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INFLUENCE OF DIETARY PROTEIN, CARBOHYDRATE, AND FAT ON COGNITIVE PERFORMANCE AND APPETITE IN HEALTHY ELDERLY PERSONS

by

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A thesis submitted in conformity with the requirements
for the degree of Doctor of Philosophy
Graduate Department of Nutritional Sciences, Faculty of Medicine
University of Toronto

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ABSTRACT

The primary hypothesis of this thesis was that energy ingestion from carbohydrates (glucose, potato, and barley), protein (whey), and fat (safflower oil) enhances cognitive performance compared with a placebo in healthy elderly persons. The secondary hypothesis was that carbohydrates influence satiety differently from each other, and protein (whey) induces higher satiety than carbohydrate (glucose) or fat (safflower oil) in this population. Experiment 1 examined the effects of 50g of carbohydrate as glucose, potatoes or barley, compared with a non-energy placebo on cognition, subjective appetite, and food intake over 120min in healthy subjects (aged 60-82y) after an overnight fast. Experiment 2 examined the effects of isoenergetic, equal volume drinks of pure whey protein, glucose, and safflower oil compared with a placebo on the same variables and age group as experiment 1, over 90min. The relationship between glucose regulation and these variables was determined. Experiment 1 showed that poor baseline memory was associated with poor glucose regulation, but this was not reproduced in experiment 2. All carbohydrates improved memory in subjects with poorer glucose regulation and baseline memories independently of plasma glucose concentration in experiment 1. Glucose, whey protein, and safflower oil improved memory in experiment 2

independently of plasma glucose concentration, however, the effects were not related to glucose regulation. Each macronutrient also had unique effects on cognition. In both experiments, the strongest effects occurred 15min after ingestion and on declarative memory. The appetite measures showed that carbohydrates had different effects on subjective appetite and food intake but glycaemic index (GI) did not predict these effects (experiment 1). Experiment 2 showed that whey protein and safflower oil induced higher subjective satiety than placebo, but only whey protein decreased food intake. These findings showed that glucose regulation may be associated with cognition in healthy elderly subjects, that energy ingestion can improve memory independently of plasma glucose concentration, and that each macronutrient exerts unique effects on cognition. These findings also showed that the elderly respond to physiologic appetite signals, that GI does not predict satiety, and that protein (whey) decreases food intake more than carbohydrate (glucose) or fat (safflower oil) in this population.

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PUBLISHED MATERIAL

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CHAPTER 1
INTRODUCTION

1. INTRODUCTION

The proportion of North Americans with cognitive impairments is increasing as the population ages. Several factors increase the risk of cognitive decline during aging including those that are genetically determined such as male gender and family history of cerebrovascular disease, as well as those that can be influenced by the environment such as heart disease, hyperlipidaemia (Meyer et al., 1998), and possibly diabetes (Ott et al., 1999). It is important to understand the effects of nutrition and other environmental factors on cognition because of their potential role in decreasing the risk of cognitive decline.

Nutritional variables and physiological changes have an impact on cognitive performance in animals and humans over the short and long term (reviewed in (Greenwood & Winocur, 1999)). For instance, evidence showing that early life undernutrition, vitamin deficiencies, and hypoglycaemia in people with diabetes can impair cognition is well established (Kanarek & Marks-Kaufman, 1991; Rogers & Lloyd, 1994). In addition, the acute and chronic effects of macronutrient composition, and the chronic effects of diabetes may also influence cognitive performance, but the evidence is less clear (Rogers & Lloyd, 1994).

Recently, studies showing that type 2 diabetes is associated with memory decline (reviewed in (Strachan et al., 1997; Stewart & Liolitsa, 1999)) and the development of dementia (Ott et al., 1999), and that memory can be improved by improving glucose regulation (Gradman et al., 1993; Meneilly et al., 1993) suggest a link between poor glucose regulation and cognitive decline. At this time, it is not clear how early in the development of glucose dysregulation cognitive impairments can be observed. Furthermore, substantial evidence indicates that an acute glucose load can improve cognitive performance in several populations, especially in the elderly and in those with poor glucose regulation. It has been suggested that these effects are related to elevations in blood glucose concentration, but because the effects of other carbohydrate foods and of individual macronutrients on cognition have not been studied extensively, the importance of alterations in blood glucose compared with energy ingestion is not known (Kanarek, 1997; Bellisle et al., 1998).

