The Influence of Macronutrients on Cognitive Performance: Effects Across Age and Task Difficulty

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ABSTRACT

The effects of pure glucose, protein, and fat ingestion on tasks of paragraph recall, word recall, and mental arithmetic were examined. These effects were also investigated with regard to the age of the participant and the task difficulty level. Twelve young and twelve older adults participated in the study. Over four separate morning sessions, participants ingested one of the four drinks (glucose, protein, fat, or placebo), and completed easy and hard versions of the paragraph recall, word recall, and mental arithmetic tasks. The between-group factor was Age of the participant (young or older adult). The within-group factors were type of Nutrient ingested (glucose, protein, fat, or placebo), and Difficulty Level (easy or hard). No effects of Nutrient were found in regard to overall task performance, collapsing across Age and Difficulty Level. There was no effect of Nutrient on the different performance levels of both age groups, or for the two task difficulty levels. However, post-hoc analyses did reveal a significant Nutrient x Age interaction for the elderly after ingestion of the protein drink. Trends in the data also pointed towards an enhancement effect of glucose for the paragraph recall and mental arithmetic tasks. Trends associated with performance levels after fat ingestion showed that fat tended to enhance mental arithmetic accuracy performance for the older adult age group. Protein did not appear to differ from placebo on any of the tasks, with the exception of the deficit in performance seen with the elderly on the mental arithmetic accuracy task. In addition, a post-hoc analysis of the effects of Nutrient on mood-state showed a significant Nutrient x Mood x Time interaction. These results were discussed in light of task-specific effects of nutrients and nutrient metabolism.

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PROLOGUE

The present study is one component of a larger work. In the work presented here, the effects of three macronutrients, carbohydrate (glucose), protein, and fat are examined with regard to their effects on cognition, specifically memory. These effects are related to the difficulty of the task as well as the age of the participant. The collaborators of this study, the New Zealand Institute for Crop & Food Research Limited (CFR), were also interested in analyzing heart rate, and gut hormone changes, specifically cholescystokinin (CCK), that may play a role in mediating cognitive function after ingestion of specific macronutrients. Both these possible mediators will be briefly outlined here.

Increased heart rate is associated with increased physical and mental arousal of an organism. Recently, it has been demonstrated that an individual's heart rate will increase during a cognitively demanding task, over and above that needed for somatic requirements of the task. Kennedy and Scholey (2000) found that heart rate increased more following glucose ingestion than following placebo ingestion. It appears that during cognitively demanding tasks and under conditions of increased glucose availability, the heart works faster to move the blood glucose to the brain for quicker uptake and (assumed) utilization. The effects of fat and protein on heart rate have not yet been elucidated.

The effects of carbohydrate, protein, and fat also have not yet been determined in regard to CCK. CCK is secreted by the gut in response to a meal (Benton & Parker, 1998). This hormone has been associated with enhancing memory retention, perhaps through utilization of the ascending fibers of the vagus nerve (Flood, Smith, & Morley, 1987). The vagus nerve is the 10^{th} cranial nerve that extends up from the abdominal regions to the brain (Flood et al., 1987). Evidence for this pathway comes from findings that when the vagus nerve is cut, the memory-enhancing ability of CCK is blocked (Flood et al., 1987). In the research programme of which the present study is a part, CCK is being analyzed to ascertain if its concentration or behaviour is in any way affected by the consumption of pure carbohydrate, protein, and fat. If so, it may be an important mediator in the relationship between pure macronutrient intake and subsequent cognitive performance. However, the results of the CCK analysis are not reported in the present work.

Thus far, much of the research on macronutrient effects on cognition has focused specifically on glucose consumption effects on declarative, verbal memory. To date, there are many inconsistencies in the data. The present study aimed to clarify the role of carbohydrate, protein, and fat on memory and reasoning performance, as well as examine the roles of these nutrients across age and task difficulty. The outcome of the present investigation will provide the basis for continuing research into the effects of diet on cognitive functioning.

INTRODUCTION

The focus of the present study is on the effects of carbohydrate, protein, and fat on short-term memory, working memory functions, and reasoning. Specifically, the effect on immediate recall tasks, as well as arithmetical reasoning capacities are examined.

Working Memory

The concept of short-term memory has evolved to that of working memory, changing in nature from merely a short-term information store to that of a system capable of simultaneously storing and manipulating information. Like the short-term store proposed by Atkinson and Shriffin's (1968) modal model, there are considered to be both time and capacity limitations to working memory (Baddeley, 1995). Lezak (1995) states that short-term memory is of limited capacity, holding up to an average of only seven pieces of information at a time. She notes that information can be held for up to several minutes in this memory store, although only under conditions of uninterrupted rehearsal. However, the capacity attributed to short-term memory may not be an adequate description of the capacity of working memory. In a study by Baddeley and Hitch (1974) participants were asked to perform a verbal reasoning task while concurrently repeating a certain number of digits that ranged in number from zero to eight (differential memory load). The hypothesis here was that the greater the digit load, the less the capacity of working memory for the reasoning task. Hence, reasoning performance should slowly worsen as the digit load approached the maximum capacity of working memory. It was found that there was an impact of digit counting on reasoning performance, although the impact of 50% less speed for a digit load of eight was smaller than expected. Also, the number of errors remained constant at approximately 5%, regardless of the digit load. However, faced with growing evidence that concurrent span tasks do interfere with a vast array of activities using working memory, Baddeley (1995) continues to support the idea of a limited-capacity general working memory. This capacity can vary from person to person, rather than being the same value for everyone.

Baddeley and Hitch (1974) propose that working memory is a system comprised of three parts. These are the central executive, the visuospatial sketchpad, and the phonological loop. The central executive oversees the other two systems and is responsible for the organization and planning of working memory as a whole. The central executive is believed to be a limited capacity system, which is also charged with providing a link between the two smaller systems and long-term memory (Baddeley, 1995).

While the visuospatial sketchpad is proposed to hold and manipulate visual and spatial images, the phonological loop is responsible for storing and manipulating memory for sounds. The phonological loop is comprised of two components; a memory store that can hold phonemic information for a period of one or two seconds, and an articulatory control process (Baddeley, 1992). This articulatory control process manipulates the storage of sounds through subvocal rehearsal; however, this rehearsal can be disrupted by irrelevant spoken material entering the phonological store (Baddeley, 1995).

As mentioned previously, Baddeley and Hitch (1974) showed that when participants were required to remember irrelevant information for a short time while concurrently performing a reasoning task, there was a decrement in reasoning performance. This suggests that working memory influences reasoning capacities in that, in some way, use of working memory detracts from a person's ability for optimal reasoning performance. This result suggests that working memory is not only involved in the manipulation of incoming information, but also in the organization and manipulation of higher cognitive processes. In line with this argument, Newell (1990) proposes that the working memory system is involved both with information that needs to be retrieved or encoded, and in the intermediate stages of processing; for example, mental computations that are needed in higher cognitive processes. Further evidence for this hypothesis comes from the finding that variations in the capacity of working memory are correlated with variations in performance on reasoning and language tasks (Just & Carpenter, 1992).

Working Memory and the Brain

The prefrontal cortex, within the frontal lobes, is an area implicated in the operation of working memory. The prefrontal cortex covers a large area anterior to the primary and premotor cortices (Shimamura, 1995). Two of its parts are often distinguished from each other, these being the orbital frontal region, believed to be involved in social and moral regulation of behaviour, and the dorsolateral region, possibly involved in certain cognitive and memory functions (Gazzaniga, Ivry, & Mangun, 1998; Shimamura, 1995). The prefrontal cortex receives extensive projections from most of the parietal and temporal cortices, regions of the occipital cortex, and various subcortical areas including thalamic nuclei, the basal ganglia, the cerebellum, amygdala, hippocampus, and brainstem nuclei (Gazzaniga et al., 1998; Shimamura, 1995). Essentially, this structure communicates with and/or sends information to vast areas of the brain.

Impairment of short-term memory is believed to result from damage to the subcomponents of the working memory system (Gazzaniga et al., 1998). Phonological short-term memory can be damaged by lesions to the lateral frontal and inferior parietal lobes (Gazzaniga et al., 1998). Lesions to this area produce deficits on tests of immediate recall of word lists.

Visuospatial short-term memory is impaired by damage to the parieto-occipital region of both hemisphere's, although damage to the right hemisphere leads to more severe deficits (Gazzaniga et al., 1998).

Damage to the dorsolateral prefrontal cortex results in dysexecutive types of disorder. People with damage to this region show deficits in problem-solving, use of strategies, monitoring and modifying behaviour, temporal organization and planning (of both mental and motor activities) (Robin & Holyoak, 1995). Thus, damage to this region likely affects the central executive functions of working memory. Impairment of the central executive results in carry-over effects for the two subcomponents of working memory. For example, deficits in strategy selection could result in the loss of the ability to process or manipulate phonological or visuospatial information. One requirement of working memory is to maintain representations of several items over time until all the relevant information has been presented (Robin & Holyoak, 1995). When there is damage to the dorsolateral prefrontal cortex, a burden is placed on working memory. The representation may be unable to be held until all the information has been presented and/or manipulated to accurately fulfill the task.

Patients with frontal lobe lesions exhibit impairment on free recall tasks, especially those of delayed recall (Robin & Holyoak, 1995; Shimamura, 1995). Short-term memory is believed to be demonstrated through tasks of immediate recall (Lezak, 1995). Both immediate word and paragraph recall are used to directly test forms of declarative verbal memory, a type of memory that refers to the conscious acquisition of information (Butters & Delis, 1995; Kaplan, Greenwood, Winocur, & Wolever, 2001). Performance of these tasks is mediated by the prefrontal cortex (Shimamura, 1995).

Immediate Free Recall

Lesions to the prefrontal cortex impair free recall performance. Janowsky, Shimamura, Kritchevsky, and Squire (1989) found that patients with prefrontal lesions performed poorly on a digit span task that assesses short-term memory. However, this poor performance did not significantly impair the capacity to learn new information or acquire long-term memories.

Differences between recall and recognition memory have also been found in patients with prefrontal lesions. Janowsky et al. (1989) found that performance of those with prefrontal lesions on the recall task of the Rey Auditory Verbal Learning Test was significantly impaired, despite near perfect performance on a test of recognition. Performance will be poor on free recall if the information has been encoded but, for some reason, is unable to be retrieved successfully. Poor retrieval processes will be shown if recall performance is disproportionally worse than recognition performance, which places minimal demands on retrieval (Butters & Delis, 1995). Following this reasoning, patients with prefrontal lesions show poor recall performance because of a deficit in their retrieval processes or strategies. This outcome is in line with observations of patients with frontal lobe dysfunction who were found to be able to learn newly presented information but could not keep track of what they had learned (Spreen & Strauss, 1998).

A similar theory posits that free recall is impaired in those with prefrontal damage because it puts large demands on internally generated memory strategies and, therefore, requires extensive use of search and retrieval processes (working memory) (Shimamura, 1995). Hence, damage to the prefrontal cortex and, consequently, working memory may mean that the person cannot retrieve information after a time delay, or indeed even retrieve the strategies required to encode the information leading to impaired performance. However, it appears that when confronted with a cue (as in recognition testing) this retrieval deficit can be overcome.

In summary, working memory is a system that oversees the storage of short-term memories while simultaneously supervising encoding, consolidation, and retrieval processes. The central executive of working memory runs its supervisory functions from the prefrontal cortex. The other two components of working memory, the visuospatial sketchpad and the phonological loop, are believed to be largely housed in the lateral frontal, parietal, and occipital regions. Due to the prefrontal cortex's vast connections to and from various areas of the brain, these distinct areas can easily work as one system. However, when there is damage to the prefrontal cortex, working memory is impaired due to the loss of functions such as organization of incoming information and loss of strategies needed to retrieve previously learnt information. Evidence suggests that damage to the prefrontal cortex results in impaired free recall performance because of deficits in retrieval functions. Recent evidence suggests that working memory is not just involved with memory aspects but also with interrelated higher cognitive processes such as language and reasoning abilities (Baddeley & Hitch, 1974). Therefore, damage to the prefrontal cortex may not only mean short-term memory deficits, but perhaps impairment of aspects of language and reasoning skills that rely on working memory.

One such reasoning task, used in this present study, is that of mental arithmetic. Lezak (1995) sees mental arithmetic purely as a reasoning task, but there are also organizational, planning, and strategy selection components involved.

Mathematical Reasoning

Many separate components make up the mental arithmetic subtask of the Wechsler Adult Intelligence Scale-III (WAIS-III), a task used in the current investigation. Impairment of performance on this task could reflect any number of different problems. For example, there could be deficits in immediate memory, concentration abilities, verbal functions, or conceptual manipulation and tracking (Spreen & Strauss, 1998).

Age of the participant and the difficulty of the mental computations are also believed to exert effects on mental arithmetic performance. One study that looked at the contribution of processing speed to differences in cognitive performance across age, found that a large part of the age-related variance in working memory capacity could be accounted for by processing speed measures (Salthouse, 1996). As the mental arithmetic task in the present study was measured both as a function of accuracy and as a function of time, it may follow, therefore, that any deficits seen in performance of the elderly group in comparison to the young adult group may be due to impairment of processing speed abilities. However, in a study by Verhaeghen, Kliegl, and Mayr (1997), no age differences in time-accuracy functions were found in regard to an easy version of a mental arithmetic task (sequential complexity – simple mental computations). However, the more difficult task (coordinative complexity – mental computations which involved the placement and organization of brackets) resulted in an Age x Complexity effect. Here, the older adults performed at a slower speed, and a lower level of accuracy than their young counterparts.

Another study that tested participants' performance on mental arithmetic chain tasks (single-digit additions and subtractions without brackets) found conflicting results. The tasks used were similar to the easy sequential task used by Verhaeghen et al. (1997). A main effect of age was found on the accuracy measure of this task, with young adults performing better than older adults (Oberauer, Demmrich, Mayr, & Kliegl, 2001). When the task was made more difficult by the addition of a memory load, a significant interaction was found between Memory Load and Age in regard to processing speed. Here, the older adults' processing speed slowed while the younger adults showed no effect of memory load. Thus, the Age x Complexity interaction found by Verhaeghen et al. (1997) was replicated.

These different aspects of working memory bring forth the idea of a system of interacting and yet distinct brain regions making a specific contribution to one system, which processes and organizes all the incoming information into a coherent memory trace. The prefrontal cortex (thought to be the central site of working memory) has vast connections throughout the brain (Shimamura, 1995). It is not unlikely, therefore, that different aspects of the working memory system may be processed in different regions of the brain. For instance, Kesner, Bolland, and Dakis (1993) found that there was a dissociation between brain regions on different tests of working memory. They note that different aspects of working memory are processed separately and, in part at least, by different brain regions.

Different components of working memory, in relation to the mental arithmetic task, are processed in different brain regions. Damage to different regions of the brain has been noted to impair what is assumed to be different components of the one task. For example, McFie (1975) found that patients with left parietal lesions tended to have significantly impaired mental arithmetic scores. Damage to this area may cause impairment of mental arithmetic because of its spatial component. Mental arithmetic calls for the spatial arrangement of numbers and functions in working memory while they are manipulated. Impairment with spatial arrangement leads to an inability to organize the elements of a problem into a sequence that makes sense and is able to be solved. Thus, the deficits seen in final performance. Impairment in arithmetic ability was also noted for those with left temporal lobe lesions (Long & Brown, 1979). Temporal lobe lesions lead to an impairment of storage of information for a length of time if there is interference with rehearsal strategies (Lezak, 1995). Because interference will occur due to the simultaneous manipulation of other information, lesions to the temporal region may mean that the information to be held is lost, leading to impairments in arithmetic ability. For example, by mentally working on one aspect of the arithmetic question, the person may lose memory for the next part of the question.

Memory and Aging

As humans and other animals age, deficits in memory appear. The deficits often described are the inability to form new memories, or enhanced forgetting of recently learned information. Age-related deficits are associated with the declarative memory system (Craik, 1991). Craik has also found that healthy elderly humans usually show memory impairment on tasks that rely primarily on free recall, rather than those that rely on outside cues as seen in recognition memory (Craik, 1985).

One theory for this age-related deficit was put forth by Grady et al. (1995) who found that elderly people were less likely than their young counterparts to use effective strategies to encode the information, resulting in less retention and learning. For example, younger people employ rehearsal and categorizing strategies to remember information more often than older people do. Hence, memory differences may reflect age-related qualitative changes in the strategy selected to deal with the problem, rather than a quantitative memory loss (Gold, McIntyre, McNay, Stefani, & Korol, 2001). Along similar lines, Grady et al. (1995) found that in elderly participants the appropriate neural network for encoding failed to respond, while in the young participants it did so. This implies that while the structures are still there and may function perfectly, they are not being used anymore.

Korol and Gold (1998) proposed two theories of neural changes that may result in age-related memory decline. The first is that specific brain elements, structures, or processes essential for learning and memory may be absent or have diminished in their abilities over time. An example given is the loss of, or change in, specific structural, chemical, or electrical components of neurons. The second theory is that while the structures necessary for learning and memory have stayed intact, the processes that regulate their function are impaired. For example, hormones that may signal specific structures to activate the processes necessary for memory formation may be absent or diminished. Both these theories could be combined to form the idea that age-related memory decline is a combination of loss of structures necessary for learning and memory (or loss of use of such structures), along with a diminished capacity of the chemical regulators needed to initiate and regulate memory and learning processes.

Strengthening the idea of a decline in neuroendocrine regulators of memory, age-related deficits have been shown to be reversed (albeit in the short term) through exogenous administration of precursors of neurotransmitters thought to enhance memory. One neurotransmitter believed to have a modulatory role on memory formation is acetylcholine (Kennedy & Scholey, 2000; Wurtman, 1982). The immediate precursors of acetylcholine are choline and acetyl co-enzyme A (Wurtman, 1982). Cherkin (1987) found through a study with mice that choline administered regularly as part of a habitual diet resulted in significant improvement in memory retention performance. Cherkin's study consisted of a choline deficient group (<1mg of choline per gram of chow), a choline enriched group (12-15mg) and a control group (1.6 mg); all groups were fed these amounts for a period of four and a half months. The mice were trained on a one-trial passive avoidance task and later tested for memory retention. The choline-enriched mice performed significantly better than their deficient counterparts. The control group had intermediate scores.

These results show two main outcomes. Firstly, while removing choline from the diet resulted in decrements in performance, performance of controls was also not optimal. This suggests that increasing choline availability may enhance memory performance beyond that seen with dosages of choline normally found in the diet. This improved performance is, however, dose-dependent. Many studies produce an inverted-U dose-response curve with regard to effects of dosage of a memory-enhancing agent on memory performance (Essman, 1987; Parsons & Gold, 1992). This means that low doses have little or no effect, but as dosage increases, memory performance increases until an optimal point is reached beyond which higher doses result in decreased performance (Parsons & Gold, 1992).

The second main outcome of this study was the interaction seen with age. The mice were 13 months old at the time of testing (equivalent to older humans). If the mice were in the enriched choline condition they showed memory retention scores equivalent to that of normal young mice. However, the mice in the deficient choline condition showed memory retention scores equivalent to those seen in even older (23-31 month old) mice. While these results cannot be directly applied to humans, it is interesting that animals can show a reversal of the memory impairing effects of aging through exogenous administration of choline. Thus, one theory of impaired memory performance with age involves decreased synthesis or usage of acetylcholine.

Another theory behind age-related decline in memory involves individual glucose regulation capacities. Glucose is the brain's primary fuel (Berdanier, 2000). Craft, Murphy, and Wemstrom (1994) have found that the decline in memory performance seen with age is more pronounced in those individuals with poor glucose regulation (the ability to extract glucose from the blood for use in the body and/or brain). Indeed, Bellisle (2001) identifies impaired glucose regulation as a factor involved in the cognitive impairment seen with age. There is a widespread prevalence of adult-onset diabetes among the elderly, which presumably reflects the disruption of glucose regulation that becomes more common with age (Craft et al., 1994). Also, Craft, Zallen, and Baker (1992) noted that patients with senile dementia of the Alzheimer's type (SDAT) showed more extreme abnormalities of the glucose system than did age-matched healthy controls. Knowing of the severe memory impairments seen with SDAT, this study suggests that impairments of the glucose system may be a direct factor influencing the appearance of memory deficits.

