

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 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 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 these 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.

Cleophas TJ: A simple method for the estimation of interaction bias in crossover studies. *J Clin Pharmacol* 1990, 30:1036-1040

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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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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 these 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.

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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

Running head: ACUTE MACRONUTRIENT INGESTION ENHANCES COGNITION

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

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Abstract

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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 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 increased ratings of alertness. The findings suggest that macronutrients: i) have different windows of opportunity for effects ii) target different cognitive domains.



## Introduction

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3           The effects of nutrition on brain and behaviour and more specifically the  
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5 cognitive effects of foods, food components and nutritional interventions are very  
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7 much on the public agenda. More specifically, it has been demonstrated that acute  
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9 administration of glucose (a simple carbohydrate), can facilitate verbal declarative  
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11 memory in healthy young adults and adolescents (e.g. Foster, Lidder & Sünram, 1998;  
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13 Smith & Foster, 2008; Smith Hii, Foster & van Eekelen, 2009; Sünram-Lea, Foster,  
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15 Durlach & Perez, 2001; 2002a; 2002b) and older populations (e.g. Craft, Murphy &  
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17 Wemstrom, 1994; Kaplan, Greenwood, Winocur & Wolever, 2001; Messier, Gagnon  
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19 & Knott, 1997; Riby, Meikle & Glover, 2004). In addition, administration of a  
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21 glucose drink has been shown to improve working memory performance in healthy,  
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23 young adults (e.g. Scholey, Harper & Kennedy, 2001); to ameliorate impairment of a  
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25 secondary, psychomotor task during a divided attention (encoding plus psychomotor)  
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27 paradigm (Scholey, Sünram-Lea, Greer, Elliott, & Kennedy, 2009) and to enhance  
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29 prospective memory (Riby, Laws, McLaughlin & Murray, 2011). Hoyland, Lawton  
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31 and Dye (2008) conducted a comprehensive review of the literature and concluded  
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33 that the most robust glucose-mediated enhancement has been demonstrated on  
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35 memory although there are numerous examples of glucose facilitation of other  
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37 cognitive tasks (see Hoyland et al., 2008; Messier, 2004; Riby, 2004 for reviews).  
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46           Glucose index (GI) is the rate at which an ingested substance increases and  
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48 maintains blood glucose levels. Pure glucose has a high GI as it increases levels  
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50 quickly with a fast return to baseline, whereas foodstuff with lower GI ratings tend to  
51  
52 elicit a slower rise, smaller peak and are slower to return to baseline. Glycaemic Load  
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54 (GL) is another way of describing response to a carbohydrate and takes into account  
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56 both GI and the quantity of food. It has been found that foods with different GIs and  
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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

1 reduced rate of forgetting when assessed 15 min after consumption. Fischer,  
2 Colombani, Langhans and Wenk (2001) administered carbohydrate-, protein- and fat-  
3 containing meals to healthy, young adults and tested cognitive performance at various  
4 time-points over the course of 180-minutes. Their data indicated that different  
5 macronutrients influence cognitive performance in a different manner, with the best  
6 performance usually observed after fat ingestion. More specifically, faster reaction  
7 times, improved short-term memory and improved attention were observed at all time  
8 points (60-, 120- and 180 minutes) following fat ingestion. In a placebo-controlled  
9 study Jones, Sünram-Lea and Threadgold (2005) compared the effects of glucose and  
10 protein administration and observed glucose-mediated enhancement of reaction times  
11 following glucose administration. However, this study failed to replicate the  
12 immediate beneficial effects of macronutrients observed by Kaplan et al. or the well-  
13 documented glucose facilitation of memory. Overall, findings to date tentatively  
14 suggest that macronutrients may differentially influence cognition and mood and that  
15 the effects are time-dependent (e.g. Fischer et al., 2001; Kaplan et al., 2001).  
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36 In addition to cognitive facilitation, previous research has also demonstrated  
37 complex macronutrient-specific effects on mood. For example, Fischer et al. (2001)  
38 observed a reduction in depression scores on the Profile of Mood Scale (POMS)  
39 following carbohydrate ingestion compared to protein. Conversely, Gibson et al.  
40 (1999) found that 120-minutes post ingestion, a meal high in protein (and low in  
41 carbohydrate) led to increased positive affect on the PANAS mood scale compared to  
42 a meal low in protein (and high in carbohydrate). In our own laboratory we have  
43 previously observed an increase in negative affect following protein ingestion (Jones  
44 et al., 2005). These findings appear to be contradictory but methodological differences  
45 between studies may account for these discrepancies. For example, Fischer et al.  
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1 (2001) administered pure macronutrients whereas Gibson et al. (1999) administered  
2 macronutrient combinations, which preclude a clear conclusion as to whether the  
3 mood effects are due to specific macronutrients or interaction effects of multiple  
4 macronutrients. In addition, the use of different mood rating scales further impedes  
5 direct comparison.  
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11 It is evident from this brief review of the literature that the effects of glucose  
12 on cognitive functioning and mood are relatively well described whereas those of  
13 protein and fat still remain to be explored in detail. It is also important to note that  
14 although investigating the effects of combined administration of different  
15 macronutrients (for example Benton & Sargent, 1992) is important to for our  
16 understanding of macronutrient interaction they do not inform us about the  
17 contributions of individual macronutrients and their potential underlying mechanisms.  
18 It is important to establish whether different macronutrients influence cognitive  
19 performance via a specific mechanism or whether they exert their influence via a  
20 shared, generalised mechanism. Kaplan et al. (2001) found evidence to indicate a  
21 generalised effect of macronutrients on cognitive function in older adults (e.g. effects  
22 15 and 60 minutes post ingestion - earlier than would be expected if metabolite-  
23 mediated) suggesting a pre-digestive influence possibly due to the release of gut  
24 hormones such as cholecystokinin (CCK), gastrin-releasing peptide or amylin which  
25 have all been demonstrated to influence memory in animals (Flood and Morley, 1989;  
26 1992; Flood, Smith & Morley, 1987; Morley, Flood, Silver & Kaiser, 1994). Whether  
27 such effects have nutrient and/ or domain specificity is yet to be examined. There is  
28 now growing interest in the potential of specific foods to influence mood and  
29 cognitive performance. The main components of our diet that can be readily  
30 manipulated are the macronutrients, glucose (carbohydrates), protein and fat.  
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1 Therefore, the aim of the current research was to further explore the effects of acute  
2 macronutrient ingestion on cognitive performance and mood in healthy young adults.  
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4 Specifically, the effects of acute fat, protein, glucose and placebo ingestion on a range  
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6 of cognitive tests and mood scales were examined.  
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## 8 9 10 Methods and Materials