The primary objectives of the present research were to determine the relationship between glucose regulation and baseline cognitive performance in healthy elderly persons and to

determine the acute effects of ingesting carbohydrate foods varying in glycaemic index (GI) and other macronutrients on cognition.

In addition to examining cognition, the present experiments examined the influence of macronutrients on another brain-mediated behaviour, appetite and food intake regulation. It is important to study this in the elderly because of the initial increase in body weight followed by the decrease in body weight that commonly occurs after age 60. Although the macronutrients have a physiologic and non-physiologic impact on hunger, satiety and energy intake in young adults (Anderson, 1994; Stubbs, 1999), the importance of the physiologic component in the elderly is unclear, as it has been shown to be somewhat impaired in this population (Roberts et al., 1994; Rolls et al., 1995).

It has been suggested that there may be a hierarchy among the macronutrients such that on a per joule basis, protein has the strongest effect on satiety, followed by carbohydrate, and then fat, however, this has not been examined in the elderly. Furthermore, different types of each macronutrient may also differentially affect appetite regulation. For instance, it has been hypothesized that a low GI food may increase satiety more than a high GI food, however, these data are inconclusive. Nevertheless, it is important to understand these effects in the elderly because the incidence of diabetes is high (Harris et al., 1987), and consuming low GI foods has been used as part of the treatment for these conditions (Wolever, 2000).

The secondary objectives of the present studies were to determine the effects of carbohydrates varying in GI, and the effects of pure macronutrients on subjective appetite and food intake in healthy elderly persons.

CHAPTER 2

LITERATURE REVIEW

2. LITERATURE REVIEW

2.1 Introduction

The objectives of the research presented in this thesis were to determine the relationship between glucose regulation and baseline cognitive performance in healthy elderly persons with normal fasting plasma glucose, and to determine the acute effects of carbohydrates varying in GI, and of each macronutrient on cognitive performance, subjective appetite, and food intake in these individuals.

The literature review is divided into three sections. The first section describes the relationship between nutrition and cognitive function, with a specific emphasis on the relationship among glucose regulation, glucose ingestion, and cognition in the elderly. The second section examines appetite and food intake regulation in the elderly and the effects of protein, carbohydrate, and fat on appetite and food intake regulation. Finally, a brief discussion of the relationship among nutrition, cognition, and appetite is presented.

2.2 Nutrition, glucose regulation and cognitive performance

The proportion of North Americans with memory loss and related cognitive impairments is increasing as the population ages. A number of factors increase the risk of cognitive decline during aging including transient ischaemic attacks, hypertension, heart disease, hyperlipidaemia, smoking, heavy alcohol consumption, male gender, low educational status, family history of cerebrovascular disease, and absence of estrogen replacement therapy among women (Meyer et al., 1998). Clearly, many of these factors are primarily genetically determined, however, others can potentially be reduced by environmental influences such as nutrition.

Nutritional variables have an impact on cognitive performance in animals and humans over the short and long term (reviewed in (Greenwood & Winocur, 1999)). The data implicating physiological changes that lead to cognitive impairments are well established for certain dietary conditions such as early life undernutrition, chronic semi-starvation, thiamin and iron deficiencies, hypoglycaemia in people with diabetes, and alcohol-induced intoxication (Kanarek & Marks-Kaufman, 1991; Rogers & Lloyd, 1994). The influences of caffeine ingestion and withdrawal on cognitive functioning have also been well studied. In contrast, the acute and chronic effects of dieting, short-term fasting, macronutrient composition, and the

chronic effects of diabetes on cognitive performance are less well established (Rogers & Lloyd, 1994).