It has been found that impaired glucose regulation may contribute to dysfunction of central cholinergic systems. Glucose acts in ways that resemble those of a cholinergic agonist (Hall, Gonder-Frederick, Chewning, Silveira, and Gold, 1989), which means that reductions in glucose availability may directly or indirectly impair the synthesis of acetylcholine. As with choline, exogenous administration of glucose has been shown in both human and animal studies to be beneficial to performance on memory, as well as some other cognitive tasks (Benton, Owens, & Parker, 1994; Craft et al., 1994; Gold, 1987; Hall et al., 1989; Kaplan, Greenwood, Winocur, & Wolever, 2000; Kaplan et al., 2001; Kennedy & Scholey, 2000; Korol & Gold, 1998; Martin & Benton, 1999; Parsons & Gold, 1992; Ragozzino, Unick, & Gold, 1996; Scholey, Harper, & Kennedy, 2001).

Glucose Effects on Cognition

Glucose administration is believed to be beneficial for immediate and delayed word and/or paragraph recall tasks which assess both short term and long term memory capacities (Bellisle, 2001; Benton et al., 1994; Benton, Parker, & Donohoe, 1996; Craft et al., 1994; Hall et al., 1989; Kaplan et al., 2000, 2001; Korol & Gold, 1998; Parsons & Gold, 1992). Glucose has also been found to be beneficial with regard to focused and sustained attention (Kaplan et al., 2001), spatial memory (Benton & Parker, 1998), choice reaction time (Fischer, Colombani, Langhans, & Wenk, 2001), spontaneous alternation performance (Ragozzino et al., 1996), concentration (Benton et al., 1994), and mental arithmetic and verbal fluency (Kennedy & Scholey, 2000; Scholey et al., 2001). Much of the work to date, however, has concentrated on the effects of glucose on memory.

Glucose Effects on Memory

There are many inconsistencies in the literature regarding the effects of ingestion of glucose on memory and other cognitive tasks. Craft et al. (1994) state that the effect of glucose is limited to declarative memory tasks. A study by Manning, Hall, and Gold (1990) found that improved performance in elderly humans was limited to a declarative verbal memory task (the Logical Memory subtest of the Wechsler Memory Scale [WMS]), with no effects seen on a test of short-term memory (digit span), or indeed, non-memory tasks. Another study by Manning, Parsons, Cotter, and Gold (1997) found that glucose failed to facilitate performance on an implicit (non-declarative) memory task. However, when the same word list was used to examine explicit (declarative) memory, glucose facilitation was found to influence performance.

The belief that glucose only facilitates declarative memory tasks is supported by the persistent finding of enhanced performance on tests of word and story recall (measures of verbal declarative memory) after ingestion of glucose. Hall et al. (1989) found that performance on the logical memory task of the WMS (paragraph recall) improved in elderly subjects after glucose ingestion. The elderly participants had performed significantly poorer than their young counterparts at baseline levels (before glucose consumption). However, after glucose ingestion, this performance deficit was reversed, with elderly participants' performance levels approaching those of the young.

In another study by Korol and Gold (1998), glucose was found to enhance performance on both immediate and delayed recall of a paragraph in young participants. Participants recalled on average 35% more items correctly from the paragraph after glucose consumption than they did after consuming saccharin (control drink). Furthermore, Manning et al. (1990) not only showed that glucose once again improved recall performance of a paragraph, but also that recall of a word list (a selective reminding task) was enhanced by, on average, 12% after glucose ingestion. Benton and Owens (1993) as well as Gonder-Frederick et al. (1987) also report results of enhanced recall of word lists or a paragraph after consumption of glucose.

The view that glucose only facilitates performance on measures of declarative, verbal memory conflicts with other aspects of the literature. Performance on declarative memory measures such as word recall tasks have been found in some studies not to be affected by the ingestion of glucose. Glucose was found to be ineffective on immediate word recall in a study by Kaplan et al. (2001). Also, Azari (1991) found that neither word recall nor recognition tasks were facilitated by the ingestion of glucose.

Glucose facilitation effects have also been found on spatial and working memory tasks. Ragozzino et al. (1996) injected glucose directly into the hippocampal formation of rats, which were then required to perform a spontaneous alternation task in a four-arm maze. In comparison with both saline injected and unoperated controls, the glucose-injected rats showed enhanced performance. Furthermore, both the saline and unoperated control rats had comparable scores, showing that the superior scores seen in the glucose group were indeed an enhancement from usual baseline performance. Hence, glucose can enhance spatial memory, at least in rats.

Taken together, studies of the effects of glucose on working memory have also produced mixed results. Korol and Gold's (1998) work with college students found that face recognition, word recognition, and working memory were not influenced by glucose ingestion. Craft et al.'s (1994) study which systematically examined various aspects of memory concluded that working memory was unaffected by glucose consumption. However, a series of studies by Benton and Sargent (1992) showed glucose to enhance performance on tasks of working memory in both elderly and young adults, although certain tasks such as digit span did not show any effect. Another task considered to involve working memory, mental arithmetic, has been enhanced by glucose consumption under some conditions. In a unique study, Kennedy and Scholey (2000) compared performance on two mental arithmetic tasks, one easy (serial three's) and one difficult (serial seven's). Glucose enhanced performance (measured by the number of responses made) on the serial seven's task. The glucose condition was also associated with fewer errors, ruling out the possibility of a speed-accuracy trade-off effect. Performance on the serial three's task was not enhanced by glucose administration. Therefore, this study not only showed that tasks involving working memory are open to facilitation by glucose, but also that the factor of difficulty of the task may begin to explain inconsistencies in results. For example, if

Kennedy and Scholey (2000) had only used serial three's as the mental arithmetic task, it may have led to the erroneous conclusion that mental arithmetic is a task unaffected by glucose ingestion. Methodological inconsistencies across studies, across tasks, and difficulty levels within tasks are only a few factors that may have led to the inconsistent results discussed above. Other factors are discussed in more detail in the next section.

Reasons for Inconsistencies

Recent work has found that certain subgroups of individuals are more susceptible to the influence of glucose on cognitive functions than others. One of these subgroups is the elderly. Many studies thus far have focused specifically on this group (Craft et al., 1994; Craft et al., 1992; Gonder-Frederick et al., 1987; Hall et al., 1989; Kaplan et al., 2000, 2001; Manning et al., 1990; Manning et al., 1997; Parsons & Gold, 1992). One of the main focuses has been the impact of glucose regulation and how this interacts with age, memory, and the effects of exogenous glucose administration. As noted previously, Bellisle (2001) has identified impaired glucose regulation as a predictor of cognitive impairment with age.

One of the main reasons that these proposed interactions could result in inconsistencies across studies is shown in an investigation by Kaplan et al. (2000). They found that after feeding each participant glucose, barley, or potato on three separate days (all carbohydrate foods), overall performance on the cognitive tests did not differ from placebo performance. However, when the participants' baseline memory performance and measures of glucose regulation were factored in, both these measures were associated with improvements in paragraph recall across all the three carbohydrates compared with placebo. Hence, through the separation of participants into groups based on, among other things, glucose regulatory performance, the study became sensitive to picking up the effects of glucose on that certain subgroup.

The memory-enhancing effects of glucose are further mediated by individual differences in utilization of blood glucose. When glucose is ingested into the body, one of the first indicators of its activity is a rise in peripheral blood glucose levels. These levels rise, peak (approximately half an hour after ingestion), and then fall back to baseline levels over a period of approximately two hours (Benton et al., 1996; Scholey et al., 2001). The rate at which the blood glucose levels fall has been associated with better cognitive performance; the faster the blood glucose levels fall, the better the cognitive performance. While arousal-linked mechanisms (such as an increase in heart rate) may partly account for the fall in blood glucose, it is possible that this fall also reflects an increased uptake of blood glucose by neural substrates (Scholey et al., 2001).

Glucose is removed from the blood by insulin that has been produced by the pancreas in response to high blood glucose levels (Benton et al., 1996). Insulin enables the glucose to be turned into energy for the brain and the body (Fischer et al., 2001). When the body does not have enough insulin, or cannot use the insulin effectively, it is unable to turn glucose into the energy needed by the brain, resulting in high peripheral levels of blood glucose (Benton et al., 1996; Fischer et al., 2001).

An individual whose blood glucose levels stay high after peaking or whose blood glucose level does not drop during cognitive tasks is generally assumed to have poor glucose regulation, a condition associated with poor cognitive performance. Hall et al. (1989) found that when blood glucose in the elderly remained high after it peaked, poor memory performance resulted. In a study by Craft et al. (1992) it was found that 'normal' elderly participants with good glucose regulation showed enhanced paragraph recall performance after glucose consumption. (Glucose regulation was defined as the time taken for glucose levels to return to baseline after peaking; the less time taken the better the glucose regulation). However, those participants with poor glucose regulation were found to have worse performance than their baseline scores. This study also examined the performance of SDAT patients. Interestingly, when this group was split into good regulators and poor regulators, the opposite pattern to that seen with the normal elderly was found. For example, SDAT patients deemed to have good glucose regulation were found to deteriorate in performance after glucose administration, while those with poor regulation showed a marked improvement. Thus, SDAT patients may be another elderly subgroup whose memory is affected by glucose regulation capacities. Why glucose affects the SDAT patients differently from the normal elderly is not known, but it may be linked to the abnormalities of the glucose system seen in SDAT patients (Craft et al., 1992).

Another (mostly) elderly subgroup that shows an unusual response pattern are those with poor baseline memories. Kaplan et al. (2000) found that participants with poor baseline memories performed better on recall tasks after ingestion of carbohydrates than those participants with good memories. In fact, the participants considered to have good memories actually performed worse after consumption of carbohydrates. These results could possibly be explained by a modification of the inverted-U dose response curve that is seen with many memory-enhancing drugs. This curve suggests that low dosages of a substance will result in little or no effect, while high dosages can result in a deficit in performance. Intermediate dosages, however, are optimal and will result in enhanced performance (Parsons & Gold, 1992). Thus, it is possible that memory may also follow a person-specific inverted-U curve, in that once memory is near or at optimal performance for that individual, any enhancing agent may in fact cause a decline in memory performance.

The relationship between glucose regulation and memory is seen in young adults as well as the elderly, albeit not to the same extent. Craft et al. (1994) state that these effects will only be subtle, as young adults tend to show less memory enhancement after glucose ingestion than older adults with good glucose regulation. However, glucose regulation effects with young adults have been demonstrated. Benton et al. (1994) found that young adult participants who experienced rapidly falling blood glucose levels after those levels had peaked, performed better on memory tasks, regardless of whether they were in the glucose or placebo condition. Thus, memory performance was better in those participants who were able to withdraw and utilize glucose from the blood (good glucose regulation), regardless of whether a glucose load had been administered.

Studies of the effects of glucose regulation abilities are also marred by inconsistencies in results. There is a tendency for different studies to use different operational definitions of what constitutes a measure of glucose regulation. One study that used the magnitude of the peak of the blood glucose curve as their measure of glucose regulation, reports that elderly participants whose blood glucose curve peaked high were those participants who showed poor memory (Hall et al., 1989). Although some of the young participants had comparable blood glucose levels to those of the elderly, their individual peak measures were not significantly correlated with their performance on the memory task. Also, elderly participants with good blood glucose control (low peak measure) scored significantly higher in the composite memory score than those elderly with poor blood glucose control (high peak measure). Interestingly, performance of the young, good glucose control group did not differ from that seen with the young, poor glucose control group.

Task Difficulty

Clearly, glucose is able to exert memory-enhancing effects on young adults. To exert these effects, however, tasks must be as cognitively demanding for young adults as the elderly find the tasks they are presented with. It is possible that studies that report no effect of glucose on cognitive capacities of young adults, have simply failed to include tests of sufficient difficulty to detect the differences (Craft et al., 1994). Korol and Gold (1998) used a task of greater difficulty (longer versions of the logical memory subtask of the WMS) for young participants than they had used previously with elderly participants, to see if changing the difficulty of the task was amenable to facilitation by glucose. It was found that glucose enhanced performance on these versions in comparison to the saccharin (control) condition.

The difficulty of a task as a moderating factor between glucose and performance also may apply to the elderly. While the elderly may not need as difficult a task as the young for glucose to show enhancing effects, it appears that some level of cognitive demand is needed. In an experiment on the effects of glucose on spontaneous alternation performance, rats were tested on both a three-arm radial maze and a four-arm maze (presumed to be more difficult) (Raggozzino et al., 1996). The rats were 13 months old (older adult), and while glucose did not improve their performance of spontaneous alternation on the easier three-arm maze, the more difficult four-arm version was enhanced by glucose. The implication from such animal studies is that, regardless of age, glucose not only works selectively on certain subgroups of people but also on certain tasks, and difficulty levels within those tasks.

At the biological level, the difficulty of the cognitive task is reflected in the blood glucose levels. Blood glucose levels decrease more rapidly during a period of intense cognitive processing than they do during performance of an easy task (Backs & Seljos, 1994). This relatively rapid decrease in blood glucose levels may be partly explained by physiological mechanisms. Cognitively demanding tasks have been associated with concomitant increases in heart rate, a mechanism that is believed to serve the function of increasing the delivery of glucose to active brain substrates (therefore, decreasing the level of peripheral blood glucose) (Kennedy & Scholey, 2000). Turner and Carroll (1985) reported heart rate increases during a mental arithmetic task and a video game task, which were considered to be above the level necessary for somatic performance of the task. In another study comparing the effects of glucose on an easy and a hard version of a serial subtraction task, it was found that both serial seven's (difficult) and serial three's (easy) resulted in heart rate elevation, with serial seven's eliciting the higher heart rate of the two (Kennedy & Scholey, 2000). This elevation was also higher than that seen in the somatically-matched control task. Hence, the accelerated heart rate was influenced directly by the level of peripheral blood glucose rather than just being a reflection of the somatic demands of the task. In fact, Kennedy and Scholey (2000) suggest that increased circulating glucose may actually cause heart rate acceleration. While increased heart rate may be one mechanism by which glucose reaches the brain quickly, especially during tasks of high cognitive demand, the mechanisms by which the brain may utilize glucose to exert its effects on memory are as yet largely unknown.

Possible Mechanisms of Glucose Effects on Memory

Glucose, once absorbed through the stomach and the intestine, passes into the liver and then throughout the body (Berdanier, 2000). Any excess glucose is used to synthesize glycogen that is stored in the muscles and liver (Berdanier, 2000). Glycogen can be turned into glucose for use when blood glucose drops below the normal level of 4-6 mmol/L (Berdanier, 2000). Glucose can circulate freely in the blood stream without the need for a special carrier and is readily transported from the blood to the brain (Berdanier, 2000). Brain scanning studies have demonstrated that increased cerebral demand results in localized increases in the rate of blood-to-brain glucose transport and glucose metabolism at these sites (Goldman-Rackic, 1992; Lund-Andersen, 1979). Benton et al. (1996) also report evidence from PET studies that increased neural activity is associated with increased glucose metabolism, and furthermore, that the metabolic rate when performing a cognitive task is positively associated with performance on that task. These findings have led to the notion of a brain efficiency model of intelligence, which states that the brain works efficiently rather than hard (Benton et al., 1996). Efficiency of the brain is evidenced through the localization of glucose metabolism specifically in the areas of the brain involved in the processing of the task at hand. Evidence linking glucose metabolism with cognitive disorders is found in studies of diabetics, a population with abnormal glucose metabolism. Deficits have been found in this population on measures that include verbal IQ, attention, and memory (Holmes, 1986). Furthermore, even cerebral glucose metabolism deficits that accompany normal aging have been linked to memory impairments (Hall et al., 1989). Thus, if the brain is not supplied with enough glucose, optimal brain glucose metabolism, and consequently, optimal task performance, cannot occur.

Traditionally, it has been assumed that homeostatic mechanisms supply the brain with as much glucose (the brain's primary fuel and source of energy) as is needed (Gold et al., 2001; Martin & Benton, 1999). However, the research evidence (reviewed above) challenges this assumption. At least in some tests, under some conditions, blood glucose levels are influential (Martin & Benton, 1999). One of these conditions involves the difficulty level of the task. The more cognitive demand placed on an individual, the more energy is needed by parts of the brain to obtain optimal performance of that task. For example, in the control condition, depletion of glucose is not seen in the brains of rats that are tested on an easy three-arm spontaneous alternation task. In contrast, depletion of glucose is seen when performing the more challenging four-arm maze (Ragozzino et al., 1996). This suggests that normal learning and memory are able to function at a deficit relative to optimal conditions whereas if the brain is under high cognitive demand, glucose reserves are drained from the specific brain regions activated by that demand (Gold et al., 2001). Cognitive demand, however, also follows an inverted-U curve (Scholey, 2001). If a task is too stressful or too difficult, there is likely to be a decrement in performance regardless of whether glucose has been administered or not.

While glucose metabolism may have a direct effect by making available the energy needed by neurons to carry out their functions, glucose metabolism also results in secondary products that have their own effects on memory. The synthesis of neurotransmitters such as serotonin, noradrenaline, and acetylcholine requires glucose (Dye, Lluch, & Blundell, 2000). Much of the interest to date has focused on acetylcholine, a neurotransmitter known for its influence on learning and memory. When the brain metabolizes glucose, it, among other functions, produces acetyl-CoA, which is a precursor of acetylcholine (Ragozzino et al., 1996). Therefore, the main hypothesis states that glucose enhances memory by increasing acetylcholine synthesis and release through increasing the amount of acetyl-CoA (Ragozzino et al., 1996). In other words, acetyl-CoA is believed to be the rate-limiting factor. The proposed effect on acetylcholine synthesis, however, only seems to appear when cholinergic neurons are activated. Rats that had consumed glucose showed increased acetylcholine synthesis in the hippocampus while performing a task (Ragozzino et al., 1996). However, this effect was not seen when the rats were sitting quietly in their cages. Further observations showed that extracellular brain glucose levels varied according to neuronal activity. This finding provides support for the brain efficiency model of intelligence, as an increase in circulating glucose levels (an important modulator in the synthesis of acetylcholine) only occurred in response to cognitive demand.

A different mechanism by which glucose might enhance acetylcholine release involves the blocking of opiates from binding to receptor sites in the hippocampal formation (Lapchak, Araujo, & Collier, 1989). Activation of opiate receptors in this area results in decrements in acetylcholine release (Lapchak et al., 1989). Increases in blood glucose levels have been shown to reduce the binding of opiates to their receptor sites, which suggests that glucose itself may act as an opioid antagonist (Brase, Han, & Dewey, 1987).

Glucose may also exert its effects directly through the gut-brain axis. Kaplan et al. (2001) hold that glucose enhanced memory 15 minutes after ingestion. This short time period between ingestion and evidence of effects on brain function may be due to a more direct route from gut to brain than that previously outlined. The vagus nerve (cranial nerve X) has been implicated as a pathway through which these effects might occur (Kaplan et al., 2001). Cutting this nerve results in a decrease in the memory-enhancing effects of glucose (Flood et al., 1987).

Another site of interest is the liver. The liver is important for delivering information regarding blood glucose levels via the vagus nerve to the brain stem (Rosenzweig, Leiman, & Breedlove, 1996). This information passes through the celiac ganglion (located outside the central nervous system), a region which if damaged also decreases the memory-enhancing effects of glucose (White, 1991).

Finally, insulin may also play a role in glucose enhancement of memory. Traditionally, insulin was believed to only aid in the removal of glucose from the blood for utilization in peripheral tissue, not in brain tissue (Benton & Parker, 1998). However, dense numbers of insulin receptors have been found in the hypothalamus, olfactory bulb, and specific regions of the hippocampus (Schwartz, Figlewicz, Baskin, Woods, & Porte, 1992). The effect of raising insulin levels is shown by work with Alzheimer's patients. Schwartz et al. (1992) found that if blood insulin concentrations are increased while keeping glucose levels at fasting rates, the result is a marked improvement in declarative memory functions. Raising insulin levels, causing more glucose to be removed from the blood, resulted in an increase in energy for neurons, specifically cholinergic neurons that use glucose to synthesize acetyl-CoA, the precursor to acetylcholine.