### 11 12 *Power calculation*

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14 A medium overall effect size was previously found in a meta-analytic review  
15 of the glucose facilitation effect ( $d = 0.56$ ; Riby, 2004). An a priori power calculation  
16 using G-power (Erdfelder, Faul, & Buchner, 1996) revealed that for a medium effect  
17 size, with alpha set to 0.05 (two-tailed) a sample size of 16 would be required for 95%  
18 power. The effect size of fat and protein is as yet unclear due to the relatively few  
19 numbers of studies carried out in this area, however Kaplan et al. (2001) observed  
20 effects using 22 participants.  
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### 31 32 *Participants*

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

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

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25 Three isoenergetic (145 Kcal) and isovolumic (300ml) drinks and an inert  
26 placebo were administered. All drinks were matched for volume, sweetness and  
27 flavour and administered in opaque cups, covered by lids and ingested through a  
28 straw. Drinks were flavoured with lemon juice in order to improve palatability and  
29 participants' compliance. Drinks were prepared in the laboratory and refrigerated  
30 prior to testing. The composition of test drinks is shown in Table 1.  
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40 <Table 1. here>  
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#### 43 *Blood Glucose Measurement*

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46 Blood glucose readings were obtained using the ExacTech blood glucose  
47 monitoring equipment (supplied by MediSense Britain Ltd, 16/17 The Courtyard,  
48 Gorsey Lane, Coleshill, Birmingham B46 1JA), following the manufacturers  
49 recommended procedure.  
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### *Measuring Cognition and Mood*

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3 A tailored version of the CDR System ([www.unitedbiosource.com](http://www.unitedbiosource.com)) was used  
4  
5 to assess participants' cognitive performance and mood. The battery was administered  
6  
7 on PCs and responses were made via a two-button (yes and no) response box.  
8

9 Responses for the Visual Analogue Scales (VAS) were made by mouse click.

10  
11 Completion of the whole battery took around 20-minutes and tasks were presented in  
12  
13 following order:  
14

15  
16 *Word presentation.* – A list of 15 words matched for frequency, concreteness  
17  
18 and imagery was presented on the monitor at the rate of one every two seconds for  
19  
20 participants to remember. During encoding, participants were required to perform two  
21  
22 complex hand-movement sequences (Sünram-Lea et al., 2001). Each sequence was  
23  
24 performed using both hands and contained three movements: fist – chop - slap and  
25  
26 back-slap – chop – fist. Participants were told to alternate the sequence every fifth  
27  
28 word and they were not informed when to change, only that they had to keep track of  
29  
30 this themselves. Hand-movements were performed continually during word  
31  
32 presentation.  
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38  
39 *Immediate word recall.* - Immediately after the words had been presented  
40  
41 participants were given 60-seconds to write down as many words as they could from  
42  
43 the list they had just seen. Participants' responses were marked according to total  
44  
45 number of errors, intrusions and percentage of words recalled correctly (accuracy).  
46  
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48  
49 *Picture presentation.* – Twenty photographs of objects were individually,  
50  
51 displayed in the centre of the screen at a rate of one every three seconds. Each picture  
52  
53 was displayed for one second. Participants were required to remember the pictures.  
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56  
57 *Simple reaction time.* – The word 'yes' was presented repeatedly in the centre  
58  
59 of the screen with inter trial intervals varying randomly between 1 and 3.5 seconds.  
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1 Participants were required to respond by pressing the ‘yes’ button on their response  
2 box as quickly as possible, whenever the word appeared. Reaction times were  
3  
4 recorded in milliseconds.  
5

6  
7 *Digit vigilance.* – A single target digit was randomly selected and  
8  
9 continuously displayed on the right side of the screen. In the centre a series of rapidly  
10 changing digits was displayed at the rate of 150 digits per minute. Participants were  
11  
12 required to press the ‘yes’ button as quickly as possible, whenever the digit in the  
13  
14 centre matched the target digit. The task lasted for three minutes Reaction times  
15  
16 (milliseconds), percentage accuracy and number of false alarms were recorded.  
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22 *Choice reaction time.* - The target words ‘yes’ and ‘no’ were repeatedly,  
23  
24 randomly displayed individually in the centre of the screen. The inter-trial intervals  
25  
26 varied randomly between 1 second and 3.5 seconds. Participants were instructed to  
27  
28 respond by pressing the appropriate button on their response box as quickly and  
29  
30 accurately as possible. Reaction times (milliseconds) and percentage accuracy were  
31  
32 recorded.  
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37 *Spatial working memory.* - A picture of a house was displayed on the screen  
38  
39 with nine evenly distributed windows. Four of the windows were lit up in the original  
40  
41 picture and participants were asked to remember the position of these windows.  
42  
43 Following this, the house was presented again, repeatedly but each time only one  
44  
45 window was lit up. Participants were required to answer whether the window was lit  
46  
47 up or not in the original house by pressing the appropriate button on their answer box.  
48  
49 Percentage accuracy of identifying novel stimuli (distractors) and target stimuli were  
50  
51 recorded in addition to reaction times (milliseconds) to distractors and targets and  
52  
53 overall reaction times. Sensitivity Index (SI) was calculated by combining an  
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55 individual’s ability to discriminate targets and their ability to discriminate distractors.  
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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*

1 The Bond and Lader visual analogue scales (VAS) were used to assess  
2 subjective mood (Bond & Lader, 1974). Sixteen VAS were presented on the screen  
3 immediately after the cognitive tests. Participants used the mouse to position an arrow  
4 at the point on the scale that represented their feelings at that moment. The 16 scales  
5 were combined as recommended by Bond and Lader (1974) to form three mood  
6 factors: 'alertness', 'calmness' and 'contentment'.  
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#### 14 *Cognitive Outcome Measures*

