.25)**	**-0.005 (0.10)**
Numeric working memory speed (ms)			
Placebo	546.67 (91.19)	-16.48 (79.04)	-29.14 (63.00)
Fat	530.86 (72.16)	-19.87 (43.64)	-22.71 (57.97)
Protein	532.73 (87.55)	-7.78 (37.66)	-37.78 (33.48)
Glucose	557.27 (92.22)	-37.31 (33.98)	-37.92 (72.86)
Total	**541.88 (85.78)**	**-20.36 (48.58)**	**-31.89 (56.83)**
Word recognition original item accuracy (%)			
Placebo	60.00 (16.01)	1.96 (12.19)	-0.39 (13.01)
Fat	55.55 (16.80)	4.31 (17.79)	1.18 (19.75)
Protein	61.48 (17.04)	-3.14 (15.83)	-1.57 (17.08)
Glucose	55.69 (18.25)	2.35 (11.29)	-4.71 (12.64)
Total	**58.18 (17.03)**	**1.37 (14.28)**	**-1.37 (15.62)**
Word recognition new item accuracy (%)			
Placebo	87.78 (10.30)	-1.57 (10.94)	-5.88 (10.24)
Fat	86.67 (14.64)	-0.79 (16.31)	-7.84 (15.14)
Protein	86.30 (13.23)	1.57 (8.67)	-6.67 (12.91)
Glucose	84.31 (16.32)	-2.75 (13.96)	-6.67 (11.55)
Total	**86.27 (13.62)**	**-0.89 (12.47)**	**-6.77 (12.46)**
Word recognition sensitivity index (SI)			
Placebo	0.53 (0.17)	0.00 (0.18)	-0.08 (0.19)
Fat	0.51 (0.21)	0.01 (0.30)	-0.11 (0.32)
Protein	0.53 (0.24)	0.01 (0.14)	-0.10 (0.21)
Glucose	0.49 (0.23)	-0.05 (0.21)	-0.16 (0.21)
Total	**0.52 (0.21)**	**-0.008 (0.21)**	**-0.11 (0.23)**
Word recognition reaction time (ms)			
Placebo	651.31 (129.45)	64.53 (241.18)	-21.01 (78.99)
Fat	616.47 (70.52)	-5.89 (74.50)	17.73 (88.08)
Protein	636.27 (101.26)	-2.63 (93.60)	-23.55 (85.32)
Glucose	622.28 (83.61)	13.96 (78.53)	19.30 (101.59)
Total	**631.58 (96.21)**	**17.49 (121.95)**	**-1.88 (88.50)**
Picture recognition original item accuracy (%)			
Placebo	75.00 (17.66)	0.29 (13.40)	1.76 (9.67)
Fat	77.22 (20.95)	1.18 (16.25)	-5.88 (16.23)
Protein	80.83 (17.00)	-4.41 (12.98)	-5.00 (17.05)
Glucose	80.59 (15.19)	-5.00 (15.91)	-5.29 (12.05)
Total	**78.41 (17.70)**	**-1.99 (14.64)**	**-3.60 (13.75)**
Picture recognition new item accuracy (%)			
Placebo	91.11 (6.08)	*-3.24 (9.34)*	-1.47 (8.62)
Fat	88.61 (11.09)	2.35 (12.13)	-0.29 (10.07)
Protein	90.00 (9.24)	0.29 (6.27)	-2.94 (6.14)
Glucose	89.41 (8.08)	-2.06 (12.63)	0.59 (9.50)
Total	**89.78 (8.62)**	**-0.67 (10.09)**	**-1.03 (8.58)**