Glucose Effects on Mood

Carbohydrate ingestion elevates the tryptophan (an amino acid) level in the brain; the enhanced availability of this amino acid results in increased synthesis of serotonin (Bellisle et al., 1998). Serotonin, among other functions, has effects on mood (Davis, Alderson, & Welsh, 2000). Dye et al. (2000) found that higher than usual amounts of carbohydrate caused participants to feel more drowsy, uncertain, and muddled. Wells and Read (1996) also found that high-carbohydrate, low-fat lunches resulted in feelings of lethargy, but less so than was found in the high-fat, low-carbohydrate lunch. Lower levels of depression were also reported after ingestion of a carbohydrate meal compared to that of a protein meal (Fernstrom & Wurtman, 1972). Less depression is expected due to the increase in brain serotonin that is partly a product of carbohydrate ingestion (Fernstrom & Wurtman, 1972). Acute dosages of tryptophan have also been found to increase subjective ratings of fatigue and decrease feelings of vigor (Lieberman, Corkin, Spring, Gowden, & Wurtman, 1983). Hence, carbohydrate ingestion appears to exert its effects on mood both through increasing tryptophan synthesis, and by enhancing serotonin availability.

In summary, glucose administration is believed to exert beneficial effects on working memory whose functions are largely mediated by the prefrontal cortex (Kaplan et al., 2000). However, these effects have not been consistently found, and enhancement effects have been found in tasks that need not necessarily involve memory; increased glucose uptake by the brain improves a number of other behaviours, for example, attention and concentration abilities (Benton et al., 1994; Kaplan et al., 2001).

Reasons for these inconsistencies may be that certain subgroups of individuals are more susceptible to the enhancing influence of glucose. These subgroups include elderly people, those with good glucose regulation, and those with poor baseline memories. However, glucose-enhancing effects are not limited to older adults; young adults, under certain conditions, are also susceptible to the influence of glucose. One of these conditions is that of task difficulty. The task must be difficult enough to produce sufficient cognitive demand in young adults. Tasks that produce high levels of cognitive demand in the elderly population will not necessarily produce the same level of cognitive demand in young adults.

Glucose metabolism, acetylcholine synthesis, insulin action, and possible mediatory pathways such as the vagus nerve are suggested mechanisms by which glucose modulates enhancement of memory and other cognitive functions. Serotonin synthesis as a byproduct of glucose metabolism is also linked to changes in mood. Changes in blood glucose levels are the starting point for all of the above mechanisms and these changes are positively associated with performance on those memory and non-memory tasks enhanced by glucose.

However, not all memory-enhancing agents exert their primary effects through changes in blood glucose levels. Protein and fat are two other macronutrients that, like carbohydrate, have recently been shown to enhance memory.

Protein and Fat Effects on Cognition

Only two studies, to the author's knowledge, have thus far compared the effects of pure protein or fat with the effects already found with glucose. The first study by Kaplan et al. (2001) used equal volume, isoenergetic, pure (>98% of energy) protein, fat, and carbohydrate drinks to examine their effects on cognitive performance of the healthy elderly. The only test where a main effect of drink was found was that of paragraph recall, a test consistently improved through administration of glucose. Measurements of performance on all the tasks were taken 15 minutes after ingestion, and then again 60 minutes after ingestion. All three drinks improved delayed recall on the paragraph recall task, and either improved or tended to improve immediate recall in comparison with placebo 15 minutes after ingestion. Further analyses of the difference between immediate and delayed recall showed that administration of protein resulted in less forgetting than that seen in the placebo condition. Interestingly, participants tended to remember more information during delayed recall than they had during immediate recall after consuming protein. Neither glucose nor fat produced this effect. This result suggests that some other aspect of memory, not directly measured by immediate and delayed recall scores, is possibly enhanced by protein.

With regard to word list recall, fat ingestion led to impaired performance but only on the overall score (all three word list scores combined), compared with placebo 60 minutes after ingestion. Fat ingestion, however, improved performance on Trails A (sustained attention task) 15 minutes after consumption when compared to placebo. There was also a tendency for fat to improve performance on an attention task 60 minutes after ingestion. This task consisted of attending and making note of various aspects of a well-known television sitcom. Also, as noted earlier, glucose selectively enhances cognitive performance in, among other things, those with poor baseline memory capacities. Not only did glucose have that effect in Kaplan et al.'s (2001) study but strong associations between poor baseline performance and improvement with fat were also found on several tasks. No such association was observed with protein, however.

A second study (Fischer et al., 2001) used young male participants and isoenergetic (1670 kJ of energy), pure meals of carbohydrate, protein, and fat to examine the effects on simple and choice reaction time, as well as a combi-test. The combi-test consisted of a demanding short-term memory task, which was presented in the center of a computer screen while an easier attention task was simultaneously presented around the periphery of the screen. This study therefore introduced an element of task difficulty. As expected, due to the low cognitive demand of the task, there was no effect of meal on simple reaction time performance. However, the more complex reaction time task resulted in a Meal x Time interaction. Choice-reaction time was enhanced by both glucose and protein ingestion (although protein enhanced this task to a lesser degree in the first hour of testing). Interestingly, the opposite effect occurred during the second hour with protein ingestion enhancing choice-reaction time performance more so than glucose ingestion.

The combi-test also showed an overall meal effect for both its components. The central short-term memory task consisted of recognizing defined sequences of coloured circles in the middle of a computer screen and pressing a button if they appeared. The peripheral attention task consisted of detecting rotations of four patterned circles that were displayed in the four corners of the same screen that displayed the central task. The participant was to indicate a rotation by pressing a different key on the keyboard. Scores on the central short-term memory processing task, the peripheral attention task, and the total overall score were highest after consumption of the fat meal. Accuracy scores (the number of correct responses as a proportion of all responses made) with regard to the central task were differentially affected by meal, with fat assuming the greatest effect, followed by carbohydrate and then protein. However, there was a change in this ordering with the peripheral attention task. Overall scores of central efficiency (the number of correct responses as a proportion of the total number of response opportunities) as well as accuracy scores on the peripheral attention task, showed the same effect of meal but with different ordering. Here, the order of effect was fat, followed by protein and lastly carbohydrate, with the only statistically significant difference being between the fat and the carbohydrate. Total scores on the combi-task, however, were higher after the fat meal than after the carbohydrate or protein meal, with no difference between the carbohydrate and protein meal effects.

Thus, fat ingestion consistently exerted the greatest enhancement of performance in the central short-term memory task, the peripheral attention task, and the overall combi-task score. This result is consistent with the outcome of Kaplan et al. (2001) where trends for improvement on attention tasks following fat ingestion were found. In addition, a study by Smith, Kendrick, Maben, and Salmon (1994) found that accuracy scores of a focused attention task were higher after a high fat meal than after a low fat meal.

Protein effects on task efficiency as well as its effects on attention have been noted through its enhanced improvement of both central efficiency and peripheral attention accuracy scores over that of carbohydrate (Fischer et al., 2001). The differential effects of carbohydrate and protein on central and peripheral tasks respectively is reflected here, suggesting that these two nutrients may act through different mechanisms to exert their enhancing effects. These two macronutrients may operate on different cognitive processes; hence, they may be task-specific (Dye et al., 2000).

Protein and Fat Effects on Mood

Protein and fat ingestion have also been examined in relation to their effect on mood. Fat produces effects similar to carbohydrate. Higher than usual proportions of fat have caused participants to report feelings of drowsiness, uncertainty, and feeling 'muddled' (Dye et al., 2000). Lethargy has also been reported after ingestion of a high-fat, low-carbohydrate meal (Wells & Read, 1996). Fat ingestion was also reported to cause a depression of alertness and mood in the morning (Wells & Read, 1996), suggesting that the same mechanisms that work to produce feelings of lethargy, drowsiness, and uncertainty after carbohydrate ingestion may also be involved after fat ingestion.

The opposite effect is seen after protein consumption. Protein ingestion is believed to enhance concentrations of tyrosine, a precursor to catecholamine neurotransmitters such as dopamine, norepinephrine, and epinephrine (Fernstrom, 2000). By elevating catecholamine synthesis and simultaneously acting to decrease brain serotonin, protein ingestion results in an alert and tense state (Lieberman, Spring, & Garfield, 1986). A greater reduction in fatigue occurs after a protein-rich breakfast in comparison to that of a carbohydrate-rich breakfast (Bellisle et al., 1998), possibly because of its effects on decreasing serotonin levels in the brain.

Proposed Mechanisms of Protein and Fat Effects on Cognition

All three macronutrients – protein, fat, and carbohydrate - enhanced memory performance 15 minutes after ingestion (Kaplan et al., 2001). This is interesting, as while glucose may have had enough time to exert effects through raising blood glucose levels directly, protein and fat do not necessarily work in the same way. Indeed, fat is not absorbed within a 15 minute time period and even at 60 minutes minimal absorption of fat into the blood stream has occurred (Kaplan et al., 2001). Hence, a more direct route through the gut-brain axis is suggested. Each macronutrient releases a specific pattern of gut hormones whose presence is signaled to the brain via the vagus nerve. The brain changes induced by these signals result in enhancement of memory performance (Kaplan et al., 2001). Evidence for this role comes from findings with CCK, a hormone found in the duodenum (a part of the small intestine) which influences memory through stimulation of the vagus nerve (Flood et al., 1987). When the vagus nerve in human participants has been electrically stimulated, improvements in performance of declarative memory tasks resulted (Clark, Naritoku, Smith, Browning, & Jensen, 1999). CCK also functions separately as a neurotransmitter in the brain. Nomoto, Miyake, Ohta, Funakoshi, and Miyasaka (1999) found that learning and memory functions were impaired in rats whose brains were devoid of a certain type of CCK receptor. Flood et al. (1987) note that CCK may act as a reinforcer in the brain. These authors believe that a link between CCK release and memory-enhancement evolved due to the survival advantages of an animal being able to remember the details of a successful hunt for food. Thus, other happenings in the environment at the same time (for example, performance on memory tasks) may also be enhanced by the increased activity of CCK.

Another hormone associated with the metabolism of all three macronutrients is epinephrine (Berdanier, 2000). Epinephrine cannot cross the blood brain barrier; however, it is believed to exert its memory-enhancing effects through elevating blood glucose levels (Korol & Gold, 1998). Epinephrine is released from the adrenal medulla in response to a stressor, for example, a cognitively demanding task. Once epinephrine is released, it stimulates the liver and muscles to change their glycogen stores into glucose (Berdanier, 2000). The consequent increase in glucose availability to the blood and brain may then result in the enhancement of mental performance.

Glucose is believed to exert its effects primarily through increasing glucose levels in the blood, thereby enhancing the availability of glucose to the brain. However, fat and protein have been shown to enhance memory task performance without any effect on the level of glucose in the blood (Fischer et al., 2001; Kaplan et al., 2001). The enhancing effect of fat and/or protein may be due to the energy content of these drinks. Kaplan et al. (2001) state that energy ingestion improves memory independent of blood glucose levels. It is hypothesized that when food is ingested, the energy it contains acts to enhance brain functions via the gut-brain axis.

While fat ingestion leads to approximately stable blood glucose levels, it also may still act through glucose mechanisms. Elevated circulating fatty acids after fat ingestion could be used as fuel for muscle and other peripheral cells, essentially freeing glycogen stores (and other glucose) to provide energy for the brain (Fischer et al., 2001). However, this would presumably happen only after fat absorption; therefore, the gut-brain axis may be the initial way through which fat exerts its effects.

Protein ingestion appears to decrease blood glucose concentrations (Fischer et al., 2001). According to the blood glucose hypothesis, increased blood glucose levels mean more glucose for possible utilization; hence, decreased levels from baseline should result in a decrement from baseline performance. However, in Fischer et al.'s (2001) study, protein was shown to enhance performance on reaction-time tasks. The idea that protein and carbohydrate ingestion are task specific and thereby work through different mechanisms was strengthened by the finding that protein resulted in higher peripheral performance and lower central performance, with the opposite pattern seen for carbohydrate (Fischer et al., 2001). Since these two macronutrients also have the opposite effect on blood glucose levels, it may be that they work through very different mechanisms. Finally, protein ingestion has been associated with an overall higher state of metabolic activation (resulting in higher arousal) than that seen after glucose ingestion, perhaps explaining the greater efficiency seen after protein ingestion when compared with carbohydrate ingestion (Fischer et al., 2001).

However, protein ingestion can cause increases in blood glucose under certain circumstances. When the body is fasted, glucose reserves are depleted (Linder, 1991a). Early in the fasting period, the body begins to utilize protein from the muscles. This protein provides a major source of carbon to be converted into glucose by the liver for use in the body and the brain. However, as fasting continues, fat is broken down in place of the protein to keep blood glucose levels within the normal range until the next carbohydrate meal appears (Linder, 1991b). Therefore, protein can increase glucose levels through the secondary mechanism of conversion to glucose, but only when the individual is in an early state of fasting.

In conclusion, glucose, protein, and fat ingestion have been shown to have beneficial effects on cognition. However, protein and fat appear to contribute to enhanced cognitive performance using different mechanisms than that used by glucose. Glucose exerts its effects on memory and other abilities primarily through increasing glucose availability to the brain. Protein and fat, however, are thought to exert their main effects by increasing the energy available to the brain via increased signaling through the vagus nerve. The type of tasks used, and the age of the participants are factors remaining to be clarified as to their importance with protein and fat. Further research is also needed on the mechanisms by which protein, fat, and glucose exert their effects.

The Present Study

The present study is a partial replication and extension of the work by Kaplan et al. (2001). Kaplan et al. (2001) set out to determine the influence of pure, isoenergetic protein, fat, and carbohydrate drinks on cognitive performance, and whether the time period after ingestion affected cognition. Their study used 22 participants, 11 men and 11 women, aged between 61 and 79 years. These participants ingested either a protein, carbohydrate, or fat drink at three separate sessions. Each session tested performance on paragraph recall, word recall, Trail Making, and attention tasks at 15 minutes after ingestion, and again 60 minutes after ingestion. The study found that all three of the macronutrients enhanced performance on tasks of immediate and delayed paragraph recall. Protein exerted an extra effect by reducing the rate of forgetting from the immediate to delayed versions of the paragraph recall task. The word recall test showed no effect of drink. However, further analyses showed that both fat and glucose ingestion tended to impair performance under certain conditions. Specifically, fat led to impaired performance on the word recall task only when the scores from the three repetitions of the list were combined, and then only at the 60-minute mark. Glucose led to impaired performance on this task, but only when scores from the 15 and 60-minute sessions were combined. However, in regard to the Trail Making Test and the attention task, effects were nutrient-specific. Carbohydrate and fat improved performance on the Trail Making Test, especially for those participants who had poor baseline scores. Fat also improved performance on the attention task. Kaplan et al. (2001) can be criticized for analyzing their data in as many ways as necessary to produce statistically significant results. Their analyses went well beyond the tests necessary to obtain answers to their original research questions. To some extent, this criticism could be avoided by taking a planned comparisons approach to the analysis (Hays, 1973; Keppel & Zedeck, 1989). The present study sought to replicate the effects of these nutrients on declarative memory functions using a planned comparisons approach, and to extend these findings by investigating the effects of participant age and task difficulty.

Age and Gender

Age of participants is a factor known to be important when considering nutrient effects on cognitive performance (Craft et al., 1994; Hall et al., 1989; Korol & Gold, 1998). Many studies, including all those that have looked at effects of pure protein, fat, and glucose, have used either young adults or older adults as their sample. The present study was made up of two age groups; a group of young adults aged 19-31 years, and a group of older adults aged 70-79 years. Only male participants were used because hormonal differences between males and females could have potentially confounded the hormonal analyses of the blood samples (not reported here).

Cognitive Tasks

The present study used the same paragraph recall task as Kaplan et al. (2001); that is, parallel versions of the logical memory subtask of the WMS-III. The word recall task used in the present study was based on the Rey Auditory Verbal Learning Test (RAVLT). The RAVLT is composed of a list of 15 nouns, which are read aloud to the participant (Spreen

& Strauss, 1998). The participant is then encouraged to immediately write down or repeat back every word they can remember. This procedure is repeated for five trials. The word recall task in the present study was made up of 30 two-syllable words – 15 nouns and 15 verbs. Four parallel versions of this task were constructed. The two types of word (nouns and verbs) were used to introduce an element of task difficulty. The hypothesis here was that nouns would be easier to recall than verbs. Also, the number of words in the list was influenced by the need for removal of possible floor and/or ceiling effects. A series of pilot studies resulted in no person scoring either zero out of 30, or 30 out of 30. Both the paragraph and word recall tasks measure immediate recall and, especially with the paragraph recall task, have been shown to be affected by the ingestion of glucose, protein, and/or fat.

In addition to paragraph and word recall, the present study included a test of mathematical reasoning. This test is thought to involve higher cognitive processing and working memory. A study by Verhaeghen et al. (1997) showed that on an easy version of a mental arithmetic task, performance measured as both a function of accuracy and as a function of processing speed, resulted in equivalent scores across both young and old adult age groups. However, upon introducing a more complex mental arithmetic task, the older age group showed deficits in both accuracy and processing speed in relation to the young adult group. Therefore, age of the participant appears to affect mental arithmetic performance only in relation to the complexity of the task. Performance on this specific subtask of the WAIS-III has, to the author's knowledge, never been assessed in regard to the effects of glucose, protein, and fat on performance.

Task difficulty

Studies that have used both older adults and younger adults in their samples have tended to use tests of insufficient difficulty to allow for an effect to be seen, particularly in the young adult group. In the present study, each of the tests outlined above were constructed in such a way that two levels of difficulty (one easy, one hard) were included. Actual difficulty levels were set through a series of pilot studies. Every attempt was made to set the higher level of difficulty such that the older group of participants could perform above chance levels, yet at the same time have the task challenging enough for the younger participants.

Findings to date suggest that glucose enhances memory for both easy and hard tasks in the elderly, while young participants' memory is only enhanced when performing hard tasks (Kennedy & Scholey, 2000; Scholey et al., 2001). These previous findings are based on *between-task difficulty*. However, in the present study, the interest was in varying difficulty levels *within* the same task. The main reason for this was to avoid confounding a change in difficulty with a change in task. As shown by Kaplan et al. (2001), some carbohydrate, protein, and fat effects on cognitive performance are task-specific.

Objectives

The present study aimed to determine:

(a) The effects of a drink containing either pure glucose, protein, or fat (equal in energetic value, and volume) on cognitive performance. More specifically, the effects of these three nutrients on paragraph recall, word recall, and mental arithmetic were investigated. On the basis of previous findings, it was expected that glucose, protein, and fat would enhance performance on the paragraph recall, word recall, and mental arithmetic tasks (Kaplan et al., 2001; Kennedy & Scholey, 2000).

(b) If cognitive effects of nutrients are influenced by age of the participant. Previous findings have shown that glucose, protein, and fat enhance older and young adults' performance on cognitive tasks (Fischer et al., 2001; Kaplan et al., 2001). It was expected that both the elderly and the young adult participants' performance would be enhanced by ingestion of the nutrients.

(c) If cognitive effects of nutrients are influenced by the difficulty of the task. It was expected that a Nutrient x Age x Difficulty Level interaction would be seen, but only for the young participants. This is based on previous findings where an effect of Nutrient was only found for the young adult age group on tasks with a high degree of difficulty (Kennedy & Scholey, 2000; Korol & Gold, 1998).

METHOD

Participants

Young Participants

Twelve males aged between 19 and 31 years ($M = 24.17$, $SD = 3.72$) volunteered to participate in the study. The young participants were evenly split between those in professional positions ($n = 6$), and those who were either undergraduate students ($n = 5$), or post-graduate students ($n = 1$). All young people were recruited from advertisements placed around the Massey University campus and the Palmerston North community, an article in a local community newspaper, or by word-of-mouth.

Older Participants

Twelve males aged between 70 and 79 years (M = 72.33, SD = 2.60) volunteered to participate in the study. All of the participants were retired, with the exception of one who was engaged in professional employment. Older participants were recruited through advertisements placed around the Palmerston North community, and through an article in a local community newspaper.

All materials and procedures used in the present study were approved by the Massey University Human Ethics Committee, Palmerston North (Protocol 02/31).

Group Assignment

The present study was a mixed design. The between-groups factor was Age, while all the other factors were within-groups. Thus, each participant took part in each nutrient condition of the study. Therefore, a 4 x 4 Latin Square design was used to balance the nutrients, test versions, and test order over the four sessions for each participant. The Latin Square design was repeated six times resulting in 24 balanced combinations of drink, test version, and test order for each of the four different sessions. One of these 24 combinations was then randomly assigned to each participant. So, each participant received a unique combination of order of drink, test version, and test order over the four sessions. This controlled for the possibility of order effects.