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16 Scores from individual measures were combined to form seven secondary  
17 outcome measures ('power of attention', continuity of attention', 'quality of working  
18 memory', 'quality of episodic secondary memory', 'quality of memory', 'speed of  
19 memory' and 'combined speed') derived from factor analysis of the Cognitive Drug  
20 Research computerised test battery (Wesnes, Ward, Ayre, & Pincock, 1999; Wesnes,  
21 Ward, McGinty & Petrini, 2000), and previously used (e.g. Wesnes et al., 1997; 1999;  
22 2000; Kennedy, Scholey, & Wesnes, 2001; 2002; Kennedy, Scholey, Tildsley, Perry  
23 & Wesnes, 2002; Sünram-Lea, Birchall, Wesnes & Petrini, 2004). See Figure 1.  
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36 *Power of attention factor (also referred to as 'speed of attention')*: Derived by  
37 combining reaction times of three attention tasks: simple reaction time, choice  
38 reaction time, and digit vigilance (units are summed milliseconds for the three  
39 tasks).  
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46 *Continuity of attention factor (also referred to as 'accuracy of attention')*:  
47 Derived by calculating the combined percentage accuracy across choice reaction  
48 time and digit vigilance tasks (with adjustment for false alarms on the latter  
49 test). 100 percent accuracy across the two tasks would result in a maximum  
50 score of 95.  
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*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.

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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-

1 minutes post and 60-minutes post). For the visual analogue scales the levels of  
2 assessment were: 10-minutes post, 35-minutes post and 80-minutes post. In order to  
3  
4 minimise the risk of type 1 errors planned comparisons were only conducted when  
5  
6 significant main effects or interactions (or trends,  $p < 0.1$ ) were observed.  
7  
8

## 9 Results

### 10 *Blood Glucose Levels*

11  
12 For mean blood glucose levels ( $\pm$ SE) see Figure 2. There was a main effect of  
13  
14 drink on blood glucose levels [ $F(3,39)=59.42$ ,  $p < 0.01$ ]. Post hoc analyses indicated  
15  
16 that the glucose drink lead to significantly higher blood glucose levels than the other  
17  
18 drinks (all p-values  $< 0.01$ ). A main effect of time [ $F(4,52)=37.43$ ,  $p < 0.001$ ] was due  
19  
20 to increasing blood glucose levels over the course of the experimental session(all p-  
21  
22 values  $< 0.01$ ) and a significant time\*drink interaction [ $F(12,156)=15.74$ ,  $p < 0.001$ ]  
23  
24 showed that blood glucose levels were significantly higher at each post dose time  
25  
26 point following a glucose drink compared to any of the other treatments.  
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### 33 *Cognitive Outcome Measures*

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35 Mean ( $\pm$ SD) baseline and post-drink change from baseline scores on the  
36  
37 cognitive outcome factors and VAS, on which significant drink effects were observed,  
38  
39 are displayed in Figures 3 and 4.  
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### 43 *Power of Attention*

44  
45 The main effect of drink just failed to reach significance [ $F(3,48)=2.30$ ,  
46  
47  $p=0.09$ ]. Planned comparisons between treatment drink and placebo demonstrated a  
48  
49 significant performance enhancement following glucose compared to placebo  
50  
51 ( $t(48)=2.34$ ,  $p < 0.05$ ) particularly 15-minutes post ingestion ( $t(48)=-3.71$ ,  $p < 0.01$ ). See  
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53 Figure 4(b).  
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### 58 *Continuity of Attention*

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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

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

### 19 *Speed of Memory*

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

### 38 *Combined Speed*

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

### 52 *Subjective Mood Measures*

53 There was a main effect of time on alertness [ $F(1,16)=7.23$ ,  $p < 0.01$ ] with  
54 participants reporting significantly higher alertness levels 10-minutes post drink  
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1 compared to either 15-minutes or 60-minutes post ingestion ( $p < 0.05$  and  $p < 0.01$ ,  
2 respectively). See Figure 4(c).  
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4 <Figures 3 and 4 here>  
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## 7 Discussion