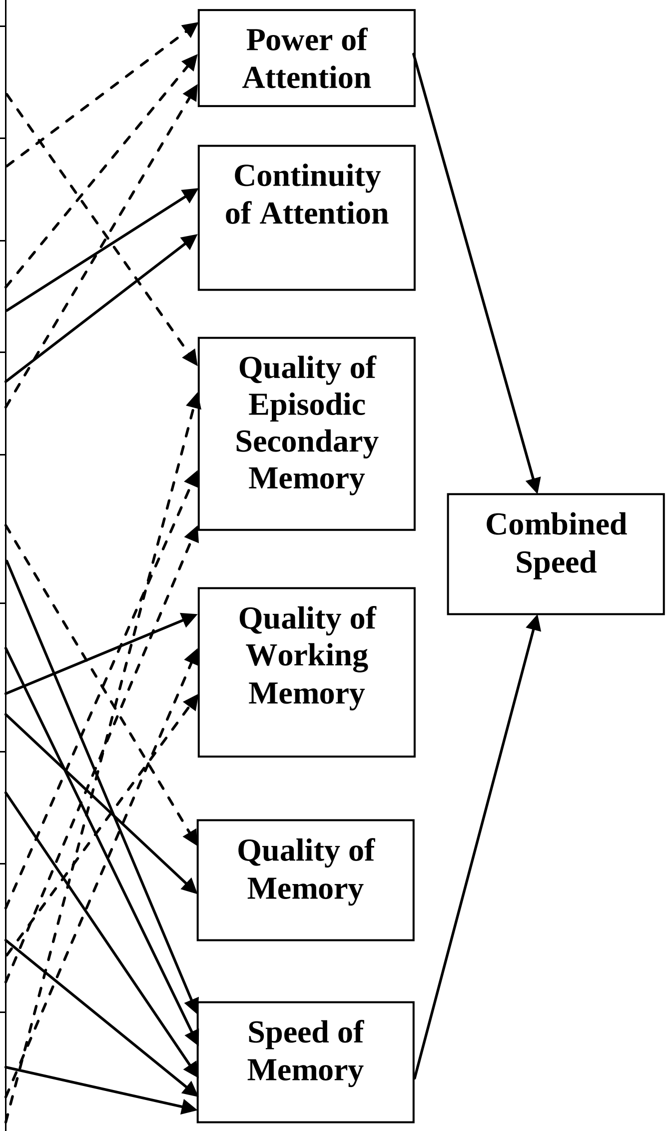
Measure	Baseline score	Post-drink change from baseline	
		15-minutes	60-minutes
Picture recognition sensitivity index (SI)			
Placebo	0.69 (0.17)	-0.04 (0.17)	0.00 (0.13)
Fat	0.67 (0.29)	0.048 (0.21)	-0.05 (0.21)
Protein	0.72 (0.24)	-0.03 (0.14)	-0.08 (0.16)
Glucose	0.72 (0.17)	-0.07 (0.21)	-0.04 (0.13)
Total	**0.70 (0.22)**	**-0.02 (0.18)**	**-0.04 (0.16)**
Picture recognition reaction time (ms)			
Placebo	718.72 (91.13)	23.15 (60.94)	0.38 (48.44)
Fat	709.04 (87.31)	-1.38 (67.94)	-6.70 (74.57)
Protein	705.53 (105.12)	41.10 (153.71)	-1.82 (76.48)
Glucose	725.84 (87.80)	-26.72 (76.48)	-17.75 (85.09)
Total	**714.78 (92.84)**	**9.04 (89.77)**	**-6.47 (71.15)**

Difference between treatment and placebo significant at: * 0.05 level and ** 0.01 level

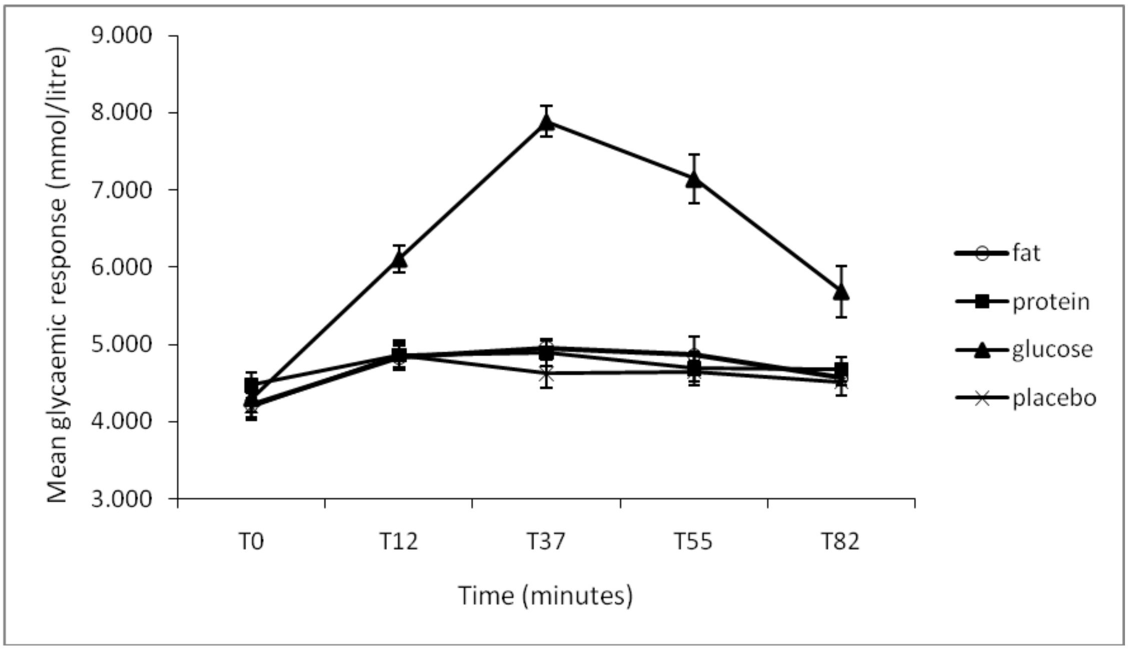
Figure(s)