The criteria for inclusion in the study were the absence of any pre-existing medical condition, or medication, that may have caused interference with the functions of the nutrient or performance of the cognitive task. Any questionable medical conditions were checked for possible interference activities by checking with pharmaceutical staff at a local chemist, and discussions with staff at the School of Psychology, Massey University, and CFR. Criteria for inclusion also required the absence of any psychological condition, any type of head injury, anticoagulation medication, diabetes, or any significant problems with hearing abilities. These factors were assessed through a screening questionnaire during initial contact with the participant over the telephone.

Apparatus

The cognitive tasks were read to the participant via a voice-recording on a Sony 60 minute tape. The tasks were transferred onto an audiotape to provide consistency of presentation. Two Sony tape recorders, one with an attached microphone, were used to either playback the instruction and cognitive task tape, or to record participant responses. The participants' responses to the paragraph recall task were recorded to ensure accurate scoring of this task at a later date. The volume of the playback tape recorder was adjusted during each session to a level comfortable for each participant.

A DSE Stopwatch was used to time responses to the mental arithmetic task items. As each mental arithmetic question had a time limit on response time, a conference timer was used to unobtrusively alert the participants when they were nearing the end of the time limit. The conference timer was a small black box with two lights on the top, one red, and one green. A switch on the side allowed the setting of the timer to different time limits. The conference timer was modified by the School of Psychology Workshop to allow the green light to flash to alert the participant that they had ten second of response time left. The red light only began to flash when the time limit was up. The side switch allowed the time limit to be changed by the experimenter depending on the difficulty of the item.

A Polar S610 Heart Rate Monitor was used to measure heart rate throughout the session. This monitor consisted of a band that the participant wore against the skin, across the heart. A device similar to a watch was placed on the participant's wrist, which, through use of a sensor, recorded the heart rate throughout the 90-minute session. The heart rate data were downloaded to a computer at a later date to be analyzed by CFR (the data are not reported here).

A one-touch glucose monitor allowed measurements of blood glucose levels. A blood sample was obtained from the participant by finger-prick, and the blood transferred to a glucose-sensing strip that was fed into the monitor. The monitor then returned a reading of the amount of glucose in the peripheral blood supply.

The four blood samples needed every session were taken intravenously. Vacutainer needles were used to transfer blood into 10ml vials. These vials were then injected with a preservative, packed in ice, and taken to CFR for hormone analyses at a later date (the results are not reported here).

Tasks and Stimuli

A mood questionnaire was administered at the beginning and at the end of each session to ascertain the participant's state of mood. The 16-item questionnaire (see Appendix A) used in the current investigation was the Bond & Lader Visual Analogue Scale (Bond & Lader, 1974).

The three cognitive tasks used in the present study consisted of a paragraph recall task, a word recall task, and a mental arithmetic task (see Appendix B). Each task was designed with two levels of difficulty, one easy, and one hard. The paragraph recall task was based on the Logical Memory subtask of the Wechsler Memory Scale – III (WMS-III). Story one of the Logical Memory subtask was used as one of the easy paragraphs in the present study. Three parallel versions of this story were created through changing the names of places, and the context, but leaving the structure of the story the same.

The more difficult paragraph recall tasks were derived from summaries of four of the "Working Knowledge" sections of Scientific American magazines. These paragraphs described technical aspects of everyday activities, such as how cleaning agents, and compact disc players work (see Appendix B). All the difficult paragraphs were equal in word length and the amount of technical language used.

The word recall tasks consisted of 30 words each. Fifteen of these words were two-syllable nouns (easy), and 15 were two-syllable verbs (hard). The words were derived from a word frequencies book based on the British National Corpus (Leech, Rayson, & Wilson, 2001). Words were randomly picked from this book. However, in an earlier pilot study (to confirm two distinct difficulty levels between the two forms of words) it was observed that nouns high in meaningfulness returned high recall scores. On the basis of this, all selected words were screened for meaningfulness value using the ratings of Toglia and Battig (1978). All words with a high rating on meaningfulness were discarded, and replaced with other randomly selected nouns. The pilot study also found that the order of presentation of the words influenced whether nouns or verbs were recalled. For example, the random allocation of words in the pilot study resulted in more nouns at the beginning and end of the list, than verbs. Thus, the nouns at the beginning and end of the list were recalled more often than the verbs at either end, or the nouns or verbs in the middle of the list. Therefore, the present study structured the word lists to start with a verb and to alternate from noun to verb to noun from then on. The choice to begin with a verb was made randomly. In addition, the placement of each particular noun or verb in each list was decided through random allocation.

The mental arithmetic task in the present study was based on the mental arithmetic subtask of the Wechsler Adult Intelligence Scale – III (WAIS –III). The first three items of this scale were not retained in the present experiment, due to their simplicity. As the present study was investigating effects of nutrients on normal, healthy individuals, it was judged that these items were too simplistic to be of any use with this sample. Therefore, these items were replaced with mathematical calculations taken from a third-form school mathematics textbook (Joyce, Kibblewhite, & Nightingale, 1990). The rest of the mental arithmetic items were retained. As the mental arithmetic subtask of the WAIS-III includes items that become more difficult as the participant moves from item to item, the first 10 items were designated to be the easy version, while the further ten items made up the hard version. Three parallel forms of this task were constructed by changing the numbers and names used in the items. For example, "three pears" may become "two apples". All attempts were made to keep the numbers used in the parallel forms within the same range as those in the original version. For example, if a number were less than 10 in the original, a number less than 10 would be used in the parallel forms.

All the parallel forms of the cognitive tasks were developed and pilot tested by the researcher to determine similar baseline performance levels across the type of task, as well as adequate separation between the difficulty levels for each type of task.

The four drinks in the present study replicated the nutrients used by Kaplan et al. (2001). The nutrients and their ingredients are outlined in Table 1.

Table 1
Ingredients, volume, and energy values of the placebo, glucose, protein, and fat drinks used in the present study

Drink	Water (ml)	Lime Juice (ml)	Nutrient (g)	Volume (ml)	Energy (kJ)
Placebo	292.5	7.5	Nil	300	0
Glucose	260	7.5	46.82 (glucose)	300	774 (100% as CHO)
Protein	260	7.5	48.08 (whey protein isolate)	300	774 (98.4% as protein, <0.38% as CHO, 1.17% as fat)
Fat	248.44	7.5	41.66 (microlipid, 50% safflower oil emulsion)	300	774 (100% as fat)

Design and Analysis

The design was a 4 x 2 x 2 repeated-measures ANOVA, in which Nutrient (placebo, glucose, protein, and fat), and Difficulty Level (easy and hard) were the within-subjects factors, and Age (young adult and older adult) was the between-subjects factor (see Table 2).

A planned comparisons approach was taken to analyze the data (Hays, 1973; Keppel & Zedeck, 1989). This means that sets of objectives (the primary research questions) were formulated before the study began. Therefore, when the data had been collected, they were analyzed specifically with the primary research questions in mind. This approach not only lessens the risk of a chance significant effect due to a large number of multiple comparisons, but also increases the statistical power with which to detect an effect for the primary objectives (Hays, 1973; Keppel & Zedeck, 1989).

The present study also investigated post-hoc comparisons, which are comparisons suggested to be important by the data themselves. These comparisons, however, carry a higher risk of a Type-I error.

All statistical analyses were completed using the statistical package SPSS for Windows, version 11.0. The *F* values and associated statistics are reported in the text. The effect size (η^2) and statistical power (*SP*) statistics were calculated in accordance with the guidelines set by Cohen (1988). The alpha level of all analyses was set at .05. ANOVA tables can be found in Appendices E and F.

Table 2
A visual representation of the design with Nutrient (glucose, placebo, protein, and fat), and Difficulty Level (easy and hard) as the within-subjects factors, and Age (young and old) as the between-subjects factor

DIFFICULTY LEVEL	Glucose	Placebo	Protein	Fat
Easy	Young Old	Young Old	Young Old	Young Old
Hard	Young Old	Young Old	Young Old	Young Old

NUTRIENT

Procedure

Screening/Training Session

Participants were individually tested in the Electrophysiological Laboratory at the School of Psychology, Massey University, Palmerston North. This room was quiet and free from distraction. All unnecessary and potentially distracting equipment was removed from the room before the study began.

After initial telephone contact, where the potential participant expressed interest in the study and was screened for eligibility, an information sheet (see Appendix C) and a general health questionnaire were mailed out. Upon the return of the questionnaire, the potential participant was contacted and an appointment made for a screening and training session. Participants were informed that a blood glucose measurement by finger-prick would be taken, and were therefore asked to fast for at least four hours before their appointment. Upon arrival at the screening/training session, the participant was given a consent form to read and sign (Appendix D).

The participant was then asked to put on the heart rate monitor and it was explained that the device would measure changes in heart rate throughout the session. The heart rate monitor was not turned on for the screening/training session, as its presence was merely to allow the participants to become accustomed to wearing it.

A blood glucose measurement by finger prick was taken next. After sterilization with an alcohol swab on the participant's least preferred hand, a lancet was used to make a small puncture wound. The blood was then transferred to a glucose-sensing strip, which had been previously placed in the glucose monitor. The reading of the blood glucose level was then noted. The blood glucose measurement was necessary to ensure that the participant was not diabetic. However, no participant showed abnormal blood glucose readings.

Participants were next asked to complete a series of questionnaires. The first questionnaire was the biographical checklist. This contained questions about possible covariates, such as years of formal education, occupation, sporting activity, and eating patterns. Following this the Mini-Mental State Examination was administered. This is a short screening measure for dementia and memory problems. No participant scored below the cut-off point of 27 points out of 30. Finally, the participants were presented with the Bond & Lader Visual Analogue Scale. They were informed that this test would measure their mood-state at this particular moment in time. The experimenter showed the participant how to fill out the scale through use of an example scale at the top of the test.

Training versions of the cognitive tasks followed the questionnaires. The participant was asked to perform these tasks, and was informed that they constituted smaller versions of the tasks that would be used in the four experimental sessions. Participants were given a brief description of each test.

The first task presented was Paragraph Recall. Participants were asked to listen carefully to the tape, which first played a series of instructions for the task, followed by the paragraph itself. After the instructions were played, the tape was paused and participants were asked if they understood the instructions. The tape then continued with the paragraph. Immediately after the paragraph was finished, the participant was asked to recall everything they could remember from the story. Their responses were recorded on a separate tape recorder for confirmation of scoring at a later date. The instructions used in this task were identical to those used with the Logical Memory subtask of the WMS-III (Wechsler, 1997b). One point was given for every correctly remembered unit of the story. At the end of the task, these points were added up, and converted into a percentage of the paragraph recalled for that session.

The second task presented was word recall. Participants were given a blank sheet of paper and a pen before the task began. The instructions were then played to them over the tape recorder. These instructions are identical to those used on the Word Recall subtask of the WMS-III, with the exception that instead of the participant repeating the words back to the experimenter at the end of the list, the participant was asked to write them down. When the participants had written down all the words they could think of, their piece of paper was taken away and they were given a fresh piece. The above procedure was then repeated. Participants had a two-minute recall limit on this task, although no participant needed the entire time available. Scores for this task consisted of the number of words recalled correctly out of 30 overall, as well as the number of nouns and verbs correctly recalled, each out of 15.

The final cognitive task was mental arithmetic. Instructions were read out on the tape. These instructions were identical to those for the mental arithmetic subtask of the WAIS –

III (Wescher, 1997a), in that participants were asked to do the problems mentally, without any aids whatsoever. An addition was made to these instructions by the experimenter. This was that the experimenter could not tell participants if the answer provided was right or wrong, but could repeat the question once if requested. Participants were also informed that their responses would be timed, and every item had a different time limit within which to answer. The lights on the conference timer were explained to participants. It was explained that when the green light was flashing, there was 10 seconds left to answer the question, but once the red light began to flash, the time limit for that item was up. If participants responded accurately after the time limit had expired, the answer was given a zero score.

The first mental arithmetic item was played on the tape. Immediately after the item was read aloud, the tape was paused and the stopwatch started. The stopwatch was stopped after the participant answered. The conference timer, set approximately 30 cm in front of the participant, was also started at the same time. The time limit for each question depended on the difficulty level of the question, and was reset by the experimenter before each item was read out. Easy items had a time limit of 45 seconds within which the participant had to respond accurately to receive one point. Difficult items had a time limit of 90 seconds, while one exceptionally difficult item had a time limit of 180 seconds. These time limits were set by taking the time limit provided for each item by the WAIS-III, and adding half the time again. For example, a 30 second time limit would have become 45 seconds, due to an increase of 15. This extension of the time limit was deemed necessary after observations during the pilot study. Here, some participants required more time than the WAIS-III allowed to answer the items. If the participant in the present study requested another reading of the item, the experimenter read the item to the participant, but the timing was not stopped. This procedure was repeated for all 20 of the mental arithmetic items. An item score was either 0 or 1. A zero score was given for any item answered incorrectly, any item not answered, or any item answered incorrectly or correctly outside the time limit. A score of 1 was given for every item answered correctly within the time limit. Overall mental arithmetic scores were found by taking the number of items that scored a one, and dividing this number by 20. Difficulty level scores were found by dividing the number of items correct in each difficulty category by 10. The average time taken for each difficulty level, as well as the average time taken to answer all the items, was also recorded.

At the end of the mental arithmetic task, the screening/training session was at an end. The participants were thanked and asked to make appointments for the next four experimental sessions. They were also asked to fast overnight, preferably for a minimum of nine hours, before arrival at the next session.

Experimental Procedure

On arrival at the experimental sessions, participants were asked whether they had fasted overnight. Once the participant was connected to the heart rate monitor, the first intravenous blood sample was taken. Either the trained co-researcher from CFR took this blood sample, or a registered nurse.

When the participants were comfortable, they were asked to fill out the Bond & Lader Visual Analogue Scale, identical to the scale used in the screening/training session. This was followed by the presentation of a nutrient drink. The nutrient drink was presented in a coloured coffee cup, with a lid and a black straw. All attempts had been made to make the nutrient drinks as alike in sensory properties as possible. However, the inability of the participant to see or smell the drink was an extra precaution taken against possible expectation effects if the participant was able to guess what he had been drinking. The participant was allowed five minutes to ingest the drink. No participant took the maximum time. A drink evaluation form was then presented which asked for the participant's rating of the drink in regard to perceptions such as 'sour', 'fatty', or 'satiating'. The participant was then provided with magazines to read quietly for 30 minutes while the nutrient was digested.

After 30 minutes, another blood sample was taken. When the participant was comfortable the cognitive testing began. The procedure was identical to that followed in the screening/training session for each test. However, in the experimental sessions, the paragraph recall tasks always came first and last. This was because the paragraph recall task was split into two tests, one easy, and one hard. This was due to the inability to combine the two levels of difficulty into one presentation of the test. Therefore, to reduce the possibility of interference effects between the two versions, either the easy or the hard version was always presented first, with the other version presented last. The order of the versions was balanced to ensure that the easy version was presented first as often as the hard version. The word recall and mental arithmetic tasks were presented in between the two paragraph recall tasks with their order also being balanced across presentations.

After the cognitive testing, a third blood sample was taken. The participant was then asked to read quietly for approximately 30 minutes. Thus, 90 minutes after ingestion of the nutrient drink, the final blood sample was taken.

Another Bond & Lader Visual Analogue Scale was then given to the participant. The purpose of this was to determine if any changes in mood had occurred since the baseline measurement, an effect that might have been attributable to the nutrient. Also, the participant was asked to state which drink they thought they had consumed that session. This was to ascertain whether the participants had knowledge of the contents of the drink, which, if so, could lead to expectancy effects on performance.

When the drink form had been completed, participants were thanked, their appointment for the second session was checked, and they were offered a continental breakfast. This procedure was followed for all the experimental sessions for each participant. Each experimental session was conducted in the morning, with a minimum of 3 days and a maximum of 7 days between each session for a given participant. This inter-session interval was primarily to ensure that interference effects from previous exposure to the cognitive tests did not carry over into the next session.

RESULTS

A planned comparisons approach was used to analyze the data for the present study. The effect of the type of nutrient on paragraph recall, word recall, and mental arithmetic performance was examined in the first planned comparison. The second planned comparison examined the effects of age of participant in regard to task performance, and the influence of the type of drink. Finally, the third planned comparison investigated the effects of type of nutrient on task difficulty.

The two recall conditions of the word recall task were found to have similar results in all analyses. Therefore, the analyses presented here for the word recall task collapse across recall version. In addition, mental arithmetic performance was assessed on two dimensions - accuracy and response time.

Planned Comparisons

Nutrient

The first planned comparison investigated the effects of three drinks containing glucose, protein, or fat, on cognitive performance. Specifically, the effects of these nutrients on performance of tasks of paragraph recall, word recall, and mental arithmetic (collapsing across Age and Difficulty Level) were determined. It was expected that all three nutrients would result in enhancing effects, compared to placebo, on performance of all three tasks. Table 3 shows the means and standard deviations for the three tasks after ingestion of each Nutrient, collapsed across Age, and Difficulty Level.

It can be seen from Table 3 that the glucose and fat drinks produced a marginal improvement in performance on the paragraph recall task. The glucose and fat drinks also resulted in a small performance increase on the mental arithmetic accuracy task, but only the glucose nutrient improved the response time measure for this task. No type of nutrient had an effect on word recall performance.

Table 3

Means (M) and standard deviations (SD) as a function of Type of Task, and Nutrient (collapsed across Age and Difficulty Level)

	Type of Task							
	Paragraph Recall		Word Recall		Mental Arithmetic $_A$		Mental Arithmetic $_T$	
Nutrient	*M*	*SD*	*M*	*SD*	*M*	*SD*	*M*	*SD*
Protein	47.68	14.41	32.15	16.50	75.42	17.50	12.05	7.07
Glucose	49.84	16.58	32.08	15.70	78.75	14.61	10.86	6.66
Fat	48.17	16.11	31.94	16.07	77.50	18.59	12.60	8.46
Placebo	47.19	15.86	32.43	15.92	76.46	15.21	12.07	7.13

Note. Table mean values are given in percentages except for Mental Arithmetic$_T$ which is given in seconds. Mental Arithmetic$_A$ is the mental arithmetic accuracy function. Mental Arithmetic$_T$ is the mental arithmetic time function.

Paired-samples *t*-tests[1] (*t*-test statistics for each pair are shown in Table 4) were calculated to determine any statistically significant differences between the placebo and the glucose, protein, and fat drinks with regard to performance on the three tasks. A Bonferroni correction value of $p = .004$ ($p = .05 / 12$) was used to control for the multiple comparisons being done. No statistically significant differences were found between performance on any task after ingestion of a placebo and performance after ingestion of the nutrients (all p's $\geq .14$).

[1] Dunnet's test would have been a better test to use here. However, SPSS 11.0 only allows use of this test for between-subjects factors, and then only through an ANOVA procedure (Pallant, 2001).

Table 4

t-test statistics for each Nutrient compared to placebo by Type of Task (collapsed across Age and Difficulty Level)

Nutrient Pair	*t* (23)	Sig. (2-tailed)	r^2_{pb} [a]	*SP*
PR Protein	0.18	0.86		
PR Glucose	0.88	0.39		
PR Fat	-0.31	0.76		
WR Protein	-0.19	0.85		
WR Glucose	-0.24	0.82		
WR Fat	0.32	0.75		
MA_A Protein	-0.42	0.68		
MA_A Glucose	1.04	0.31	0.04	0.17
MA_A Fat	-0.37	0.72		
MA_T Protein	-0.01	0.99		
MA_T Glucose	-1.52	0.14	0.09	0.30
MA_T Fat	-0.56	0.58		

Note. PR = Paragraph Recall; WR = Word Recall; MA_A= Mental Arithmetic Accuracy Function; MA_T= Mental Arithmetic Time Function. All Nutrients were compared against the placebo for each particular task. Effect sizes, statistical power statistics, and r^2_{pb} values were not included for values of $t \leq 1$, as any results would have been uninterpretable.