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9 The present study further demonstrated the ability of macronutrients to affect  
10 cognition and mood and that the effects are time-dependant and vary between  
11 macronutrients. Protein facilitated working memory performance 15-minutes post  
12 ingestion, and enhanced episodic memory 60-minutes post ingestion. Glucose  
13 ingestion enhanced attentional processes (power of attention), speed of processing  
14 (combined speed) and speed of memory 15-minutes post ingestion. Fat ingestion was  
15 associated with enhanced speed of processing 15-minutes post drink. However,  
16 glucose was also associated with impaired working memory (quality of working  
17 memory) 60-minutes post ingestion.  
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31 Beneficial effects of protein (compared to placebo) were observed 15 and 60-  
32 minutes post drink and were specifically targeting memory processes with enhanced  
33 working memory 15-minutes post drink and enhanced episodic memory 60-minutes  
34 post drink. Given the significant effects of protein on these memory factors, it is not  
35 surprising that protein ingestion was also associated with enhanced memory accuracy  
36 (quality of memory) as this factor is a combination of accuracy scores from all of the  
37 memory tasks.  
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48 Beneficial effects following fat and glucose ingestion were also observed.  
49 Fifteen minutes after fat ingestion cognitive processing speed (combined speed) and  
50 speed of memory were faster than following placebo. Cognitive processing speed,  
51 speed of memory and the ability to allocate attentional processes ('power of attention'  
52 factor) were also faster 15-minutes following a glucose drink compared to placebo.  
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1 Furthermore, 60-minutes after glucose ingestion working memory was significantly  
2 impaired compared to placebo. This latter finding is not surprising since glucose is  
3 metabolised quickly and enhancement is observed up to 20-minutes post ingestion  
4 (e.g. Foster et al., 1998; Owens & Benton, 1994; Sünram-Lea et al., 2001; 2002a;  
5 2002b; 2004) so enhancement at a later time point would not be expected. And indeed  
6 impairments might be explained by a drop in blood glucose levels subsequent to  
7 ingestion of a glucose load due to increased insulin output.  
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17 In general, the findings show beneficial effects on cognition soon after glucose  
18 and fat ingestion (15-minutes post ingestion), whereas protein enhanced cognition at  
19 later time points. There are some notable exceptions e.g. protein also enhanced  
20 working memory 15-minutes post drink. These findings suggest that i) different  
21 macronutrients have different windows of opportunity for performance improvements  
22 and ii) different macronutrients target different cognitive domains. More specifically  
23 it appears that protein has a beneficial effect on general memory processes, whereas  
24 fat and glucose apparently target attentional processes and speed of processing.  
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36 In terms of glycaemic response the observed trajectories were nutrient-  
37 dependent. As expected glucose ingestion led to significantly increased blood glucose  
38 levels compared to fat, protein or placebo ingestion. This further supports the notion  
39 that ingestion of energy, regardless of source, appears to improve certain aspects of  
40 cognition and that at least some of the effects are independent of increases in blood  
41 glucose levels (Kaplan et al., 2001).  
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51 The current findings are consistent with those of Kaplan et al. (2001) who  
52 demonstrated macronutrient-mediated cognitive enhancement 15-minutes following  
53 protein, glucose and fat. However, in contrast to the current findings, they also  
54 observed maintained performance improvements following glucose ingestion 60-  
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1 minutes post ingestion. Moreover, the current study employed a repeated measures  
2 design in order to reduce inter-participant variability. Steps were taken to minimise  
3 the effects of drink order. Despite this a drink\*time\*drink order interaction was  
4 observed on working memory. There were no main effects of drink order on any of  
5 the measures. Future work should consider potential order effects perhaps studies  
6 could employ between-participant designs.  
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14 The glucose facilitation effect of cognition has been widely reported in the  
15 literature and has previously been reliably demonstrated with dosages of 25g glucose  
16 in healthy, young adults (e.g. Foster et al., 1998; Kennedy & Scholey, 2000; Sünram-  
17 Lea et al., 2001; 2002a; 2002b, 2004) and dosages of 25g and 50g in elderly adults  
18 (e.g. Craft et al., 1994; Messier et al., 1997; Riby et al., 2004). The most robust  
19 facilitation appears to be on memory (see reviews by Riby, 2004; Hoyland et al.,  
20 2008); however glucose-mediated enhancement of other tasks including attention and  
21 speed of processing (those that were enhanced in the current study) has also been  
22 reported in previous research (e.g. reaction times: Owens & Benton, 1994;  
23 information processing: Benton, Owens & Parker, 1994; Donohoe & Benton, 1999).  
24 Taken together these findings suggest that glucose administration may not specifically  
25 target memory processes and the effects of glucose may be more widespread.  
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43 Some previous papers have reported fat-mediated cognitive impairments (e.g.  
44 Cunliffe, Obeid & Powell-Tuck, 1997; Kaplan et al., 2001; and Wells & Read, 1995).  
45 However, Fischer et al. (2001) observed a beneficial effect of fat on a variety of  
46 cognitive tasks which was not replicated in the current study. This could be due to the  
47 optimal time-frame of fat metabolism which may have been missed as a result of the  
48 relatively short experimental sessions employed in the current study (final testing was  
49 60-minutes post ingestion). However, Fischer et al. (2001) observed faster reaction  
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1 times, improved short-term memory and improved attention at all time points (60-,  
2 120- and 180 minutes) following fat ingestion. In the current study we observed faster  
3 processing 15-minutes following a fat drink. The discrepancies between findings may  
4 be a result of different dosages, time-frames and tasks that were employed by  
5 different research groups. Further research is required to establish the effects of  
6  
7 different fat dosages on a variety of cognitive domains.  
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14 The current experiment attempted to separate nutritionally-mediated effects on  
15 cognition and the effects of food perception on mood by assessing mood prior to  
16 nutrient metabolism (10-minutes post drink) and 35-minutes and 80-minutes post-  
17 drink. However, it is important to note that although the 10-minutes post drink time  