2.2.1 Diabetes, cognitive performance, and dementia

Conditions that alter glucose regulation and utilization could also affect cognitive function. Glucose is the primary substrate used by the brain for cognitive function (Craft et al., 1996). Because brain neurons are unable to store or synthesize glucose, the brain is dependent on glucose obtained through systemic circulation. Certain regions of the brain, such as the hippocampus, which is important for declarative memory (conscious recollections about facts or events (Squire, 1987)), are especially sensitive to changes in glucose availability (McCall, 1992). Thus, when glucose dysregulation affects the hippocampus and surrounding medial temporal lobe, declarative memory can be impaired (Craft et al., 1996).

The notion that changes in glucose concentration influence cognitive function has important consequences for patients with diabetes because glucose fluctuations occur frequently in this population in response to meal consumption, activity level, and insulin injection (Holmes et al., 1986). Consequently, the influence of transient increases in blood glucose, or hyperglycaemia, and transient decreases, or hypoglycaemia, on cognitive function has been examined in this population. In addition, the long-term impact of diabetes on cognitive function has been studied. Because the aetiology and characteristics of type 1 and type 2 diabetes markedly differ, these two populations will be discussed separately. In general, the data suggest that cognitive impairments in individuals with type 1 diabetes may be limited to transient hypoglycaemic episodes, whereas there appears to be a longer-term impairment in cognitive function associated with type 2 diabetes. Some evidence presented here indicates that diabetes may be a significant risk factor for the development of dementia and Alzheimer Disease (AD).

2.2.1.1 Type 1 diabetes mellitus and cognition

Cognitive deficits in individuals with type 1 diabetes, a condition caused by an absolute deficiency in insulin secretion (American Diabetes Association, 1997), have been reported. Cognitive dysfunction has been observed in both children (Jyothi et al., 1993) and adults with this condition during episodes of severe hypoglycaemia (Blackman et al., 1992; Lingenfelser et al., 1992; Sachon et al., 1992; Draelos et al., 1995; Gold et al., 1995). Others have shown that

even mild hypoglycaemia in subjects with type 1 diabetes impairs cognitive performance (Holmes et al., 1986; Bischoff et al., 1992; Gschwend et al., 1995). Moreover, hypoglycaemia in subjects without diabetes also impairs cognitive function (Gold et al., 1995). The reasons for this are likely related to the fact that acute hypoglycaemia can produce cerebral energy failure, loss of ion homeostasis, membrane depolarization, alterations in amino acid metabolism, depressions in protein synthesis, increases in phospholipid hydrolysis and concentrations of free fatty acids, and alterations in cyclic nucleotide metabolism (reviewed in (McCall, 1992)).

Although the acute effects of hypoglycaemia are consistently observed, the majority of evidence indicates that recurrent instances of hypoglycaemia may not be damaging. Duration of diabetes has been shown to be unimportant (Holmes et al., 1986; Jyothi et al., 1993) and it has been demonstrated that there is no cumulative cognitive dysfunction due to recurrent hypoglycaemic episodes (The Diabetes Control Complications Trial Research Group, 1980; Blackman et al., 1992; Austin & Deary, 1999). Young adults with type 1 diabetes performed as well as non-diabetic controls on several learning and memory tasks (Ryan & Williams, 1993). Furthermore, patients with diabetes performed as well as their healthy siblings, which controls for environmental factors, on several tasks (Crawford et al., 1995), and nocturnal hypoglycaemia did not impair cognitive performance the following morning in diabetics (Bendtson et al., 1992). Others have observed that adults with long duration type 1 diabetes may have cognitive deficits, but these are related to degree of polyneuropathy and suggest that recurrent hypoglycaemia may not directly impair cognition but may magnify an existing dysfunction (Ryan et al., 1993).