[a] r_{pb} is the point biserial correlation coefficient and is calculated by the formula $r_{pb} = \sqrt{t^2 / t^2 + df}$. This statistic gives an indication of the magnitude of the relationship, and is recommended for assessing variance accounted for (r^2_{pb}) when comparing group means (Polit, 1996).

Performance on both functions of the mental arithmetic task after ingestion of glucose were associated with small effect sizes (assessed by r^2_{pb}, a measure of variance accounted for). This suggests that with a larger sample (thereby increasing statistical power) a significant effect of glucose, relative to placebo, on both functions of this task may become apparent. It can be noted that the statistical power available to produce a statistically significant outcome for accuracy and time on the arithmetic task was very low (*SP* = .17 and .30).

Age

While no significant effects of Nutrient were found for any of the tasks, collapsed across Age and Difficulty Level, there was a possibility that by collapsing across Age, Nutrient effects specific to certain age groups may have been cancelled out. Therefore, the second planned comparison was to determine if cognitive effects of nutrients were influenced by the age of the participant. It was expected that both the older and younger groups' performance on all three tasks would be enhanced by all three nutrients relative to placebo.

It can be seen in Table 5 that the elderly group's performance on the paragraph recall task marginally improved only after ingestion of protein.

Table 5
Means (M) and standard deviations (SD) as a function of Type of Task, Nutrient, and Age

		Type of Task							
		Paragraph Recall		Word Recall		Mental Arithmetic $_A$		Mental Arithmetic $_T$	
Age	Nutrient	*M*	*SD*	*M*	*SD*	*M*	*SD*	*M*	*SD*
Young	Protein	53.93	9.27	42.36	17.65	81.25	13.34	10.85	6.47
	Glucose	58.75	13.66	42.08	13.24	80.83	13.11	10.11	5.57
	Fat	55.89	13.57	42.78	15.53	76.67	18.63	12.71	9.11
	Placebo	53.52	17.29	43.06	14.79	80.00	13.98	12.37	8.13
Elderly	Protein	41.43	16.23	21.94	5.50	69.58	19.71	13.26	7.72
	Glucose	40.93	14.66	22.08	11.04	76.67	16.28	11.62	7.78
	Fat	40.44	15.11	21.11	6.56	78.33	19.35	12.49	8.17
	Placebo	40.85	11.79	21.81	8.05	72.92	16.16	11.77	6.34

Note. Table mean values are given in percentages except for Mental Arithmetic$_T$ which is given in seconds. Mental Arithmetic$_A$ is the mental arithmetic accuracy function. Mental Arithmetic$_T$ is the mental arithmetic time function.

Glucose ingestion showed a similar improvement effect on the word recall task for this age group and also showed an improvement effect on the mental arithmetic accuracy function. A relatively marked improvement in accuracy performance was noted after fat ingestion. No improvement effects were noted for any of the nutrients for response speed for the elderly age group.

Paired-samples *t*-tests with a Bonferroni correction set at $p = .002$ ($p = .05 / 24$) were used to determine if any significant differences existed between the means of the placebo condition, and the means of the three nutrient conditions for the elderly and young adult age groups. The statistics for each *t*-test are shown in Table 6. No statistically significant differences were found between performance after ingestion of a placebo and performance after ingestion of the nutrients on any of the tasks for the elderly age group (all p's $\geq .15$).

Calculation of the point biserial correlation coefficients showed that performance of the elderly on both functions of the mental arithmetic task showed small to moderate effect sizes with the nutrients. Glucose ingestion explained 9% of the variance in performance on the mental arithmetic accuracy task, while fat ingestion explained 18% of the variance. Protein consumption explained 10% of the variance in the performance scores seen for the elderly age group on the mental arithmetic time function. Thus, with a larger sample size, glucose and fat may elicit significant effects on the accuracy component of the mental arithmetic task for the elderly age group, while protein may elicit a significant effect on the mental arithmetic time function for this age group.

However, these effects are very small and the power available to detect them very low (see Table 6). It was calculated that 87 participants in each group would be necessary to detect a significant effect of glucose on mental arithmetic accuracy performance for the elderly age group, with an alpha level of .05 and a statistical power of .80. Fat would require 42 participants per group to detect a significant effect on this same task, and protein would require 77 participants per group to acquire a significant effect for response time.

Table 6
t-test statistics for each Nutrient, compared to placebo, as a function of Type of Task and Age

Age	Nutrient Pair	*t* (11)	Sig. (2-tailed)	r^2_{pb} [a]	*SP*
Young	PR Protein	0.09	0.93		
	PR Glucose	1.15	0.27	0.11	0.19
	PR Fat	-0.42	0.68		
	WR Protein	-0.28	0.79		
	WR Glucose	-0.46	0.66		
	WR Fat	0.12	0.91		
	MA_A Protein	0.35	0.73		
	MA_A Glucose	0.31	0.76		
	MA_A Fat	0.80	0.44		
	MA_T Protein	-0.90	0.39		
	MA_T Glucose	-2.23	0.05	0.31	0.57
	MA_T Fat	-0.37	0.72		
Elderly	PR Protein	0.16	0.88		
	PR Glucose	0.02	0.98		
	PR Fat	0.12	0.90		
	WR Protein	0.08	0.94		
	WR Glucose	0.13	0.90		
	WR Fat	0.34	0.74		
	MA_A Protein	-0.94	0.37		
	MA_A Glucose	1.04	0.32	0.09	0.16
	MA_A Fat	-1.54	0.15	0.18	0.29
	MA_T Protein	1.13	0.28	0.10	0.18
	MA_T Glucose	-0.13	0.90		
	MA_T Fat	-0.42	0.68		

Note. PR = Paragraph Recall; WR = Word Recall; MA_A= Mental Arithmetic Accuracy Function; MA_T = Mental Arithmetic Time Function. All nutrients were compared against the placebo for each particular task. Effect sizes and statistical power statistics were not included in this table for values of $t \leq 1$, as any results would have been uninterpretable.

Analysis of the means for the young adult age group (see Table 5) showed that both glucose and fat ingestion improved paragraph recall performance. No improvement in performance was noted for any of the nutrients on the word recall task. A slight improvement could be seen for the mental arithmetic accuracy task after ingestion of protein. Finally, both protein and glucose ($M = 10.85$ and $M = 10.11$, respectively) showed an improvement in response time compared to placebo ($M = 12.37$) on the mental arithmetic time function.

Paired-samples *t*-tests (see Table 6) were calculated to ascertain any significant differences between each nutrient and the placebo for each task. No significant differences were found between the placebo condition and any of the nutrient conditions on any of the tasks for the young adult age group (all p's $\geq .05$). However, the point biserial correlation coefficient did show that glucose accounted for nearly 11% of the variance seen in paragraph recall performance, and also accounted for 31% of the variance seen in the speed of response to the mental arithmetic items for the young age group. This suggests that, given a larger sample size, a significant improvement effect of glucose would be seen for both these tasks for the young age group.

Task Difficulty

The final planned comparison was to determine if cognitive effects of nutrients were influenced by the difficulty level of the task. It was expected that a Nutrient x Age x Difficulty Level interaction would be found for all the tasks, with the young participants' performance being enhanced by all the nutrients, but only on the difficult version of each task.

As can be seen in Table 7, all the nutrients, especially the glucose nutrient, improved performance for the difficult version of the paragraph recall task for the young adult age group. Glucose also marginally improved the difficult version of the mental arithmetic accuracy task. Glucose and protein ingestion were also seen to improve response times for the mental arithmetic items. No improvement effects were noted on performance of the difficult version of the word recall task with the young adult age group.

Table 7

Means (M) and standard deviations (SD) as a function of Type of Task, Nutrient, Age, and Difficulty Level

			Type of Task							
			Paragraph Recall		Word Recall		Mental Arithmetic$_A$		Mental Arithmetic$_T$	
Age	Nutrient	Task Difficulty	*M*	*SD*	*M*	*SD*	*M*	*SD*	*M*	*SD*
Young	Protein	Easy	68.54	14.52	48.33	16.97	90.00	11.28	8.48	4.96
		Hard	39.32	10.48	36.39	19.41	72.50	23.79	13.23	8.74
	Glucose	Easy	72.98	16.63	49.44	14.62	87.50	11.38	8.47	5.29
		Hard	44.53	13.04	34.72	13.89	74.17	18.81	11.75	6.33
	Fat	Easy	72.69	17.74	50.00	16.88	86.67	17.75	9.54	8.06
		Hard	39.09	13.36	35.56	17.02	66.67	22.29	15.88	11.35
	Placebo	Easy	68.37	21.37	48.33	13.82	86.67	9.85	10.08	8.16
		Hard	38.67	17.54	37.78	18.33	73.33	21.03	14.65	8.90
Elderly	Protein	Easy	54.11	24.20	22.78	8.02	76.67	20.15	10.17	7.25
		Hard	28.90	11.20	21.11	9.36	62.50	22.61	16.35	9.61
	Glucose	Easy	51.33	15.27	22.50	13.79	90.00	7.39	8.35	7.01
		Hard	30.54	17.85	21.67	11.85	63.33	28.71	14.88	9.57
	Fat	Easy	52.46	22.43	23.06	6.74	89.17	16.76	7.83	7.18
		Hard	28.42	12.44	19.17	9.11	67.50	26.67	17.16	9.72
	Placebo	Easy	55.87	17.18	24.72	12.51	82.50	11.38	8.13	6.22
		Hard	25.83	13.03	18.89	8.21	63.33	24.98	15.41	8.60

Note. Table mean values are given in percentages except for Mental Arithmetic$_T$ which is given in seconds. Mental Arithmetic$_A$ is the mental arithmetic accuracy function. Mental Arithmetic$_T$ is the mental arithmetic time function.

A 4 x 2 x 2 repeated-measures ANOVA was conducted to determine if a significant Nutrient x Age x Difficulty Level interaction was apparent for each task. The within-subjects factors were Nutrient (glucose, protein, fat, or placebo), and Difficulty Level (easy or hard). The between-subjects factor was Age (young adult or older adult). Unexpectedly, no significant interaction was found for paragraph recall ($F < 1$), word recall ($F < 1$), the mental arithmetic accuracy function F (3, 66) = 1.06, p = .37, η^2 = .05, SP = .27, or the mental arithmetic time function (F < 1). Thus, Nutrient did not interact with Age to produce an enhancement of performance on the difficult version of any of the tasks. However, the small effect size and low statistical power associated with the mental arithmetic accuracy task, suggests that a Nutrient x Age x Difficulty Level effect may become apparent for this task, given a much larger sample size.

Overall, the planned comparisons showed no significant effects of Nutrient, either collapsed across Age and Difficulty Level, or measured in regard to Age and Difficulty Level as factors in the analysis. However, small to moderate effect sizes were associated with some of the tasks and nutrients. The first planned comparison showed that glucose was associated with performance on both functions of the mental arithmetic task. When the analysis was split by age in the second planned comparison, it was seen that the association between glucose and the mental arithmetic time function was attributable to the young adult group's scores, while the association between glucose and the accuracy function was attributable to the older adult group's scores. Accuracy and time performance were also associated with fat and protein ingestion, respectively, for the elderly age group. Glucose was also associated with paragraph recall performance for the young group. Finally, the third planned comparison showed that while there was no significant Nutrient x Age x Difficulty interaction for any of the tasks, a small effect size and low statistical power associated with the mental arithmetic accuracy function was apparent. Thus, the possible age-specific effects of Nutrient, and the possibility of a Nutrient x Age x Difficulty Level interaction may reach significance with a larger sample size. However, due to the non-significance of any of the results and the small effect sizes, these results suggest that the three nutrients used have only small effects, if any, on performance.

Post-Hoc Comparisons

The post-hoc comparisons included investigations of results, important to the analysis, that reached significance, and results that were associated with large effect sizes.

Nutrient x Age Interaction on the Mental Arithmetic Accuracy Function

The series of 4 x 2 x 2 ANOVA's that were conducted to determine if any significant Nutrient x Age x Difficulty Level interaction effects were apparent for any of the tasks, showed a moderate interaction between Nutrient and Age for the mental arithmetic accuracy function, F (3, 66) = 2.61, p = .059, η^2 = .11, SP = .61. This interaction accounted for 11% of the experimental variance, thus warranting further investigation.

The Nutrient x Age interaction is illustrated in Figure 1. Here, a large difference in the mean scores can be seen between the two age groups with regard to the protein drink. This figure also shows that the elderly group's performance on this task was best after fat ingestion, and worst after protein ingestion. However, the opposite is true for the young adult group, with protein ingestion resulting in the young group's highest levels of performance on this task, and the fat drink resulting in the lowest levels of performance.

Paired-samples t-tests were calculated to investigate the Nutrient x Age interaction effect suggested by the ANOVA for the mental arithmetic accuracy function. A Bonferroni correction set at p = .006 (p = .05 / 8) was used to control for the multiple comparisons. A moderate difference between the mean accuracy performance of the elderly across the drinks and the mean performance of this age group after ingestion of protein was found, t (11) = -2.30, p = .042, r^2_{pb} = .32, SP = .56. This means that the elderly group showed a deficit in performance on the mental arithmetic accuracy task after ingestion of the protein drink. The moderate point biserial correlation statistic shows that protein ingestion accounted for 32% of the variance seen in performance of the accuracy function by the elderly group. Therefore, the strength of the relationship between protein ingestion and consequent deficits in accuracy performance for this age group, suggests that with a larger sample size, a significant deficit in performance after ingestion of the protein drink may become apparent.

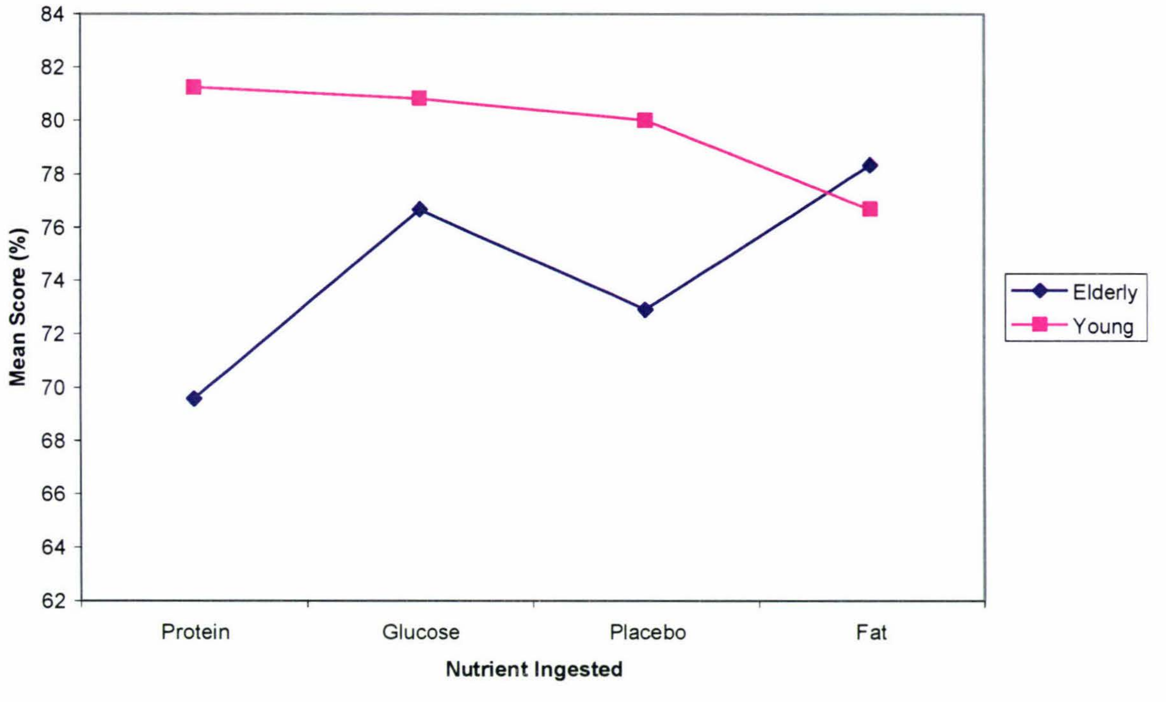

Figure 1. Interaction between Nutrient and Age on mental arithmetic accuracy performance.

Figure 1 also shows possible differences between Nutrient's within each age group in respect to the protein and fat effects on mental arithmetic accuracy performance. Paired-samples t-tests were conducted to examine the differences in average performance for each nutrient, within each age group. The Bonferroni correction was again used, set at the $p = .004$ ($p = .05 / 12$) level. The young adult age group showed no significant differences between the protein and the fat drinks effects on mental arithmetic accuracy performance, t

(11) = 1.51, p = .16, r^2_{pb} = .17, SP = .28. The older adult age group showed a stronger trend towards a significant difference between the protein and the fat drinks, t (11) = -2.43, p = .033, r^2_{pb} = .35, SP = .61. Here, protein ingestion resulted in a large decrement in mathematical accuracy performance (M = 69.58), when compared with performance after the consumption of fat (M = 78.33).

Figure 1 also shows a difference between the age groups in mental arithmetic accuracy levels after ingestion of protein. The elderly group performed at a much lower level on this task after protein ingestion (M = 69.58), than their younger counterparts (M = 81.25). An independent-samples t-test was used to examine this difference. No significant difference was found between the young and the elderly group's performance after consumption of the protein drink, t (22) = -1.70, p = .104, r^2_{pb} = .12, SP = .37. However, the point biserial correlation suggests that with a larger sample size, the statistical power may be increased enough to detect a significant difference between the two age groups' means.

Apart from this interaction and the moderate Nutrient x Age x Difficulty Level interaction effect seen on the mental arithmetic accuracy task (discussed under the Task Difficulty section of Planned Comparisons), the mental arithmetic time function is the only other task that shows main effects and interactions with effect sizes warranting reporting. Here, a non-significant main effect of Nutrient shows a small effect size and low statistical power, F (3, 66) = 1.23, p = .31, η^2 = .05, SP = .31. Also, the interactions between Nutrient and Age, and Nutrient and Difficulty Level showed similar or higher effect sizes, F (3, 66) = 1.15, p = .34, η^2 = .05, SP = .30, and F (2, 52) = 1.53, p = .22, η^2 = .07, SP = .34, respectively. Thus, with a larger sample size (increased power), the main effect of Nutrient and the interaction effects outlined here may reach significance. Analysis of the ANOVA's calculated for the paragraph recall and word recall tasks, found no statistically significant effects (All *F*'s < 1).

Mood Analyses

The 16 Bond & Lader Visual Analogue items were combined, as recommended by the authors, to form the three factors of Alert, Contented, and Calm (Bond & Lader, 1974). A high score on these factors indicates a more depressed state of mood, for example, low scores on the alert factor would mean the participant was moderately to highly alert, whereas high scores would imply a state of drowsiness.

A 4 x 3 x 2 x 2 repeated-measures ANOVA was conducted to determine the effects of Nutrient (protein, glucose, fat, or placebo), type of Mood (alert, contented, or calm), Age (elderly or young), and Time (pre-nutrient or post-nutrient) on self-reported mood scores. Age was the between-groups variable, with Nutrient, Mood, and Time as the within-groups variables.

A significant main effect of Age was found, F (1, 22) = 9.27, p = .006, η^2 = .30, *SP* = .83. Analysis of the means revealed that (collapsed across Mood and Time) the elderly reported a more positive mood state (M = 24.94) than the young adults did (M = 38.41).

A significant main effect of Mood was also found, F (2, 38) = 16.97, p = .0001, η^2 = .44, *SP* = 1.00. Significant differences were found between the means for Alert and Contented (*M's* = 36.00 and 27.52, respectively, p = .0001), and for Alert and Calm (*M's* = 36.00 and 31.50, respectively, p = .005). The difference between Contented and Calm also resulted in a significant difference, (*M's* = 27.52 and 31.50, respectively, $p = .057$). Thus, overall, participants' reported feeling more contented than calm and, more calm than alert.

A significant interaction was also found between Nutrient, Mood, and Time, $F\ (5, 116) = 2.55$, $p = .029$, $\eta^2 = .10$, $SP = .79$. All possible two-way interactions of this relationship are illustrated in Figure 2.