18 point would precede absorption of both fat and protein, glucose would be  
19 metabolically active so any observed effects could be nutritionally-mediated. Macht,  
20 Gerer, & Ellgring (2003) found that increasing energy content of food was associated  
21 with increased negative emotions and increased negative perceptions of the food (e.g.  
22 more unhealthy and dangerous etc). However, in the current study no significant main  
23 effects of drink type on self-rated alertness, contentment or calmness were observed  
24 suggesting that energy content does not influence mood in healthy adults. However,  
25 increased alertness was observed immediately post-drink, regardless of drink-type.  
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27 The fact that all drinks increased alertness suggests a general mechanism, possibly the  
28 impact of hydration from all drinks. For example, Neave, Scholey & Emmett et al.  
29 (2001) and Rogers, Kainth & Smit (2001) have shown that, compared to no drink,  
30 water ingestion increases subjective alertness in both fasted and non-fasted, healthy,  
31 young adults.  
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56 Alternatively, the experimental situation may have been responsible for the  
57 immediate post-drink increase in alertness observed. The 10-minutes post-drink mood  
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1 scale was administered in isolation whereas the other mood scales were all  
2 administered immediately after the cognitive test battery. It may be the case that  
3 participants felt significantly less alert at these times because they had just been  
4 engaged, for 20-minutes, in cognitive tasks.  
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9       Macronutrient-specific mood effects demonstrated in previous research (e.g.  
10 Fischer et al., 2001; Gibson et al., 1999) have not been replicated in the current study.  
11 Fischer et al. (2001) found that carbohydrate ingestion led to an overall reduction in  
12 depression scores on the POMS, which was not related to time of testing. Gibson et  
13 al. found improved positive affect 120-minutes after a meal high in protein compared  
14 to a meal low in protein. The observation of protein-mediated mood effects may  
15 depend on a longer post-dose time-frame than that employed in the current study.  
16 Alternatively, it may be that the mood scales used in the current study lacked  
17 sensitivity to these effects, for example, previous research appears to demonstrate  
18 specific improvement of subjective depression ratings associated with carbohydrate  
19 ingestion (e.g. Fischer et al., 2001; Sayegh, Schiff, Wurtman, Spiers, McDermott &  
20 Wurtman, 1995; Wurtman, Brzezinski, Wurtman, & Laferrere, 1989) but depression  
21 was not measured in the current study. Moreover, Gibson administered a more  
22 general mood scale and measured negative and positive affect. This was shown to be  
23 sensitive to protein ingestion. Furthermore, Gibson did not administer a cognitive test  
24 battery which in itself might affect mood.  
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48       In terms of mechanisms, we can only speculate as biomarkers were not  
49 measured in this study. There are, however, a number of potential mechanisms that  
50 could be responsible for the effects of macronutrient ingestion on cognitive  
51 performance and mood. Kaplan et al. (2001) suggest that carbohydrates, fat and  
52 protein may improve performance via distinct mechanisms that are mediated by  
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1 different brain regions. Moreover, they argue that facilitation of certain aspects of  
2 cognitive performance after administration of macronutrients which do not  
3 significantly raise blood glucose levels suggest that facilitation of cognitive  
4 performance might be due to more generic aspects of energy supply to the brain  
5 (Kaplan et al., 2001). There is substantial evidence suggesting possible hippocampal  
6 mediation for the glucose facilitation effect in both cognitive and physiological terms.  
7 However, the fact that macronutrients which do not raise blood glucose levels also  
8 improve certain aspects of cognitive functioning suggests that the enhancement effect  
9 of certain foodstuffs on cognitive function may be nutrient-specific whereby the  
10 action of glucose is on specific central mechanisms and other macronutrients have  
11 their effects on more generalised peripheral mechanisms.  
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27 It has been suggested that food-related memory enhancement may occur  
28 through release of gastrointestinal peptides in response to ingestion of fat, protein,  
29 and glucose (Flood et al., 1987; Flood & Morley, 1989). This notion is supported by  
30 animal studies which have demonstrated that gastrointestinal peptides, such as  
31 cholecystokinin (CCK; Flood & Morley, 1989; Flood et al., 1987), gastrin-releasing  
32 peptide (Morley et al., 1994), and amylin (Flood & Morley, 1992) enhance memory  
33 performance through vagus nerve stimulation. Moreover, enterostatin, an intestinal  
34 peptide produced after food ingestion, has been shown to attenuate scopolamine-  
35 induced amnesia (Takenaka, Nakamura, Jinsmaa, Lipowski, & Yoshikawa, 2001).  
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48 Furthermore, Flood et al. (1987) and Morley et al. (1994) demonstrated that  
49 CCK memory enhancement is abolished by vagotomy, suggesting that the central  
50 effects of this peptide are mediated by activation of the ascending pathways of the  
51 vagus nerve. In terms of mood effects, Cunliffe et al (1997) argue that the previously  
52 observed fat mediated reduction in alertness and flicker fusion frequency could be  
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1 due to unspecified hormonal changes. Wells & Read (1996) attributed impaired  
2 Bakkan performance to concomitant changes in mood and reduced alertness  
3 following fat ingestion to increased CCK release stimulated by the presence of lipid  
4 in the duodenum. CCK has been shown to increase sleepiness (e.g. Kapas et al., 1991;  
5 Stacher, Bauer, & Steinringer, 1979). Thus CCK is attributed to both performance  
6 enhancements and impairments. However, the findings reported in the current study  
7 did not reveal fat-mediated performance impairments or mood effects. Alternatively,  
8 cognitive enhancement could be related to circulating insulin. Previous research has  
9 shown that insulin administration can enhance cognitive performance (e.g. Benedict  
10 et al., 2004; Craft et al., 1999; Moosavi, Naghdi, Maghsoudi, & Zahedi, 2007). The  
11 ingestion of protein and glucose is associated with increased levels of circulating  
12 insulin (e.g. Nuttall, Mooradian, Gannon, Billington, & Krezowski, 1984) that could  
13 potentially act directly on the CNS. However, this would not explain why fat-  
14 mediated enhancements have been previously observed (Fischer et al., 2001) as fat  
15 does not stimulate insulin secretion. Moreover, if insulin were responsible for  
16 glucose- and protein-mediated cognitive enhancement the same or at least similar  
17 effects would be observed following these two macronutrients.