Cognitive Outcome Measure

Task	Measure
Immediate word recall	Number correct (%) Errors (#) Intrusions (#)
Simple reaction time	Reaction time (msec)
Digit vigilance	Accuracy (%) Reaction time (msec) False alarms (#)
Choice reaction time	Accuracy (%) Reaction time (msec)
Spatial working memory	Original accuracy (%) New accuracy (%) Sensitivity Index Reaction time (msec)
Numeric working memory	Original accuracy (%) New accuracy (%) Sensitivity Index Reaction time (msec)
Delayed word recall	Number correct (%) Errors (#) Intrusions (#)
Delayed word recognition	Original accuracy (%) New accuracy (%) Sensitivity Index Reaction time (msec)
Delayed picture recognition	Original accuracy (%) New accuracy (%) Sensitivity Index Reaction time (msec)

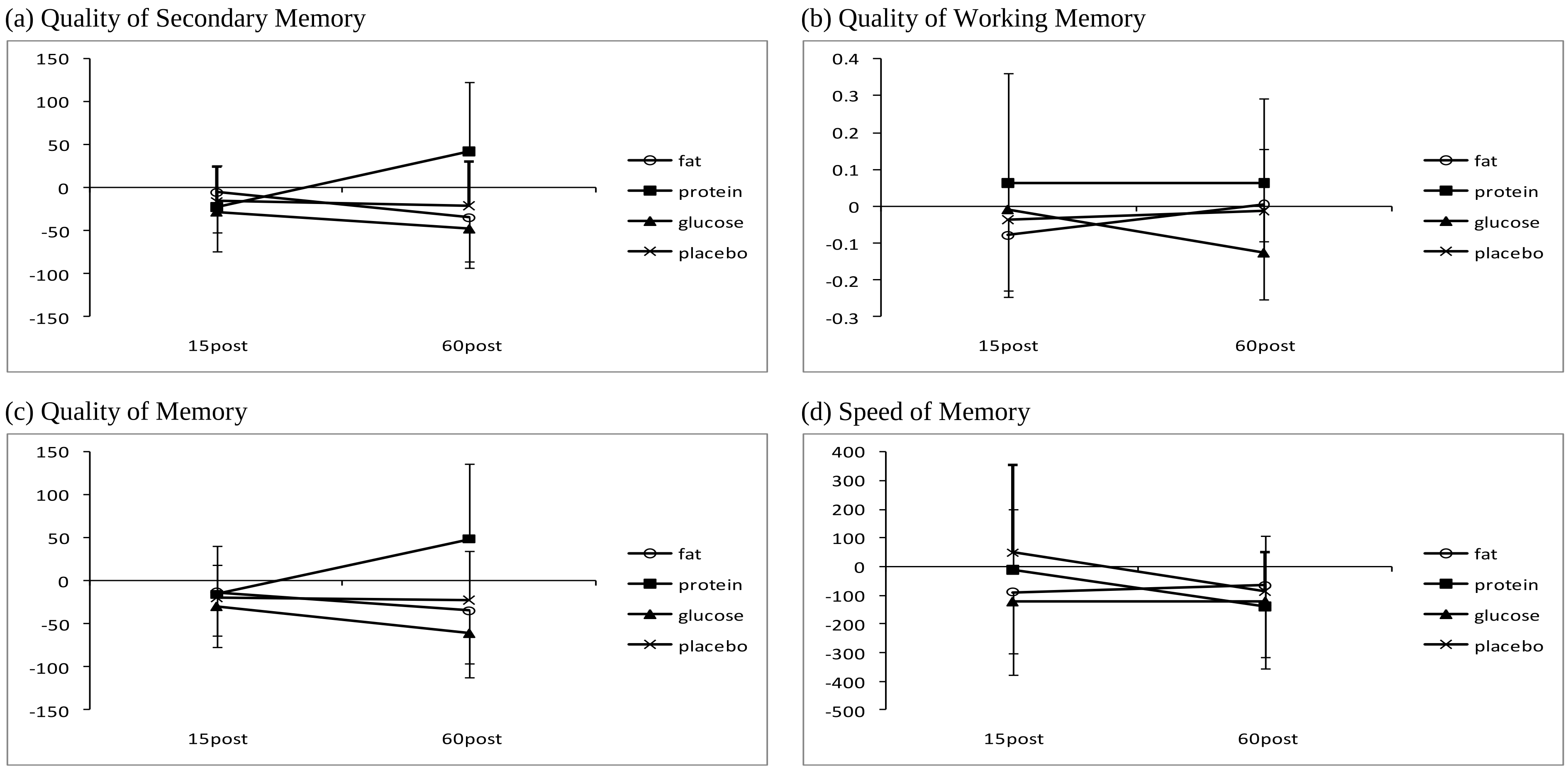


Figure(s)



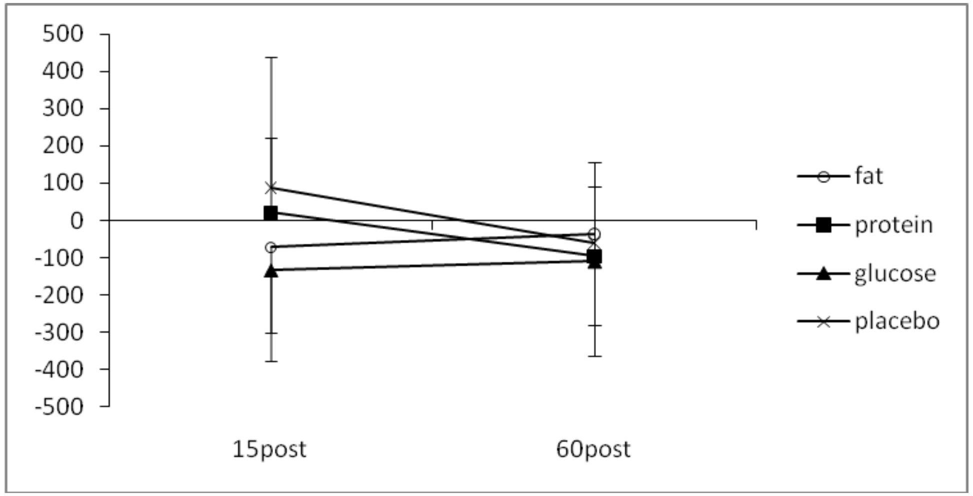
9.000
8.000
7.000
6.000
5.000
4.000
3.000
Mean glycaemic response (mmol/litre)
T0
T12
T37
T55
T82
Time (minutes)
fat
protein
glucose
placebo

Figure(s)

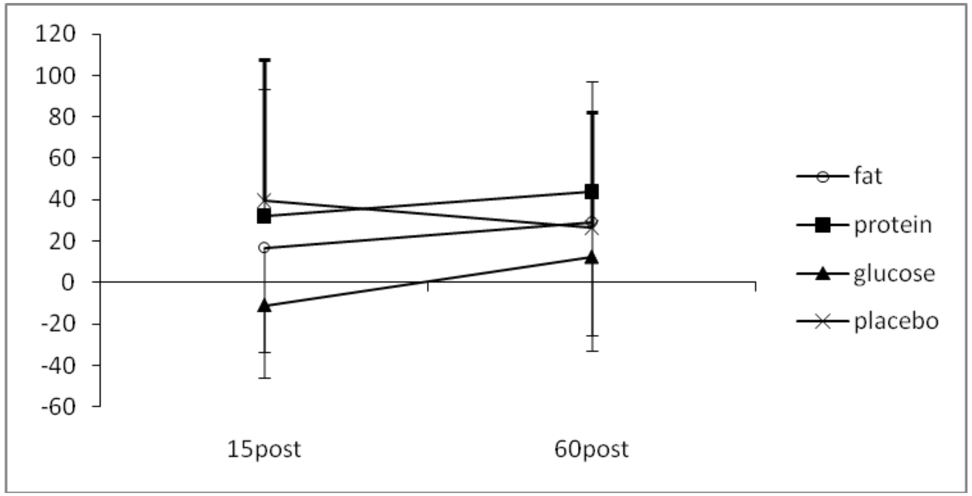

(a) Quality of Secondary Memory
150
100
50
0
-50
-100
-150
15post
60post
fat
protein
glucose
placebo
(b) Quality of Working Memory
0.4
0.3
0.2
0.1
0
-0.1
-0.2
-0.3
15post
60post
fat
protein
glucose
placebo
(c) Quality of Memory
150
100
50
0
-50
-100
-150
15post
60post
fat
protein
glucose
placebo
(d) Speed of Memory
400
300
200
100
0
-100
-200
-300
-400
-500
15post
60post
fat
protein
glucose
placebo