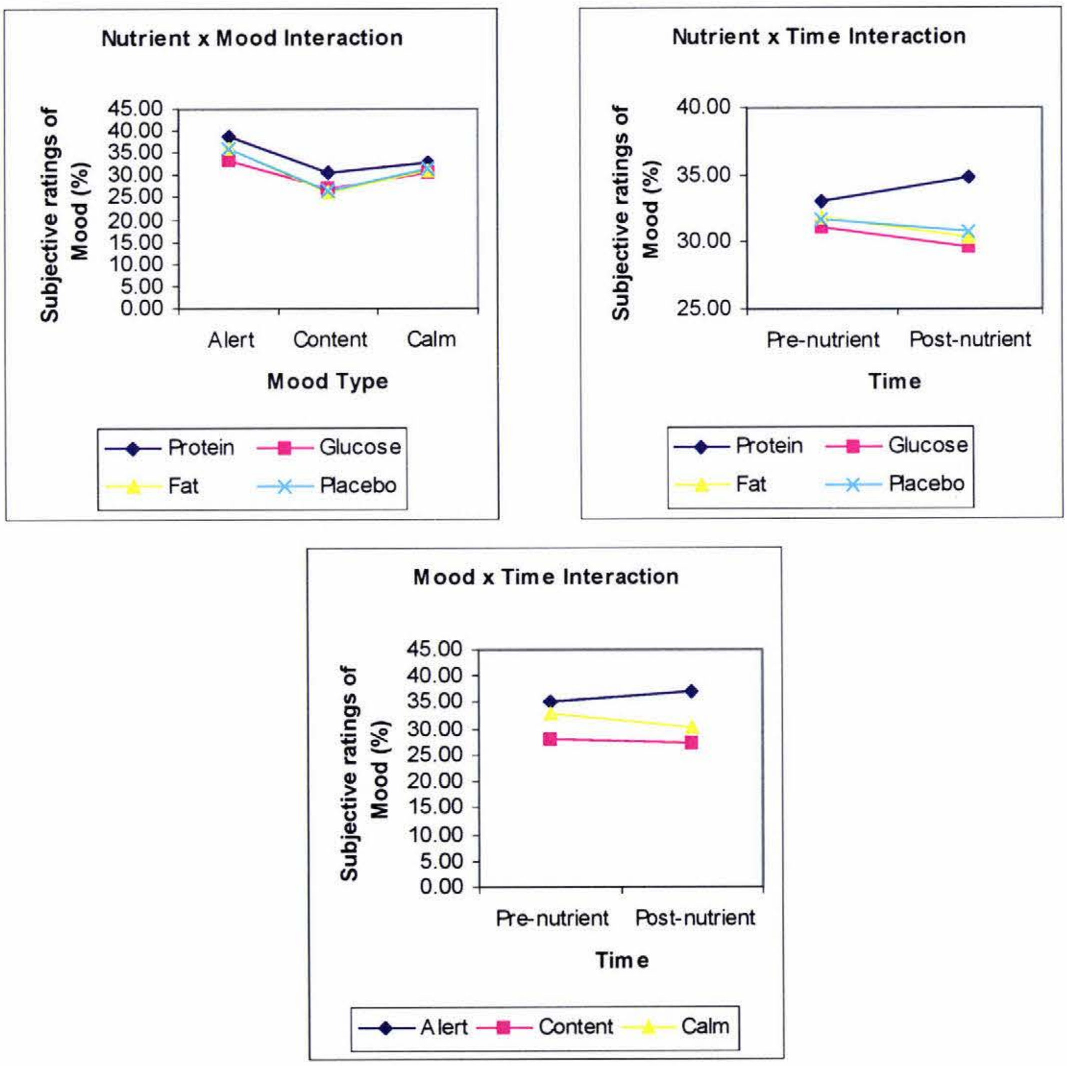

Figure 2. Interaction Effects between Nutrient and Mood, Nutrient and Time, and Mood and Time.

Speed –Accuracy Relationship on the Mental Arithmetic Task

The mental arithmetic task has two measures, accuracy and time. It is often the case that speed is traded against accuracy in the sense that participants may respond faster but only at the expense of accuracy. To check for a speed-accuracy trade-off for the mental arithmetic task, a scattergraph was constructed to show overall accuracy scores against the overall response time for each participant. It was expected that accuracy scores would increase as time to respond increased if a speed-accuracy trade-off effect was evident. Figure 3 shows that this was not the case. In general, the longer a participant took to answer, the more likely the response was to be *inaccurate*, $r = -.55$, $n = 24$, $p = .01$.

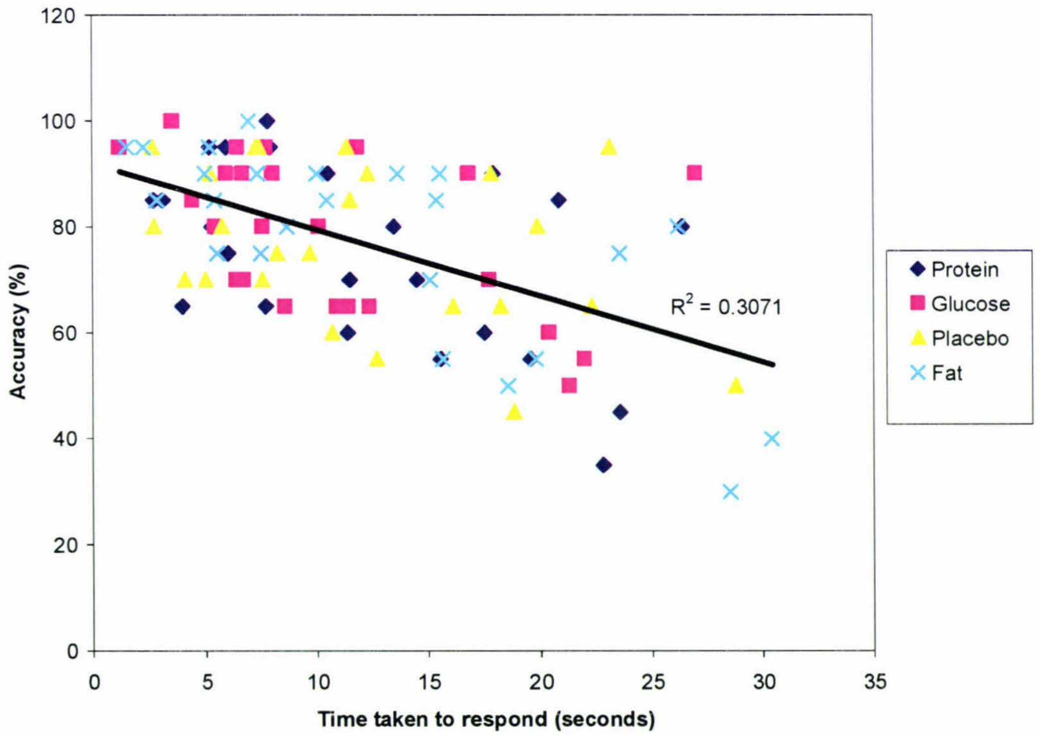

Figure 3. The correlation between accuracy and response time for the mental arithmetic task collapsed across Age and Difficulty Level.

The differences between the correlation coefficients for each pairing of nutrients, and for each nutrient compared to placebo was calculated through use of the procedure outlined in Pallant (2001). No significant differences for any of the nutrients was found between the correlation associated with the placebo drink, and the correlations associated with the nutrients. Also, no significant differences were found between any of the pairings of the nutrients. Thus, in the present study, the nutrients did not significantly differ from placebo, or from each other, in the effects they exerted on the speed-accuracy relationship.

DISCUSSION

The primary planned comparison of the present study was to determine the effects of pure glucose, protein, and fat drinks on paragraph recall, word recall, and mental arithmetic performance. On the basis of previous findings, it was expected that all the nutrients would exert enhancing effects on all the tasks. But this was not the case. There was no significant effect of Nutrient found on any of the tasks when the data collapsed across age of participant and task difficulty level. However, glucose ingestion effects on both speed and accuracy performance of the mental arithmetic task were associated with small effect sizes. Therefore, a much larger sample size may well have produced statistically significant results.

The second planned comparison of the present study investigated the effects of the nutrients when moderated by the age of the participant. It was expected that the elderly group's and young adult group's performance on the tasks would be enhanced by ingestion of all the nutrients. Again, there were no significant nutrient effects on task performance for either age group. However, small to moderate effect size statistics associated with some nutrients and tasks showed possible age-specific effects of nutrients on task performance. Glucose tended to enhance paragraph recall and speed of response of the mental arithmetic items for the young adult group. Glucose and fat tended to enhance mental arithmetic accuracy performance for the older adult group, with protein resulting in a deficit in performance for speed of response of the mental arithmetic items.

The third planned comparison investigated the cognitive effects of the nutrients on task difficulty. Based on previous research (Kennedy & Scholey, 2000; Korol & Gold, 1998), an enhancement of performance by glucose, protein, and fat was expected for the young adult participants, but only on the difficult version of each task. However, the expected Nutrient x Age x Difficulty Level interaction did not eventuate. The mental arithmetic accuracy function was associated with a small effect size that may have reached statistical significance had more power (a larger sample) been available.

Post-hoc comparisons showed a Nutrient x Age interaction for the mental arithmetic accuracy function. Interestingly, the protein drink produced significant deficits in performance for the elderly group. This result was unexpected as protein has, to date, only been associated with the enhancement of performance (Fischer et al., 2001; Kaplan et al., 2001). It must be remembered, however, that, to the author's knowledge, the effect of protein had not previously been examined with regard to a mental arithmetic task.

Post-hoc analyses also found a significant Nutrient x Mood x Time interaction. This showed that the participants were, on average, more alert after ingestion of the glucose drink and less alert after ingestion of the protein drink. Ratings of contentedness also decreased after ingestion of protein.

Nutrient

None of the three nutrients examined produced statistically significant changes in performance for any of the tasks used in the present study. Thus, there was a failure to replicate the findings of Kaplan et al. (2001), who found significant improvements on a paragraph recall task after ingesting glucose or fat, as well as a strong trend for improvement after protein ingestion. However, Kaplan et al. (2001) found these nutrients had no effect on word recall performance, a result that was replicated here. Finally, the mental arithmetic task also was not affected significantly by ingestion of glucose, protein, or fat. This result is inconsistent with previous research where enhancement of mental arithmetic performance after ingestion of glucose was found (Kennedy & Scholey, 2000).

Of special interest here is the lack of effect of the nutrients on the two declarative memory tasks, paragraph recall and word recall. The majority of studies that have looked at the effect of glucose on cognition have shown that glucose enhances declarative verbal memory performance (Bellisle, 2001; Benton et al., 1994; Benton et al., 1996; Craft et al., 1994; Hall et al., 1989; Kaplan et al., 2000, 2001; Korol & Gold, 1998; Parsons & Gold, 1992). Indeed, Craft et al. (1994) goes so far as to claim that glucose selectively influences only declarative memory tasks.

Glucose in the present study did tend to enhance performance for the paragraph recall task when the analysis collapsed across participant age and task difficulty level. Fat ingestion also tended to enhance the paragraph recall task, but only to a very small degree. Improvement of paragraph recall performance by glucose and fat were more strongly seen in Kaplan et al.'s (2001) study.

Glucose is believed to exert its effects primarily through the raising of peripheral blood glucose levels (Scholey et al., 2001). The inverted-U glucose response curve, seen over approximately a 2-hour period after ingestion of glucose, illustrates the rising of blood glucose levels to a peak, followed by their fall (Parsons & Gold, 1992). During periods of cognitive demand, such as periods of cognitive testing, the blood glucose levels have been observed to fall at a more rapid rate, which is hypothesised to be due to increased uptake of glucose from the blood by neural substrates (Scholey et al., 2001). The metabolism of glucose by the brain results in the synthesis of, among other things, acetyl-CoA, a precursor to acetylcholine (Ragozzino, 1996). Therefore, glucose is believed to exert its memory-enhancing effects by increasing the availability of acetyl-CoA, and subsequently, acetylcholine, a neurotransmitter known for its effects on memory. Thus, the increase and subsequent falls in blood glucose levels seen in both the elderly and the young age groups in the present study (see Figure 4) may reflect the mechanism outlined above, therefore resulting in a trend towards enhanced performance.

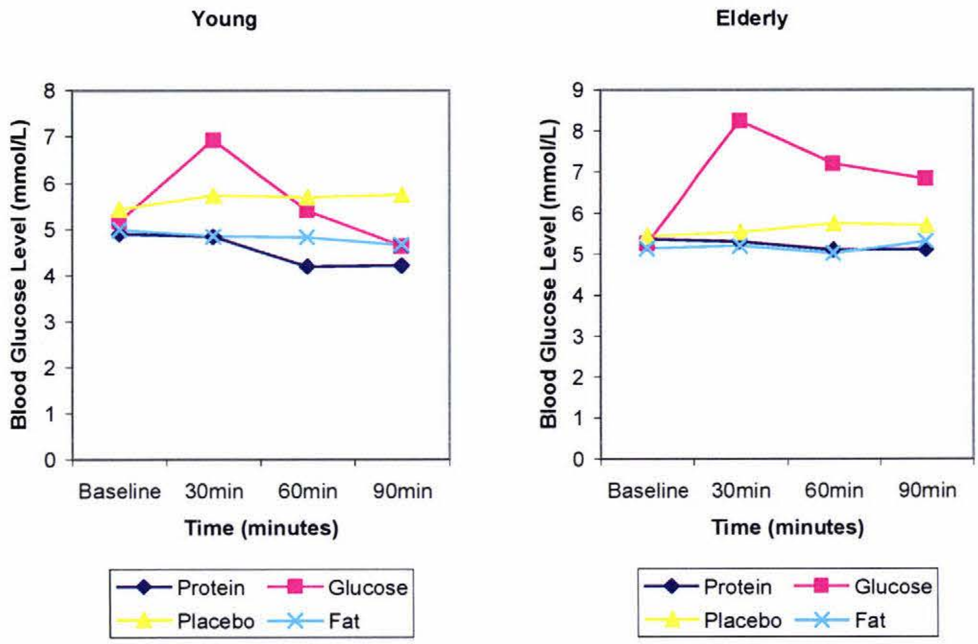

Figure 4. Comparison of the blood glucose levels for the elderly and the young adult age groups from baseline to 90 minutes after ingestion of each nutrient.

However, the increase in blood glucose levels seen in the present study (see Figure 4) did not result in a significant effect of glucose on performance of the paragraph recall task. This lack of a significant effect may have been due to a too small sample size, or differential effects in the different age groups that cancelled out any overall enhancement effect of glucose on this task. In later analyses with Age as a factor, it was seen that while glucose resulted in a large trend for enhancement in performance on the paragraph recall task for the young group, no difference from placebo was noted for the elderly group.

Therefore, when the analysis collapsed across age, the difference from the placebo seen with the young group was masked by the lack of an effect seen with the elderly.

Fat ingestion also tended to slightly improve performance on the paragraph recall task, collapsed across participant age and task difficulty. However, Kaplan et al. (2001) found that fat significantly improved performance on immediate recall (15 minutes after ingestion), and delayed recall (60 minutes after ingestion). The result of the present study suggests that the improvement attributed to fat is likely to be due to simple fluctuations in performance levels of participants over time. Kaplan et al.'s (2001) finding of a significant effect of fat on paragraph recall performance is surprising in light of the present study's results. Either result, of course, may be a chance effect. Further studies are needed on fat ingestion effects on paragraph recall performance before any conclusions on the type of effect attributed to this nutrient, if any, can be drawn.

Kaplan et al. (2001) proposed that the effect seen in their study on paragraph recall 15 minutes after ingestion of fat was due to the stimulation of the gut-brain axis. This hypothesis was brought about by the observation that protein and fat did not raise blood glucose levels as glucose ingestion did, and therefore, must elicit their effects through a different mechanism. That a different mechanism is possibly involved was further strengthened by the fact that full fat absorption cannot take place within 15 minutes, or indeed 60 minutes, after ingestion (Davenport, 1977). Thus, according to Kaplan et al. (2001), the energy of such nutrients, rather than blood glucose effects, was the important predictor of enhancement of cognitive performance. When a macronutrient is ingested, a specific pattern of hormones is released in the gut. These hormones then stimulate the vagus nerve (a cranial nerve that reaches from the gut to the brain), which is believed to result in enhancement of memory performance. CCK, a gut hormone known for its effects on satiety-mediating mechanisms in the hypothalamus via the vagus nerve (Flood et al., 1987), has been implicated in the enhancement of memory via this pathway. Flood et al. (1987) believe that the link between food ingestion and memory enhancement may be evolutionary in nature, as it allows organisms to remember successful hunts and techniques that resulted in consumption of food, which is, of course, necessary for survival. CCK has been noted to work as a neurotransmitter in the brain, and further evidence for its role with memory comes from findings that learning and memory are impaired in rats devoid of CCK-A receptors in the brain (Nomoto et al., 1999). Little is yet known of the specific mechanisms by which CCK or other gut hormones exert their effects. However, it has been observed that CCK activity is especially enhanced if the food ingested contains a high content of fat or protein (Rosenzweig et al., 1996). Thus, the initial ingestion of energy as fat or protein is hypothesised to result in activation of the gut-brain axis, which perhaps results in hormonal activated memory-enhancing effects in the brain.

Therefore, the lack of significant effects of protein or fat on cognitive performance in the present study may have been due to a lack of use of the gut-brain axis. Kaplan et al. (2001) hypothesized that when a nutrient is first ingested, hormone activity stimulates the vagus nerve, thus resulting in the significant effects of protein and fat seen in their study. However, when testing does not begin until 30 minutes after ingestion (as in the present study), it is speculated that the hormone activity and consequent stimulation of the vagus nerve may have already ceased, resulting in no energy for improvement of the cognitive tasks. Further research is greatly needed on the vagus nerve and its functions in respect to nutrient effects on cognition.

The lack of a significant effect of protein on the paragraph immediate recall task may also be due to a lack of an effect of this nutrient on this specific type of task. Kaplan et al. (2001) showed a significant enhancement effect of protein on paragraph delayed recall performance 60 minutes after ingestion. However, type of task is a confounding factor here. Immediate recall performance relies on working memory to store newly presented information while it is manipulated through the use of strategies (such as categorisation), so as to remember a large amount of the information more effectively (McNamara & Scott, 2001). Delayed recall performance, however, relies on information retrieval from long-term memory (Butters & Delis, 1995). Thus, protein may be task-specific in its effects, significantly enhancing delayed paragraph recall performance while having no effect on immediate paragraph recall performance. It is apparent that further studies in the area of protein and fat effects on cognition are needed, particularly those that take into account the possible task-specific effects, differential rates of absorption, and differential mechanisms of action that each nutrient may take. Furthermore, the present study suggests that the influence of nutrients on performance may be slight. Therefore, to detect any effects, attention must be paid to the amount of statistical power available. In general, the present study had very low levels of power.

While some studies have found word recall performance to be enhanced after glucose ingestion (Benton & Owens, 1993; Manning et al., 1990), others have found word recall performance to be unaffected by nutrient intake. Azari (1991) found no effect of glucose ingestion on performance of a word recall task. Kaplan et al. (2001) also found that neither glucose, protein, nor fat enhanced performance on a word recall task. In an earlier study, Kaplan et al. (2000) reported no effect of ingestion of three different types of carbohydrate on subsequent performance of a word recall task. Enhancement of word recall performance by Benton and Owens (1993) was merely a report of an association between rising blood glucose levels and increasing word recall scores. (Increasing blood glucose levels were noted in the present study but were not associated with improvements in word recall performance). The enhancement effect of glucose on word recall found by Manning et al. (1990) was based on performance of a selective-reminding word recall task, different from the task used in the current investigation. Considering that nutrients have been found to be task-specific in their effects, it is suggested that different types of word recall tasks may elicit different effects of glucose, protein, and fat. All the nutrients showed no difference from placebo in performance levels on the present study's word recall task. Therefore, the lack of effect found on the word recall task in the present study may be due simply to the failure of glucose, protein, and fat to exert an effect on this specific type of word recall task. The selective nature of nutrient ingestion effects on declarative memory tasks in the present study may be due to the difficulty of the task. The added complexity of the paragraph recall task (remembering by rote how a sentence was constructed, and the amount of information to remember in sequence [some of which was technical and unfamiliar]) elicited enhancement effects of nutrients, while the word recall task, perhaps was not complex enough for the nutrients to elicit any effects on performance. Further research is needed on the task difficulty levels required to produce an effect of nutrient ingestion on subsequent performance.