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

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

<sup>a</sup> The other 10% is made up of small amounts of carbohydrate, fat, fibre, sodium and calcium.

Table 2. Mean ( $\pm$  SD) Cognitive Performance Scores at Baseline and the Post-drink Change from Baseline

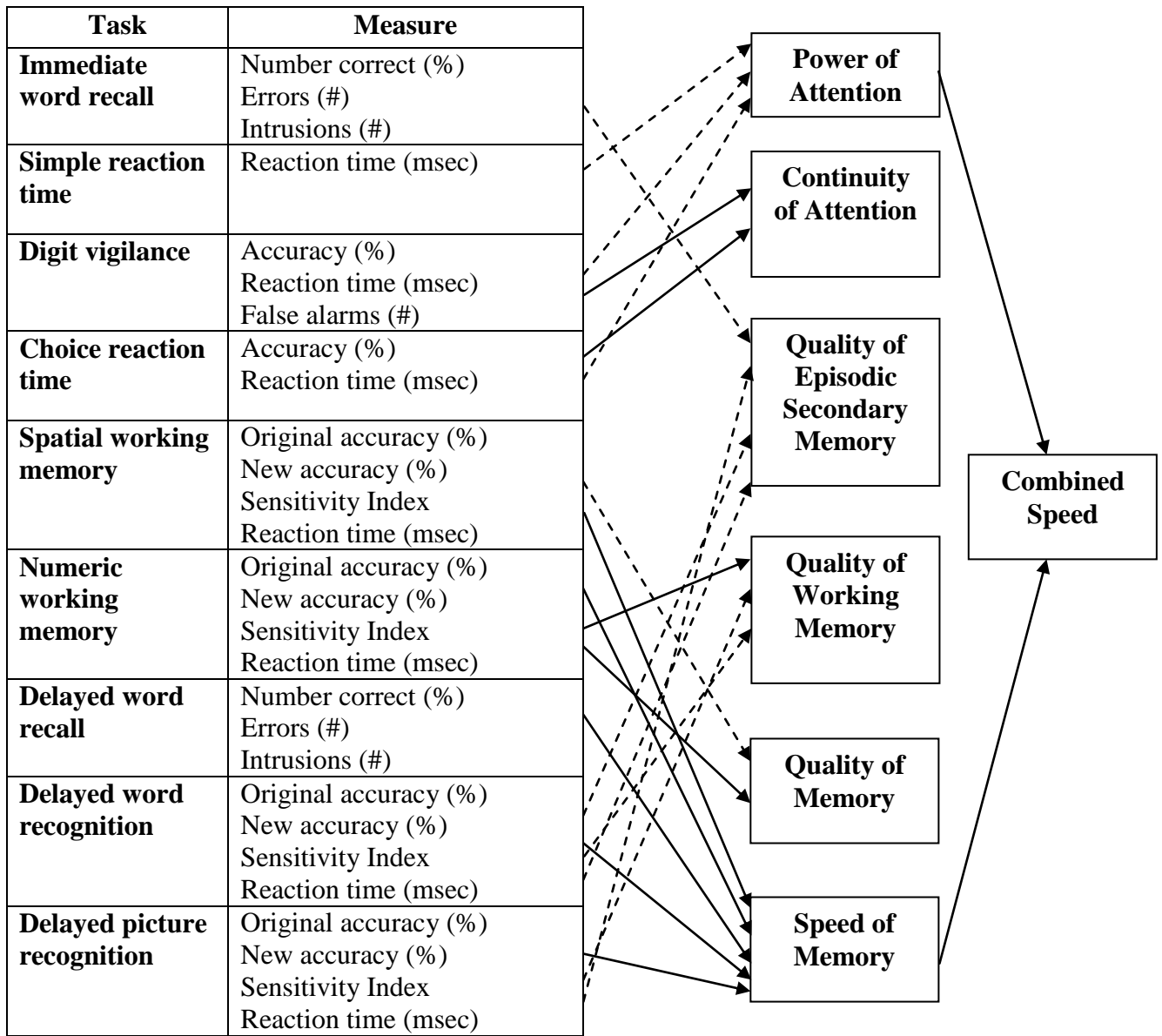
Measure	Baseline score	Post-drink change from baseline	
		15-minutes	60-minutes
<i>Immediate word recall (% accuracy)</i>			
Placebo	35.93 (11.35)	0.00 (13.54)	-4.12 (16.65)
Fat	37.78 (14.99)	-1.57 (13.29)	-6.67 (13.69)
Protein	42.78 (13.00)	-5.10 (15.42)	-4.31 (13.63)
Glucose	43.14 (10.24)	-7.26 (10.82)*	-11.57 (12.91)*
Total	<b>39.91 (12.40)</b>	<b>-3.48 (13.27)</b>	<b>-6.67 (5.90)</b>
<i>Delayed word recall (% accuracy)</i>			
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	<b>26.09 (12.76)</b>	<b>-11.42 (12.13)</b>	<b>-13.04 (13.61)</b>
<i>Simple reaction time (ms)</i>			
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	<b>266.47 (31.46)</b>	<b>12.41 (23.20)</b>	<b>15.74 (22.09)</b>
<i>Choice reaction time accuracy (%)</i>			
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	<b>94.47 (5.51)</b>	<b>-0.265 (4.35)</b>	<b>-0.06 (3.87)</b>
<i>Choice reaction time (ms)</i>			
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	<b>394.60 (44.64)</b>	<b>-1.86 (28.08)</b>	<b>4.29 (30.54)</b>
<i>Digit vigilance accuracy (%)</i>			
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	<b>95.32 (5.81)</b>	<b>0.29 (5.45)</b>	<b>-2.35 (5.41)</b>
<i>Digit vigilance reaction time (ms)</i>			
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	<b>421.70 (40.88)</b>	<b>8.643 (31.64)</b>	<b>8.09 (30.14)</b>
<i>Spatial memory original item accuracy (%)</i>			
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	<b>92.63 (7.90)</b>	<b>-0.09 (11.83)</b>	<b>0.09 (11.41)</b>
<i>Spatial memory new item accuracy (%)</i>			
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	<b>96.19 (5.87)</b>	<b>-0.29 (7.55)</b>	<b>-1.47 (9.72)</b>
<i>Spatial memory sensitivity index (SI)</i>			
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	<b>0.89 (0.32)</b>	<b>-0.0025 (0.17)</b>	<b>-0.015 (0.18)</b>
<i>Spatial memory reaction time (ms)</i>			
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	<b>591.60 (156.76)</b>	<b>-49.95 (118.65)</b>	<b>-63.748 (101.74)</b>

Measure	Baseline score	Post-drink change from baseline	
		15-minutes	60-minutes
<i>Numeric working memory original item accuracy (%)</i>			
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	<b>88.02 (13.40)</b>	<b>-2.22 (6.67)</b>	<b>-1.21 (6.55)</b>
<i>Numeric working memory new item accuracy (%)</i>			
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	<b>93.80 (9.73)</b>	<b>0.66 (4.76)</b>	<b>0.56 (5.23)</b>
<i>Numeric working memory sensitivity index (SI)</i>			
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	<b>0.83 (0.22)</b>	<b>-0.05 (0.25)</b>	<b>-0.005 (0.10)</b>
<i>Numeric working memory speed (ms)</i>			
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	<b>541.88 (85.78)</b>	<b>-20.36 (48.58)</b>	<b>-31.89 (56.83)</b>
<i>Word recognition original item accuracy (%)</i>			
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	<b>58.18 (17.03)</b>	<b>1.37 (14.28)</b>	<b>-1.37 (15.62)</b>
<i>Word recognition new item accuracy (%)</i>			
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	<b>86.27 (13.62)</b>	<b>-0.89 (12.47)</b>	<b>-6.77 (12.46)</b>
<i>Word recognition sensitivity index (SI)</i>			
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	<b>0.52 (0.21)</b>	<b>-0.008 (0.21)</b>	<b>-0.11 (0.23)</b>
<i>Word recognition reaction time (ms)</i>			
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	<b>631.58 (96.21)</b>	<b>17.49 (121.95)</b>	<b>-1.88 (88.50)</b>
<i>Picture recognition original item accuracy (%)</i>			
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	<b>78.41 (17.70)</b>	<b>-1.99 (14.64)</b>	<b>-3.60 (13.75)</b>
<i>Picture recognition new item accuracy (%)</i>			
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	<b>89.78 (8.62)</b>	<b>-0.67 (10.09)</b>	<b>-1.03 (8.58)</b>