Figure(s)

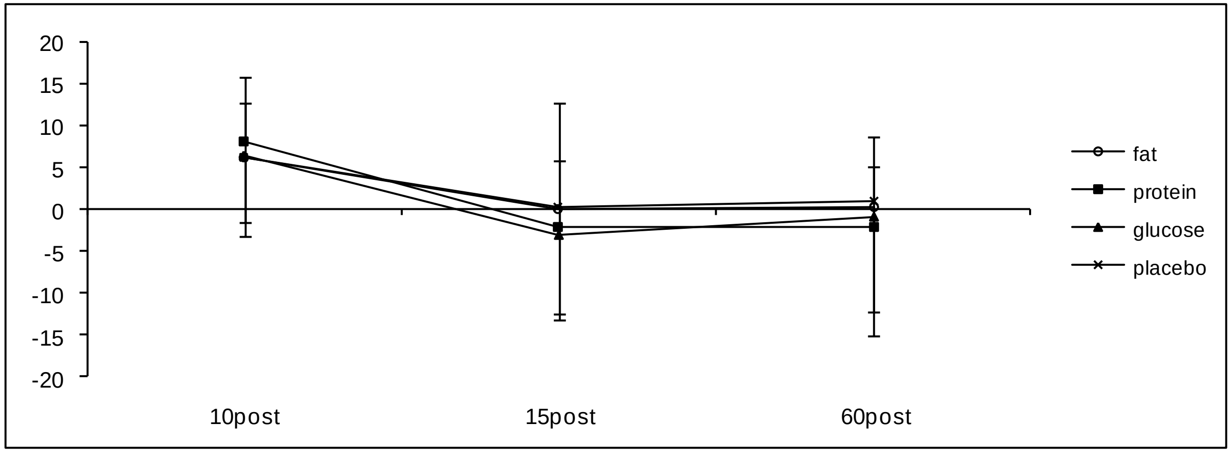
(a) Combined Speed



(b) Power of Attention



(c) Self-rated Alertness



Figure(s)

Figure 1. *Schematic representation of the CDR battery showing the cognitive tasks, individual task outcome measures and the factors derived by factor analysis. Arrows indicate that a task outcome measure contributes to the given factor: Power of Attention, Continuity of Attention, Quality of Memory and Speed of Memory. Format of figure taken from Kennedy, Scholey, Tildesley, Perry and Wesnes (2002).*

Figure 2. *Mean Blood Glucose Levels over the Course of the Experimental Sessions*

Figure 3. *Profile of effects of macronutrients on cognitive outcome measures relating to memory: a) Quality of secondary memory, b) Quality of working memory, c) Quality of memory and c) Speed of memory at the two post dose time points (15-minutes and 60-minutes). Planned comparisons revealed significant enhancement following protein ingestion, compared to placebo on Quality of secondary memory (60-minutes post, $p<0.001$), Quality of working memory (15-minutes post, $p<0.05$) and quality of memory (60-minutes post, $p<0.001$). Glucose ingestion, compared to placebo: impaired working memory (60-minutes post drink, $p<0.05$), impaired quality of memory (60-minutes post, $p<0.05$) and led to faster memory processing (speed of memory, 15-minutes post, $p<0.05$). Fat ingestion led to faster memory processing (speed of memory) compared to placebo (15-minutes post, $p<0.05$).*

Figure 4. *Profile of cognitive factors: a) Combined Speed and b) Power of Attention and c) self rated mood (measured by VAS). Power of attention was significantly improved following glucose compared to placebo (particularly 15-minutes post, $p<0.01$). Fat and glucose led to faster overall responses (combined speed) 15-minutes post drink, compared to placebo ($p<0.01$ and $p<0.001$ respectively). Self-rated alertness was significantly higher 10-minutes post drink, regardless of drink, than 15- and 60-minutes post drink ($p<0.05$ and $p<0.01$ respectively).*