The mental arithmetic task of the present study also returned a non-significant effect of nutrient for both accuracy and time functions. Glucose, however, did tend to improve accuracy performance, while fat and protein showed no difference from placebo.

Glucose was associated with a small effect size on this function, suggesting that the effect of glucose on accuracy performance may reach significance with a larger sample size. Kennedy and Scholey's (2000) study with young adults found this same effect, with glucose ingestion being associated with a higher accuracy rate on a serial-seven's task. Glucose is believed to elicit its enhancing effects on the accuracy function of the mental arithmetic task primarily through the mechanism outlined above.

Fat and protein ingestion resulted in no difference from placebo on mental arithmetic accuracy performance. Fischer et al.'s (2001) study did find an improvement of accuracy performance after fat ingestion on a short-term memory task that consisted of remembering defined sequences of circles, and pressing a key if the sequence appeared on a computer screen. Protein was also found to improve performance on this task but to a lesser degree than that attributed to glucose or fat. While Fischer et al.'s (2001) study and the present study measured the same accuracy concept, the two tasks were different and the data were gathered from two distinct samples (Fischer et al. [2001] used young adult participants, while the present study used both young and older adults) and, therefore, cannot be directly compared. In the present study, fat and protein did not enhance or decrease accuracy performance relative to placebo for the mental arithmetic task. It must be remembered, however, that this is an analysis of the overall effects, collapsed over the age of participants and task difficulty level. Therefore, it would be hasty to claim no enhancement of effect of accuracy performance on this task by these nutrients without first investigating any age-specific effects of protein and fat. Indeed, small to moderate effect sizes were associated with mental arithmetic accuracy performance when the analysis was split by age. These results are discussed in a later section.

Analysis of the time to respond on the mental arithmetic task showed a trend of the glucose drink to shorten the time taken to respond in comparison to the placebo. Kennedy and Scholey (2000) found similar albeit statistically significant results to the present study. In their study, glucose was found to facilitate performance of a serial seven's task, where performance was measured as the number of responses made. Thus, glucose significantly enhanced the speed at which responses were made on the mental arithmetic task. Fischer et al. (2001) also found that glucose enhanced reaction time performance on a complex reaction time task. Possible mechanisms producing this effect include the raising of blood glucose levels (a mechanism outlined above), and also stimulation of the vagus nerve after ingestion of the nutrient. Further research is warranted on the effects of nutrients, specifically glucose, on enhancement of response speed.

The protein drink showed no difference from placebo in regard to performance on the mental arithmetic time function. Fischer et al. (2001) showed that protein improved reaction time performance, although to a lesser degree than that seen with glucose during the first hour of testing, but improved reaction-time performance over that of glucose during the second hour of testing. These authors therefore proposed that protein tends to elicit its most beneficial effects on reaction time one to two hours after ingestion. As performance of the mental arithmetic task in the current investigation always took place before 60 minutes after ingestion of the nutrient, it is possible that the protein nutrient did not have enough time to absorb or stimulate the vagus nerve sufficiently to exert an effect on response speed performance.

Fat ingestion also resulted in no difference from placebo for performance on the time function of the mental arithmetic task. Fat and protein have thus far never been investigated as to their effects on mental arithmetic performance. Thus, the lack of effect on mental arithmetic performance seen after fat and protein ingestion may be what is to be expected on a mental arithmetic task measured 30-60 minutes after ingestion. However, further research is needed before any firm conclusions can be drawn.

Age

No statistically significant differences in task performance scores were found for either age group when performance after placebo ingestion was compared with performance after ingestion of any of the nutrients. There are no previous studies that have looked at the effects of glucose, protein, and fat on identical cognitive tasks for both age groups. Thus, the results found here are preliminary and must be interpreted with caution. However, there were some interesting trends with regard to specific nutrients.

The elderly group showed a tendency (small effect size) for protein ingestion to produce deficits in performance on both mental arithmetic functions. The mental arithmetic time function for this age group was associated with a small effect size, suggesting that with a larger sample, protein ingestion may promote a significant deficit in speed of response. Comparison with the results found with the young adult group suggested an age-specific nutrient effect (as opposite trends on both these functions was found in the young adult group).

Interestingly, post-hoc comparisons showed a Nutrient x Age interaction effect, with the elderly participants performing at a deficit on the mental arithmetic accuracy task after consuming the protein drink. The fact that protein failed to improve the performance of the elderly may be due to the susceptibility of this age group to the effects of nutrients. While protein intake in this age group appears to be adequate, protein synthesis and degradation rates are associated with decreases in functioning with age (Ausman & Russell, 1991). An elderly person may also not be utilizing protein accurately (Berdanier, 2000). In other words, an elderly person may not only fail to synthesize all the protein ingested, but also may fail to make accurate use of the protein that is synthesized. When fasting occurs, as it did in the present study, the body's glucose stores are depleted, leading to the process of gluconeogenesis (Linder, 1991a). Gluconeogenesis takes place in the early stages of fasting, and is the process by which glucose is formed, especially by the liver, from non-carbohydrate sources, such as amino acids (which come from protein) and fats (Berdanier, 2000). Hence, when protein was ingested after an overnight fast, it may have been more easily synthesized, and finally utilized, by the young participants. Therefore, protein ingestion in the young age group lead to an ability to maintain a normal level of functioning (no difference from placebo). However, the elderly participants' protein synthesis and utilization abilities may have been impaired, leading to an inability to use the protein ingested either adequately, or indeed accurately, to produce glucose for use in the body and brain. Therefore, deficits in performance were seen on the mental arithmetic task.

Thus, protein is hypothesised to have age-specific and task-specific effects. Instead of the overall enhancement effects expected of each nutrient on each task for each age group, protein showed a tendency to elicit deficits in performance, but only for the elderly and only on the functions of the mental arithmetic task. This area needs further research to determine which nutrients enhance or worsen performance for which tasks and for which age groups.

Since both fat and glucose metabolism are not generally found to be affected by age, it is suggested that these nutrients did not result in a Nutrient x Age interaction effect because both age group's metabolisms were able to absorb and utilize the nutrient in a similar way.

Glucose was associated with a small effect size for paragraph recall performance for the young adult group. Previous studies have found glucose ingestion to enhance paragraph recall performance in both young and older adults (Craft et al., 1994; Hall et al., 1989;

Kaplan et al., 2000, 2001; Korol & Gold, 1998; Manning et al., 1990). The age-specific improvement effect seen in the present study may have reflected the ability of the young group to utilize glucose more efficiently than the elderly group. An analysis by CFR found that a subgroup of the elderly sample in the present study were deficient in their ability to metabolise glucose. This inability is illustrated in Figure 4, where the elderly group's blood glucose levels are still high 90 minutes after ingestion, when they should have returned to baseline, as with the young adult group. The consistent high levels of blood glucose can be interpreted to mean that less glucose is being absorbed from the blood, and therefore, made available to the brain. The consequent deficit in performance due to a lack in energy for some elderly participants would therefore result in lower overall average scores for this age group.

Trends in the data for the elderly group also showed that performance on the mental arithmetic accuracy function was improved by glucose. This function was associated with a small effect size. The young adult group also showed tendencies for improvement on the mental arithmetic time task after ingestion of glucose, with a moderate effect size associated with the time function. Thus, an examination of effect sizes suggested that glucose tended to enhance mental arithmetic performance differentially across both age groups. Kennedy and Scholey's (2000) study with young adults found that glucose ingestion produced faster response times and also higher (although nonsignificant) accuracy rates than that seen in the placebo condition, thus ruling out a speed-accuracy trade-off effect. The present investigation also found no evidence of a speed-accuracy trade-off effect. The glucose-enhancing trend for speed of response performance on a mental arithmetic task for young adults is replicated in the present study. Further research is greatly needed to clarify why glucose has a selective age-specific enhancement effect for response speed with young adults, and for accuracy for older adults, within the mental arithmetic task.

Fat ingestion also showed a tendency for improved scores on the mental arithmetic accuracy function for the elderly age group. The mental arithmetic accuracy task was associated with a small-to-moderate effect size. Fat and protein have never been investigated as to their effects on mental arithmetic performance; therefore, these present results can only be preliminary. Nevertheless, it is interesting to note that while in the overall analysis fat and protein exerted no effect on mental arithmetic accuracy or time performance, when the analysis is split by age group, differential effects associated with small to moderate effect sizes occur. For example, protein showed no effect on accuracy or time performance when analysed across Age and Difficulty Level; however, a moderate interaction effect of protein and the elderly age group was found in the post-hoc analyses, and a small effect size was associated with the time function after protein ingestion in the elderly. While it can be hypothesised that an age-specific effect is likely for the elderly on cognitive tasks after ingestion of protein (due to their inability to utilise it adequately or completely) (Linder, 1991a), it is unknown why age-specific effects of glucose and fat should occur.

Finally, word recall performance showed no difference from placebo for either age group after glucose, protein, or fat ingestion. This result strengthens the hypothesis that glucose, protein, and fat have no effect on this type of word recall task (Kaplan et al., 2001).

Task Difficulty

The third planned comparison investigated whether a Nutrient x Age x Difficulty Level interaction was apparent for all three tasks. Glucose, protein, and fat were expected to improve performance for the young participants, specifically on the more difficult versions of each task. However, no such significant Nutrient x Age x Difficulty Level interaction was found on any of the tasks.

Trends in the data for the young age group showed that glucose ingestion enhanced performance on the difficult version of the paragraph recall task, and resulted in a small deficit in performance on the difficult version of the word recall task. Furthermore, glucose ingestion resulted in improved speed of response with regard to the hard items of the mental arithmetic task.

Glucose ingestion has previously been shown in young participants to result in performance improvements on difficult paragraph recall tasks (Korol & Gold, 1998). The trend in the present study's data suggests that with a larger sample size, a statistically significant glucose enhancement effect on difficult paragraph recall performance for this age group may have appeared. The finding that the magnitude of the enhancement effect was not as strong for young participants on the easy version of this task strengthens the hypothesis that tasks of sufficient difficulty are needed for glucose effects on young adults to be seen.

Glucose showed a tendency to enhance performance of the time function of the difficult version of the mental arithmetic task. Kennedy and Scholey (2000) have previously found this effect with glucose. In their study, glucose significantly enhanced the performance of a serial seven's task when analysed as the number of responses made. The enhancement effect of glucose seen on the tasks (with the exception of word recall) as well as the rise and fall of blood glucose levels illustrated in Figure 4 for the young age group, suggests that glucose may be acting through its primary mechanism of raising blood glucose levels (outlined above) to enhance memory and mental arithmetic performance on the more difficult versions of each task. Of course, the small trends in the present study are not strong enough to draw any firm conclusions. However, taken together with previous findings, they strengthen the possibility that glucose improves cognitive performance.

The trend after glucose ingestion for a deficit in performance on the difficult version of the word recall task could be a chance result. The three nutrients differed from the placebo in word recall performance scores by only 1-2% for the easy version. The protein and fat nutrients also showed a minimal difference in performance scores from the placebo condition for the difficult version. The deficit in performance after glucose ingestion for the difficult version is inconsistent with previous findings that have shown that glucose enhances performance for young adults on only those tasks of sufficient difficulty (Craft et al., 1994; Korol & Gold, 1998). It is also worth noting that the present study's results point towards a lack of an effect of any of the three nutrients on this type of word recall task.

Fat ingestion in the young participants led to a deficit in performance on the hard version of the mental arithmetic task for the accuracy function. The deficit in performance for the accuracy function was large. When the mental arithmetic task was easy, however, there was no difference from placebo in performance levels after fat ingestion for this age group. It is unknown why fat ingestion resulted in such a large deficit in performance, relative to placebo. It can be noted that this result is inconsistent with the overall pattern of results. Thus, it cannot be ruled out that the deficit observed was a chance effect.

The protein drink resulted in no difference from performance at baseline levels for either of the hard versions of the mental arithmetic functions, or the paragraph recall task, for the young adult age group. The lack of effect of protein on performance of the hard versions of the tasks is also unexplained. The hypothesis that all nutrients would exert enhancing effects on all tasks was based on the finding that glucose (another macronutrient) enhanced performance for young adults only on the difficult versions of cognitive tasks. Thus, while a general finding of improvement in scores for the hard versions of tasks was found after glucose ingestion, this same nutrient-task difficulty level relationship may not hold true for fat or protein. The interaction between nutrient and task difficulty level needs extensive further research before the relationship seen between glucose and task difficulty can be said to exist for fat or protein.

Glucose was the only nutrient to show a consistent pattern of enhancement across most of the tasks for the hard versions and young age group. In other words, from the overall pattern shown by the data, glucose is the only nutrient where a possible Nutrient x Age x Difficulty Level interaction might eventually be shown to exist for the paragraph recall and mental arithmetic functions, provided there is enough statistical power to detect the effect. Given the small effect sizes and low statistical power levels, it is clear that 12 participants per age group was too small a sample to detect a significant effect of the nutrients on this age group and task difficulty level.

Post-Hoc Analysis of Nutrient Effects on Mood

Analyses of the effect of Nutrient on Mood resulted in a significant interaction for Nutrient, Mood, and Time. This showed that the participants were moderately more alert after ingestion of the glucose drink, and less alert than average after ingestion of the protein drink at the end of each session. Ratings of contentedness also decreased after ingestion of protein.

The effects on mood reported here are the opposite of those seen in previous studies. Lieberman et al. (1986) claim that protein ingestion will result in an alert and tense state, due to its simultaneous elevating effects on catecholamine synthesis and decreasing effects on brain serotonin levels. However, on average, participants in the present study, after ingesting protein, reported themselves to be less alert at the end of the session in comparison to how they felt after the other drinks. Contentedness ratings also dropped after ingestion of this nutrient. One possible explanation for these findings is that the protein in the present study may not have enhanced concentrations of tyrosine (a precursor to the catecholamine neurotransmitters) as it was expected to do. Tryptophan, a precursor to serotonin, is more sensitive than tyrosine, under certain circumstances, to changes in levels of protein (K. Silvers, personal communication, November 29, 2002). Tryptophan has been found to increase subjective ratings of fatigue (Lieberman et al., 1983). Thus, it appears that protein ingestion may have led to increases in levels of tryptophan in the brain, thereby decreasing alertness. However, it is acknowledged that such an explanation is purely speculative. Research is required that directly examines such explanations by measuring changes in neurotransmitter levels or their metabolites in the presence of nutrients.

Previous studies have reported that carbohydrate ingestion results in drowsiness, uncertainty, and decreased feelings of vigour (Dye et al., 2000; Lieberman et al., 1983). In the present study glucose was found to have the opposite effect on mood. Participants reported feeling more alert at the end of the session where they had ingested glucose. A possible explanation for this inconsistency is that much of the research to date has focused on the effects of high carbohydrate meals, which have low levels of other nutrients, such as fat or protein. The combination of nutrients to determine the effect of one on state-of-mood confounds the results. For example, Wells and Read (1996) reported that high-carbohydrate, low-fat lunches resulted in feelings of lethargy. However, higher feelings of lethargy were reported after the high-fat, low-carbohydrate lunch. It is impossible to detect how much of the lethargic mood-state was attributable to carbohydrate. Thus, it is suggested that pure nutrients may elicit effects on mood-state different to those seen with mixed-nutrient meals.

Limitations of the Present Study and Suggested Further Research

In hindsight, one major limitation of the present study was the sample size used. Previous studies, including the two studies that recently found significant effects of glucose, protein, and fat on aspects of cognitive function, used *smaller* sample sizes than the present study. Kaplan et al. (2001) based their research on 22 elderly men and women, while Fischer et al. (2001) based their study on results obtained from 17 young adult men. The number of participants used in the present study was guided by these investigations. However, it is apparent that more statistical power was needed to find significant effects of nutrients on the cognitive tasks used in the present study, especially when the sample was split to analyse nutrient effects on performance of different age groups. Further research will need to evaluate effect sizes for nutrient effects on tasks from previous studies, and carefully assess the sample size needed to detect an effect. However, the present investigation shows that it may not be possible (especially within the time constraints of a Masters thesis) to run enough participants to produce enough power to obtain statistically significant results. For example, for a small effect size, a *t*-test having 80% power to detect the effect at the .05 significance level requires well in excess of 200 participants per group (Cohen, 1988).

Another limitation of the current investigation regarded the equivalency of the four test versions of each task. Paired-samples *t*-tests with a Bonferroni correction set at $p = .008$ ($p = .05 / 6$) revealed that version two of the paragraph recall task was significantly different from versions one, three, and four, t (23) = 8.43, $p = .0001$, t (23) = -2.91, $p = .008$, t (23) = -8.89, $p = .0001$, respectively. Overall, performance on version two ($M = 39.20$) returned a mean score that was significantly lower than the mean scores found with the other versions. Version three was also found to differ significantly from versions one and four, t (23) = 4.53, $p = .0001$, t (23) = -4.38, $p = .0001$, respectively. Performance on version three ($M = 44.49$) returned a mean score that was significantly lower than those found with versions one ($M = 53.43$), and four ($M = 55.76$). The paragraph recall task may be confounded with the factor of type of task, as versions two and three are significantly different in some way from versions one and four. Therefore, the confounding effect of type of task may explain the inability of the present study to replicate the significant effects of Nutrient found by Kaplan et al. (2001) on the paragraph recall task.

Version one and version four of the word recall task also produced different means, t (23) = -2.98, $p = .007$. However, due to the large number of *t*-tests that were conducted, and due to the fact that this result was the only one of all the word recall tasks to return a significant difference, it may be that this significant finding was due to chance. With regard to the mental arithmetic task, no significant differences were found between the four versions.

Another limitation of the present study was the inability to include the covariate of glucose tolerance in the analysis. This was due largely to the time constraints imposed on the present research. However, previous studies have shown that glucose tolerance is an important covariate with respect to subsequent performance on cognitive tasks after glucose ingestion (Benton et al., 1994; Craft et al., 1994; Craft et al., 1992; Hall et al., 1989; Kaplan et al., 2000, 2001).

Further research is needed on the effects of nutrients on specific components of tasks. The many inconsistencies in the literature can be partly explained by the lack of clear guidelines as to what constitutes certain tasks. For example, Lezak (1995) states that mental arithmetic is made up of many differing components of memory and executive functions. Nutrients may act on certain components of tasks in different manners. Therefore, tasks must be examined and analysed as to the components that make up the overall performance if the effect of nutrients on aspects of cognition is to ever be fully understood. Indeed, a good argument could be made for producing a well-standardised and validated set of tasks for use in research such as the present study.

The type of a certain task also needs to be taken into account in future research. Whether an effect of a nutrient is found on a mental arithmetic task may depend on the type of mental arithmetic task being used. For example, Kennedy & Scholey (2000) found a significant effect of glucose on the enhancement of serial seven's performance. This task consists of counting backwards from a predesignated number in lots of seven. This result, however, does not necessarily carry over to other forms of mental arithmetic. The verification and production tasks used by Verhaeghen et al. (1997), while not examined for effect of nutrient, may be types of mental arithmetic tasks worth examining in the future. The verification task consists of viewing a calculation followed by the viewing of a proposed answer. The participant is then required to say whether the answer is true or false. A production task, on the other hand, requires the participant to work out the answer themselves from either the viewing or the hearing of the calculation. A form of the production task was used in the present study, where no effect of nutrient was found, but interesting trends for glucose and fat did emerge. Future studies need to place more emphasis on the task-specific nature of nutrient enhancement, and to design the studies to capture the specific aspects of a task that may be affected by the nutrient in question.

The task difficulty element of the present study made use of two distinct difficulty levels, easy and hard. Some earlier studies that have looked at difficulty effects also seem to have used the easy/hard distinction (Fischer et al., 2001; Kennedy & Scholey, 2000; Korol & Gold, 1998). However, introducing a medium level of difficulty would allow examination of particular nutrients at various levels of mental demand, rather than extremes. Cognitive demand has been shown to follow an inverted-U response curve (Scholey, 2001). Thus, participants are theorised to respond to cognitive demand increasingly well until optimal performance is reached, upon which any larger amount of cognitive demand will result in decrements in performance levels. Future studies that looked at medium levels of difficulty could ascertain not only general optimal levels of cognitive demand, before performance drops, but also the effects of nutrient-specific changes on the response curve. Also, by investigating effects of nutrients on medium difficulty tasks, nutrients that are specific to enhancement of low to medium cognitive demand tasks may become apparent.