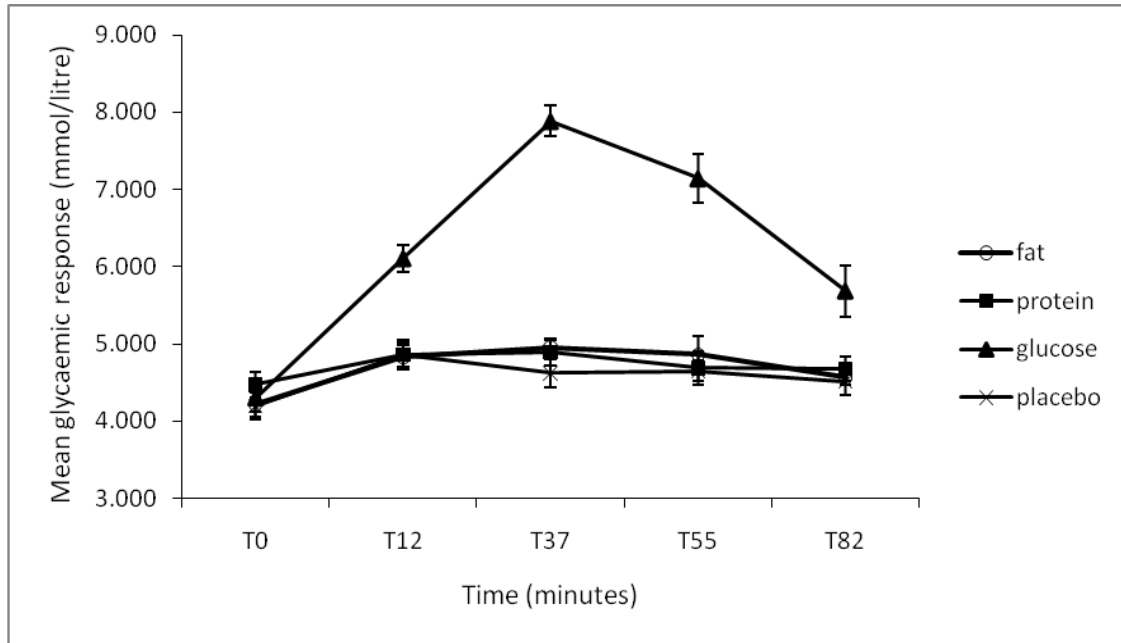
Measure	Baseline score	Post-drink change from baseline	
		15-minutes	60-minutes
<i>Picture recognition sensitivity index (SI)</i>			
Placebo	0.69 (0.17)	-0.04 (0.17)	0.00 (0.13)
Fat	0.67 (0.29)	0.048 (0.21)	-0.05 (0.21)
Protein	0.72 (0.24)	-0.03 (0.14)	-0.08 (0.16)
Glucose	0.72 (0.17)	-0.07 (0.21)	-0.04 (0.13)
Total	<b>0.70 (0.22)</b>	<b>-0.02 (0.18)</b>	<b>-0.04 (0.16)</b>
<i>Picture recognition reaction time (ms)</i>			
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	<b>714.78 (92.84)</b>	<b>9.04 (89.77)</b>	<b>-6.47 (71.15)</b>

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

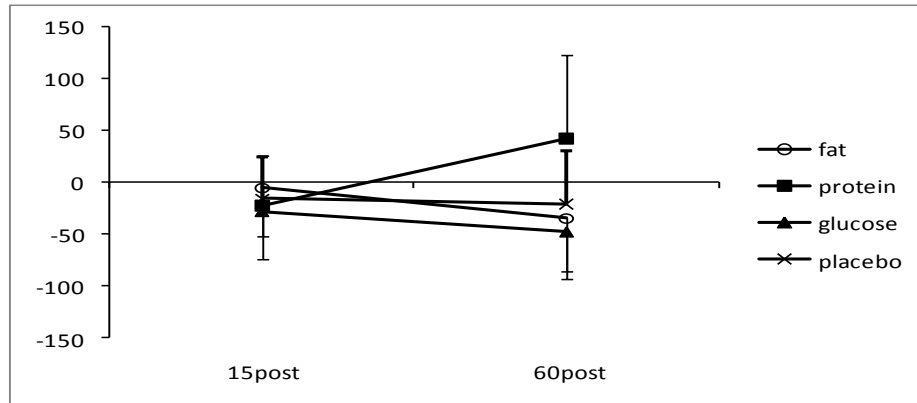
**Cognitive Outcome Measure**



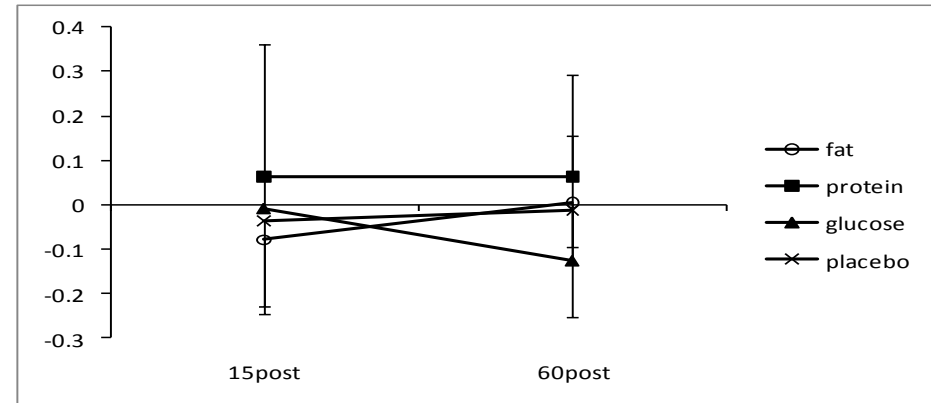
Figure(s)



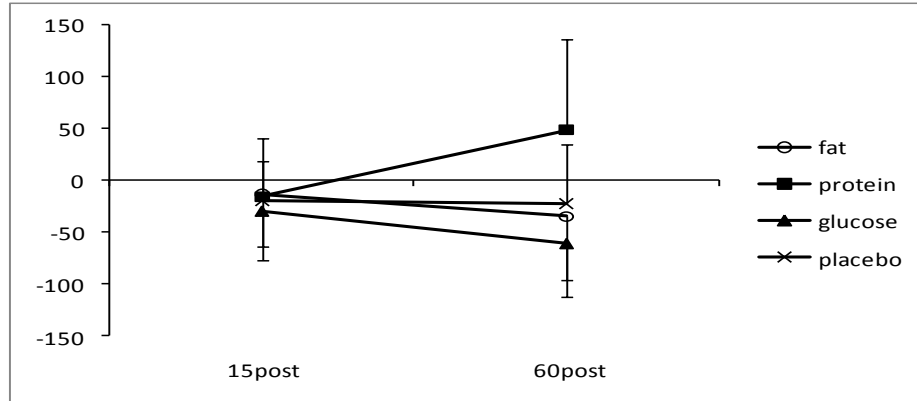
(a) Quality of Secondary Memory



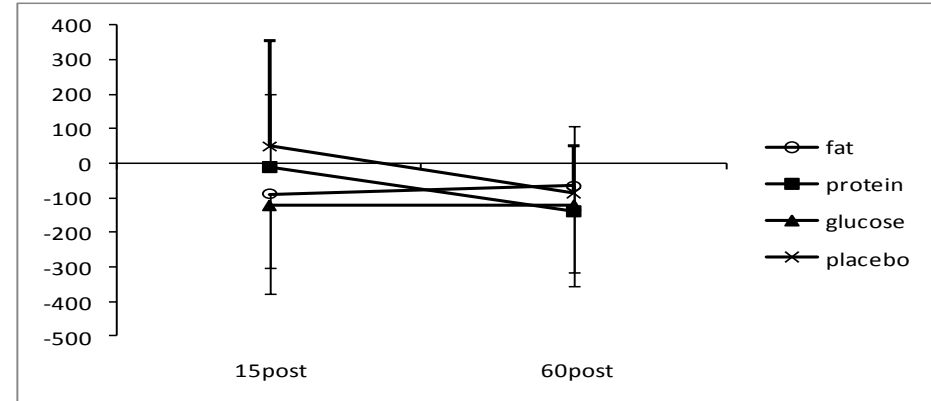
(b) Quality of Working Memory



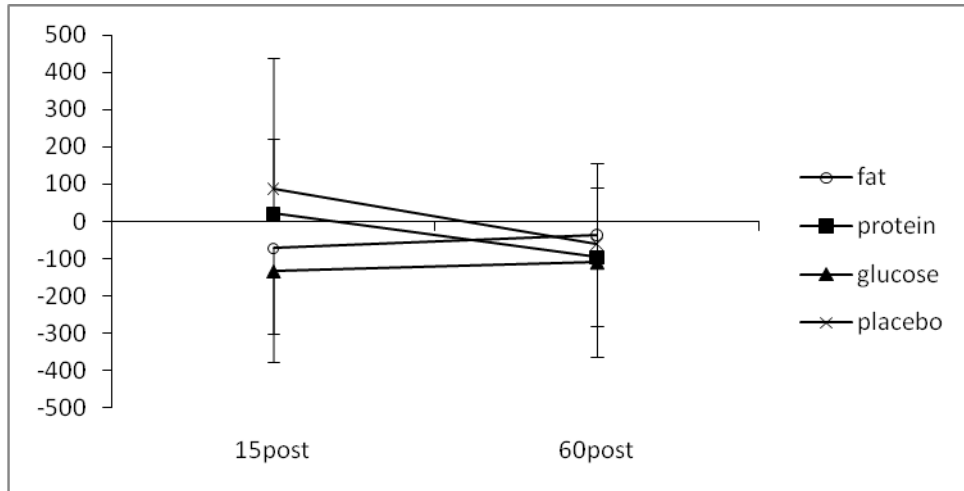
(c) Quality of Memory



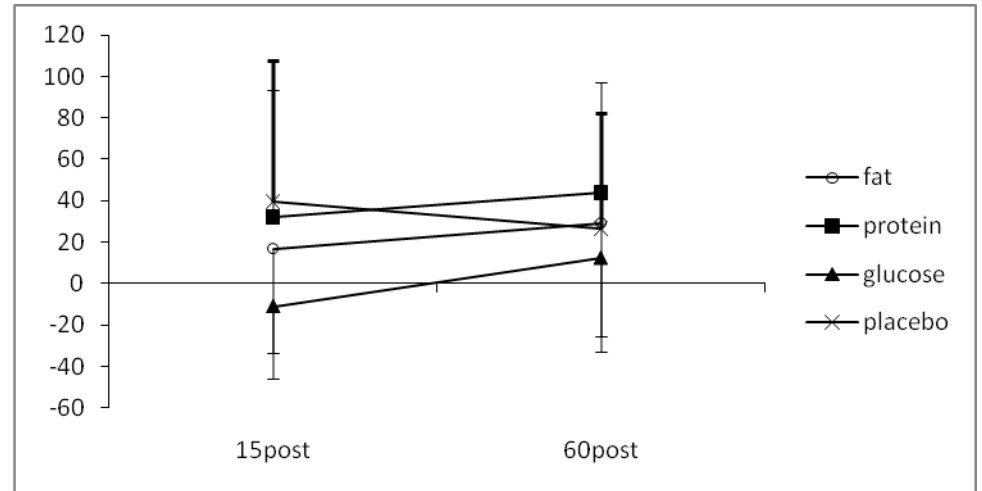
(d) Speed of Memory



(a) Combined Speed



(b) Power of Attention



(c) Self-rated Alertness

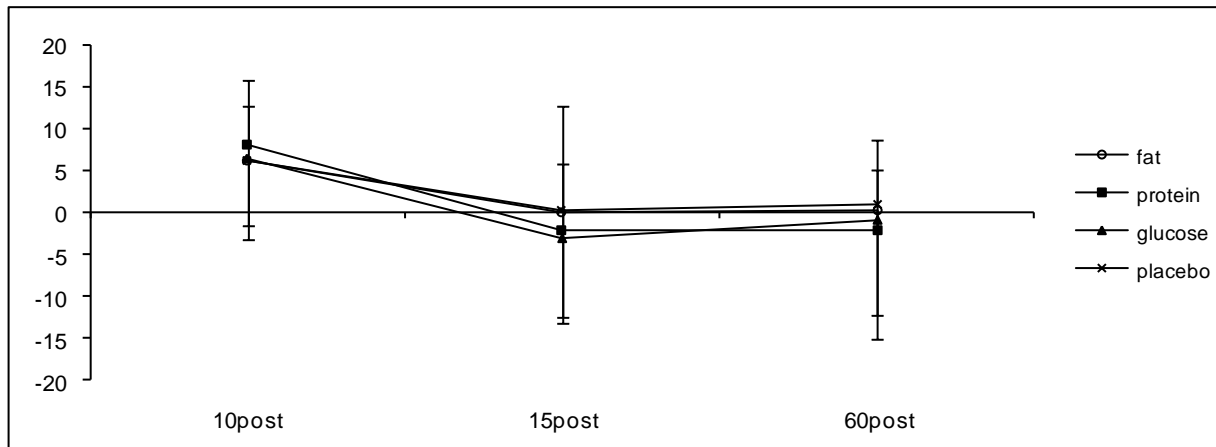




Figure 1. *Schematic representation of the CDR battery showing the cognitive tasks, individual task outcome measures and the factors derived by factor analysis. Arrows indicate that a task outcome measure contributes to the given factor: Power of Attention, Continuity of Attention, Quality of Memory 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).*