One benefit of the current investigation was its use of within-task difficulty. Previous studies by Kennedy and Scholey (2000), and Scholey et al. (2001), have used between-task difficulty levels to examine the effects of glucose on performance. While their easy and hard tasks were similar (serial three's and serial seven's respectively), the medium difficulty level task was a word retrieval task. This introduced the confounding variable of type of task. Future studies using only within-task difficulty are necessary to obtain an accurate picture of the effects of nutrients on varying levels of cognitive demand.

A further benefit of the present study was the use of both young and older adults in the sample. To date, either young adults or older adults have been used in samples to determine the effects of glucose, protein, and fat on cognitive performance (Fischer et al., 2001; Kaplan et al., 2001). Both age groups in the sample of the present study allowed the direct comparison of test results across age groups. There was no confounding of type of task, type of nutrient, or differences in experimental conditions. Further studies using both age groups are needed to determine accurate age group differences. In addition, the use of different age groups in one study allows the investigation of nutrient-specific effects on age.

Summary and Conclusions

The present study investigated the effects of glucose, protein, and fat drinks on cognitive performance, specifically, paragraph recall, word recall, and mental arithmetic abilities. Age of the participant, as well as the difficulty level of the task were analyzed to determine if these factors influenced the effect of the nutrient.

The present study was unable to replicate the finding that glucose, protein, and fat enhances paragraph recall performance as found by Kaplan et al. (2001). However, the paragraph recall task used in the current investigation may have been confounded by differences in type of task, due to the significant differences in average scores found on two of the versions. However, overall trends in the data did show that glucose and fat tended to improve performance on this task, a finding in line with the effects shown by Kaplan et al. (2001).

A non-significant effect of nutrient on the word recall task replicated the findings of Kaplan et al. (2001). Overall, the trend for no effect relative to placebo by all of the nutrients on this task suggests that glucose, protein, and fat may not exert an effect on this particular type of word recall task.

While there was a non-significant effect of nutrient for the mental arithmetic time function, a moderate effect size associated with response time after glucose ingestion for the young age group was apparent. With a larger sample size a significant result may have been obtained. Nonetheless, the trend found is consistent with the previous findings of glucose enhancement of mental arithmetic response time found by Kennedy and Scholey (2000), and Scholey et al. (2001).

Glucose and fat also tended towards improving performance on the mental arithmetic accuracy task for the elderly. However, it must be remembered that these conclusions are drawn from non-significant trends (small-to-moderate effect sizes) in the data, and therefore are only preliminary.

Protein ingestion resulted in deficits in performance (relative to placebo) for the elderly on both components of the mental arithmetic task, with the opposite effect seen for the young adult group. Post-hoc analyses showed a moderate Nutrient x Age interaction on the mental arithmetic accuracy task. Here, protein ingestion resulted in a significant deficit in performance on the mental arithmetic accuracy function specifically for the elderly age group. This result was explained through the finding that, with age, loss of efficient and accurate metabolism of protein occurs (Linder, 1991a). The young adult group's ability to synthesize protein accurately and adequately was believed to reflect itself through the maintenance of normal performance seen with the mental arithmetic task for this age group.

Finally, analyses of task difficulty did not result in a significant Nutrient x Age x Difficulty Level interaction for any of the tasks. This interaction was expected to show that glucose, protein, and fat would enhance the young age group's performance, but only for the difficult version of each task. The present study's data did show a trend towards enhancement of the hard version of each task after ingestion of glucose for the young participants. Therefore, perhaps with a larger sample, a significant 3-way interaction may be observed. However, fat and protein failed to show similar trends. Thus, it is proposed that fat and protein may not be as sensitive to changes in task difficulty level as glucose has been found to be. However, these results are preliminary; further research is necessary.

The present study is the first to integrate elements of within-task difficulty, non-memory task performance, and age into an investigation of the effects of glucose, protein, and fat. An interesting finding was the observation of small task-specific and age-specific effects of the nutrients. Another interesting finding was the significant Nutrient x Mood x Time interaction with the post-hoc mood analyses. This interaction showed that protein and glucose had opposite effects on mood than those found in previous studies. Further research focusing on the effects of pure nutrients on self-reported mood-state is needed to clarify these results.

In conclusion, the presence of significant enhancement effects of glucose, protein, and fat on paragraph recall, word recall, or mental arithmetic has not been confirmed by this study. However, the deficit found in mental arithmetic accuracy performance for the elderly after ingestion of protein, the small effect size associated with paragraph recall performance of the young age group after ingestion of glucose, and the small to moderate effect sizes associated with mental arithmetic performance after glucose and fat ingestion (seen for different functions within each age group), suggest that significant nutrient effects are likely, given a larger sample size. This study questions the conclusions drawn by Craft et. al., (1994) who claimed that only declarative verbal memory tasks could be enhanced by glucose. However, it must be remembered that few of the present results reached significance and are only preliminary in nature. Further research needs to focus on the possible interactions between nutrient and participant age, as well as interactions between nutrient type, and the type of task. Of particular importance, more informed knowledge of the components that make up those tasks is needed if the effects of nutrients on aspects of cognitive performance are to ever be fully understood.

As a final comment, this study made use of a planned comparisons approach with which to analyze the data. This approach investigated the specific questions posed by the study, rather than simply interpreting any result that happened to come out statistically significant. Furthermore, an attempt was made to interpret the data both in terms of the significance level and the effect sizes associated with each calculation (variance accounted for was used as the estimate). A number of small and medium effect sizes, coupled with very low power, suggests that more statistically significant results would have been obtained with a larger sample. However, for small effect sizes, it may not be practical to run the very large number of participants required to produce statistically significant results. An acceptable alternative would be to run a series of underpowered studies whose results must then be combined (for example, through meta-analysis) to reach an overall conclusion.

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APPENDICES

Appendix A
Bond & Lader Visual Analogue Scale

Bond & Lader

1. *Please rate the way you feel in terms of the dimensions given below.*
2. *Regard the line as representing the full range of each dimension.*
3. *Rate your feelings as they are at the moment.*
4. *Mark clearly and perpendicularly across each line.*

Example:

Happy ____ **Sad**

Alert	________________________	Drowsy
Calm	________________________	Excited
Strong	________________________	Feeble
Muzzy	________________________	Clear-headed
Well-Co-ordinated	________________________	Clumsy
Lethargic	________________________	Energetic
Contented	________________________	Discontented
Troubled	________________________	Tranquil
Mentally slow	________________________	Quick-witted
Tense	________________________	Relaxed
Attentive	________________________	Dreamy
Incompetent	________________________	Proficient
Happy	________________________	Sad
Antagonistic	________________________	Amicable
Interested	________________________	Bored
Withdrawn	________________________	Gregarious

Appendix B

Examples of Cognitive Tasks: Paragraph Recall, Word Recall, and Mental Arithmetic

PARAGRAPH RECALL

Paragraph Recall (Easy Version):

Anna Thompson of South Boston, employed temporarily as a cook in a school cafeteria, reported at the police station that she had been held up the night before, and robbed of fifty-six dollars. The rent was due, she had not eaten for two days, and she was soon to lose her job. The police, touched by the woman's story, took up a collection for her.

This is a slight modification of Story 1 of the Wechsler Memory Scale-III (Wechsler, 1997). The parallel versions were developed through changing the names, occupations, places, and general context in the above story. The structure however, remained the same.

Paragraph Recall (Hard Version):

Modern aerial fireworks displays use computer-controlled electronic ignitions, and precisely timed fuses, to synchronise the bursts to the pulse of music. The fiery five-pointed star is a firework that erupts into a heart or peace symbol with a bang. Chemical pellets called stars, which are packed into a plastic form filled with blast powder, explode out of a spherical shell. Sawdust and rice hulls fill the rest of the shell and hold the assembly in shape on its journey.

This paragraph was derived from the Working Knowledge section of Scientific American Magazine, Zambelli, (1999). The parallel versions were also derived from the Working Knowledge sections of Scientific American Magazine. The extracts used were by Pohlmann, (1998), Poynter, (1998), and Bloomfield, (2000) respectively.

WORD RECALL

Grammar, Fulfil, Aspect, Agree, Statement, Marry, Decree, Hurry, Whisper, Detect, Suppose, Behalf, Exam, Honour, Ocean, Limit, Hero, Highlight, Increase, Glory, Agent, Govern, Dining, Perform, Handle, Going, Reading, Logic, Hazard, Decrease.

Each parallel form followed this same structure; a two-syllable verb followed by a two-syllable noun and so on.

MENTAL ARITHMETIC

1. $25.50 was paid into an empty bank account. The next day $11.25 was withdrawn from the account. What is left in the account?

2. Chewing gum costs 55c per packet. How much would it cost to buy six packets?

3. A family drove 215 kilometres in five hours. What was their average speed in kilometres per hour?

4. A family bought some second-hand furniture for two-thirds of what it cost new. They paid 400 dollars for it. How much did it cost new?

5. If 8 machines are needed to finish a job in 6 days, how many machines would be needed to finish the job in one-half day?

6. Linda had eight yellow paper clips, five green paper clips, and seven orange paper clips. She picked out one paper clip without looking. What was her chance of picking out a green paper clip?

7. The price of shirts is two for 31 dollars. What is the price of one-dozen shirts?

8. What is the cost of six cans of fruit juice at $1.30 each?

9. If you buy seven 20c mints and give the shop assistant five dollars, how much change should you get back?

10. Soft drinks are sold 12 cans to a package. If you want 96 cans, how many packages must you buy?

11. John bought six pieces of chocolate for $1.60. An additional 20c sales tax was added to this price. How much did he pay for each chocolate including sales tax?

12. Chris has two times as much money as Robert. Chris has 99 dollars. How much money does Robert have?

13. How much is $4.35 and $5.65?

14. How many hours will it take a person to walk 24 kilometres at the rate of three kilometres an hour?

15. What is the average of these numbers: 10, 5, and 15?

16. If you buy $5.40 worth of petrol and pay for it with a $20 note, how much change should you get back?

17. If Peter was born on the 1st of January, 1903, how old would he be on the 1st of January, 1978?

18. If you have 18 dollars and spend seven dollars and 50 cents, how much will you have left?

19. A merry-go-round makes four complete circuits in one minute. How long will Joanna have to stay on in order to go around 32 times?

20. A coat that normally sells for 60 dollars is reduced by 15 percent during a sale. What is the price of the coat during the sale?

Each parallel version of the mental arithmetic task was formed through changing the numbers and context of each item. The structure, however, remained the same. These items were based on the mental arithmetic subtask of the WAIS –III (Wechsler, 1997a).

Appendix C
Information Sheet

The relationship between nutrient intake and cognition

INFORMATION SHEET

We are Niki Culligan and Charlotte Geluk, both postgraduate students, supervised by Associate Professor John Podd of the School of Psychology, Massey University, and Drs. Karen Silvers and John Monro of the New Zealand Institute of Crop & Food Research Limited. We are both completing the thesis requirements of our Masters Degrees.

We are trying to find out how diet effects how well people can do things like problem-solving, maths, and remembering information. We are looking for healthy people between the ages of 18-28, and 70-80, with English as their first language, and who are available for the duration of the study, about six weeks.

During the study we will ask you to come in on five different mornings, separated by at least three days, but preferably each week. Every session takes place in the morning after an overnight fast. You will be asked to eat and sleep as usual on the nights before each session, but to have no breakfast. The first session will take about half an hour where blood glucose will be measured by finger prick. We will also ask you to weigh yourself (fully clothed) and complete a couple of simple questionnaires, along with training versions of the tests that will be used throughout the rest of the study.

The other four sessions will each take approximately two hours where we will ask you to partake of a pleasantly flavoured drink containing carbohydrate, protein, fat, or a placebo. Then, over the next two hours we'll take four blood samples and get you to complete a questionnaire and three tests to measure memory and reasoning abilities. You will be offered a continental breakfast at the end of each session. We will pick you up and take you to the lab, and return you to wherever you want to go afterwards.

The outcomes of the research, which will be published in completed theses, reports, publications, and seminars, will reflect only general trends, not personally identifiable information. Your anonymity will also be protected during the study through use of a code unique to you. Information linking the codes with your name will only be available to the senior researchers and will be kept in locked storage. All information will be treated with absolute confidence and will be maintained for at least five years after publication. After this time it will be destroyed or dealt with as specified by you. If requested, a copy of your personal information can be sent to you at any time.

You have already spoken to us by phone. If you now want to go ahead (after reading this Information Sheet), please return the questionnaire in the enclosed envelope. We will make contact with you to sign a Consent Form prior to the beginning of the first session and schedule times for the rest of the study.

It is important that you know that you have the right:

- *To decline to participate;*
- *To refuse to answer any particular questions;*
- *To withdraw from the study at any time;*
- *To ask questions about the study at any time during participation;*
- *To provide information on the understanding that your name will not be used unless you give permission to the researcher;*
- *To be given access to a summary of the findings of the study when it is concluded.*

Researchers:
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Crop & Food Research
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Senior Researchers:
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Karen Silvers and John Monro
Nutrition and Health Scientists
Crop & Food Research
Palmerston North: (06) 351 7066 ext 6150

This project has been reviewed and approved by the Massey University Human Ethics Committee, PN Protocol 02/31.
If you have any concerns about the conduct of this research, please contact:
Professor Sylvia Rumball, Chair, Massey University Regional Human Ethics Committee, Palmerston North, Telephone: (06) 350 5249, E-mail: S.V.Rumball@massey.ac.nz

Appendix D
Consent Form

The relationship between nutrient intake and cognition

CONSENT FORM

I have read the Information Sheet and have had the details of the study explained to me. My questions have been answered to my satisfaction, and I understand that I may ask further questions at any time.

I understand I have the right to withdraw from the study at any time, and to decline to answer any particular questions.

I agree to provide information to the researcher on the understanding that my name will not be used without my permission. *(The information will be used only for this research, and publications arising from this research project).*

I agree to provide samples of blood for a range of hormone analyses on the understanding that I have access to my results, and that I be advised if any abnormalities are found.

At the end of the study I would like my blood samples to be:

- ❑ *Destroyed*
- ❑ *Returned*
- ❑ *Handled alternatively, namely:……………………………………………………………..*

I understand that I may request any personal information held about me at the end of the study.

I do/do not wish to be provided with a summary sheet of the general outcomes of the study.

I agree/do not agree to my responses to the cognitive tests being audio taped.

I also understand that I have the right to ask for the audiotape to be turned off at any time during the recording of my responses to the cognitive tests.

I agree to participate in this study under the conditions set out in the Information Sheet.

Signed: ……………………………………………………

Name: ……………………………………………………

Date: ……………………………………………………

Appendix E

ANOVA Tables for the Paragraph Recall Task, Word Recall Task, and Mental Arithmetic Task

ANOVA Tables for the Paragraph Recall Task

Test of Within-Subjects Effects

Source	Sum of Squares	*df*	Mean Square	*F*	Sig.	Eta Squared	Power
NUTRIENT	190.08	3	63.36	0.28	0.84	0.01	0.1
NUTRIENT*AGE	234.44	3	78.15	0.35	0.79	0.02	0.12
NUTRIENT*DIFFICULTY	188.12	3	62.71	0.54	0.66	0.02	0.16
NUTRIENT*DIFFICULTY*AGE	171.61	3	57.2	0.49	0.69	0.02	0.15
Error (NUTRIENT)	14766.79	66	223.74				
Error (NUTRIENT*DIFFICULTY)	7661.57	66	116.08				

Test of Between-Subjects Effects

Source	Sum of Squares	*df*	Mean Square	*F*	Sig.	Eta Squared	Power
AGE	10220.97	1	10220.97	11.02	0.003	0.33	0.89
Error (AGE)	20413.12	22	927.87				

ANOVA Tables for the Word Recall Task

Test of Within-Subjects Effects

Source	Sum of Squares	*df*	Mean Square	*F*	Sig.	Eta Squared	Power
NUTRIENT	6.02	3	2.01	0.03	0.99	0.001	0.06
NUTRIENT*AGE	20.84	3	6.95	0.11	0.95	0.01	0.07
NUTRIENT*DIFFICULTY	34.49	3	11.50	0.16	0.92	0.007	0.08
NUTRIENT*DIFFICULTY*AGE	129.86	3	43.29	0.60	0.62	0.03	0.17
Error (NUTRIENT)	4084.27	66	61.92				
Error (NUTRIENT*DIFFICULTY)	4791.16	66	72.59				

Test of Between-Subjects Effects

Source	Sum of Squares	*df*	Mean Square	*F*	Sig.	Eta Squared	Power
AGE	20833.38	1	20833.38	20.41	0.000	0.48	0.99
Error (AGE)	22454.57	22	1020.66				

ANOVA Tables for the Mental Arithmetic Accuracy Function

Test of Within-Subjects Effects

Source	Sum of Squares	*df*	Mean Square	*F*	Sig.	Eta Squared	Power
NUTRIENT	293.23	3	97.74	0.68	0.57	0.03	0.19
NUTRIENT*AGE	1122.40	3	374.13	2.61	0.06	0.11	0.61
NUTRIENT*DIFFICULTY	234.90	3	78.30	0.56	0.65	0.03	0.16
NUTRIENT*DIFFICULTY*AGE	447.40	3	149.13	1.06	0.37	0.05	0.27
Error (NUTRIENT)	9471.88	66	143.51				
Error (NUTRIENT*DIFFICULTY)	9305.21	66	140.99				

Test of Between-Subjects Effects

Source	Sum of Squares	*df*	Mean Square	*F*	Sig.	Eta Squared	Power
AGE	1354.69	1	1354.69	0.77	0.40	0.03	0.13
Error (AGE)	38515.63	22	1750.71				

ANOVA Tables for the Mental Arithmetic Time Function

Test of Within-Subjects Effects

Source	Sum of Squares	*df*	Mean Square	*F*	Sig.	Eta Squared	Power
NUTRIENT	77.80	3	26.05	1.23	0.31	0.05	0.31
NUTRIENT*AGE	73.05	3	24.46	1.15	0.34	0.05	0.30
NUTRIENT*DIFFICULTY	58.24	2	24.83	1.53	0.22	0.07	0.34
NUTRIENT*DIFFICULTY*AGE	5.85	2	2.50	0.15	0.89	0.01	0.07
Error (NUTRIENT)	1396.27	66	21.25				
Error (NUTRIENT*DIFFICULTY)	836.33	52	16.21				

Test of Between-Subjects Effects

Source	Sum of Squares	*df*	Mean Square	*F*	Sig.	Eta Squared	Power
AGE	28.75	1	28.75	0.08	0.79	0.003	0.06
Error (AGE)	8484.71	22	385.67				

Appendix F

ANOVA of Nutrient Effects on Mood

ANOVA of Nutrient Effects on Mood

Test of Within-Subjects Effects

Source	Sum of Squares	*Df*	Mean Square	*F*	Sig.	Eta Squared	Power
NUTRIENT	1074.18	3.00	358.06	2.22	0.09	0.09	0.54
NUTRIENT*AGE	517.22	3.00	172.41	1.07	0.37	0.05	0.28
NUTRIENT*MOOD	242.13	5.00	51.03	0.72	0.60	0.03	0.25
NUTRIENT*MOOD*AGE	109.87	5.00	23.16	0.33	0.89	0.02	0.13
NUTRIENT*TIME	271.31	3.00	90.44	0.71	0.55	0.03	0.19
NUTRIENT*TIME*AGE	302.07	3.00	100.69	0.79	0.51	0.04	0.21
NUTRIENT*MOOD*TIME	608.46	5.00	115.50	2.55	0.03	0.10	0.79
NUTRIENT*MOOD*AGE*TIME	139.45	5.00	26.47	0.58	0.72	0.03	0.21
Error (NUTRIENT)	10632.22	66.00	161.09				
Error (NUTRIENT*MOOD)	7401.79	104.00	70.91				
Error (NUTRIENT*TIME)	8435.12	66.00	127.81				
Error (NUTRIENT*MOOD*TIME)	5250.18	116.00	45.30				

Test of Between-Subjects Effects

Source	Sum of Squares	*df*	Mean Square	*F*	Sig.	Eta Squared	Power
AGE	26113.06	1	26113.06	9.27	0.01	0.30	0.83
Error (AGE)	61995.53	22	2817.98				