In contrast to the data examining hypoglycaemia, the acute effects of hyperglycaemia are less clear. Some have suggested that hyperglycaemia may not impair cognitive function (Draelos et al., 1995; Gschwend et al., 1995), or may only impair it slightly compared with hypoglycaemia (Holmes et al., 1983). Others, however, have suggested that chronic hyperglycaemia may permanently impair cognition, particularly psychomotor function, because of its association with peripheral neuropathy (Ryan et al., 1992; Sachon et al., 1992). This hypothesis argues that metabolic changes associated with peripheral neuropathy, such as alterations in Na^+-K^+-ATPase activity and the subsequent accumulation of sorbitol and depletion of myo-inositol, may trigger similar biochemical abnormalities at the neuronal level leading to a central neuropathy (Ryan et al., 1992).

Although taken together, these data indicate that cognitive impairments associated with hypoglycaemia may be limited to transient hypoglycaemic episodes with no long-term deficits, most of this work has been conducted in children and young adults. More work is needed examining older adults with type 1 diabetes who have had the condition for longer periods of time to determine whether repeated episodes of hyper- and hypoglycaemia lead to permanent cognitive deficits.

2.2.1.2 Type 2 diabetes mellitus and cognition

Type 2 diabetes, which is much more prevalent than type 1, is caused by a combination of resistance to insulin action and an inadequate compensatory insulin secretory response (American Diabetes Association, 1997).

Although it is difficult to determine the effects of diabetes on cognition because this condition is associated with hypertension, cerebrovascular disease, dyslipidaemia, depression, and obesity, which may each independently impair cognition, research generally indicates that memory is impaired in elderly individuals with type 2 diabetes compared with age-matched healthy controls (reviewed in (Strachan et al., 1997; Stewart & Liolitsa, 1999)). Although a wide range of cognitive tests and exclusion criteria for subjects have been used across studies, making it difficult to compare studies, the majority of case-control studies indicate that verbal memory, generally assessed by testing immediate and delayed recall of short stories and word lists, is significantly impaired in subjects with type 2 diabetes compared with healthy controls (Strachan et al., 1997). A recent longitudinal study showing that diabetes was associated with cognitive decline over a 6 year period, even in a relatively young 47-57 year old group, supports this relationship (Knopman et al., 2001). By contrast, mixed results have been observed on tests of attention, concentration, psychomotor speed, and frontal lobe or executive function tasks, suggesting that any deficits may be confined to verbal memory (Strachan et al., 1997).

Cognitive deficits associated with type 2 diabetes appear to be minimized when hypertension and cerebrovascular disease are eliminated as confounding variables. Although some have suggested that any cognitive deficit associated with type 2 diabetes may be explained by risk factors other than diabetes (Lowe et al., 1994), others have shown that both diabetes and hypertension are independently associated with cognitive decline, and that the effects are greatest when both diabetes and hypertension are present (Elias et al., 1997). More studies are

needed that control for confounding risk factors, such as hypertension, to clearly understand whether diabetes per se is a cause of cognitive impairment (Stewart & Liolitsa, 1999).

Impairment in glucose regulation associated with diabetes appears to be at least partly responsible for the deficits observed in individuals with diabetes. Despite the uncertainty about the specific effects of diabetes on cognitive function, two studies have found memory to improve after glucose regulation was improved with hypoglycaemic agents in elderly patients with type 2 diabetes (Gradman et al., 1993; Meneilly et al., 1993).

A number of mechanisms have been suggested to explain the cognitive deficits observed in type 2 diabetes, independently of the other risk factors associated with this condition (reviewed in (McCall, 1992; Strachan et al., 1997; Stewart & Liolitsa, 1999)). For instance, chronic hyperglycaemia can result in a significant loss of cortical neurons and a reduction of the neocortical capillary network in rodents (Jakobsen et al., 1987), and can reduce glucose transport across the blood-brain barrier inducing brain fuel starvation, or neuroglycopenia (Gjedde & Crone, 1981). The insulin resistance and resultant hyperinsulinaemia that occurs in type 2 diabetes could also be involved in the mechanism because insulin can act as a neuromodulator inhibiting synaptic activity (Strachan et al., 1997). Research with experimental diabetes in rodents suggests that the cognitive deficits associated with this condition may be related to the influence of diabetes on cholinergic neurotransmission (Messier & Gagnon, 1996). These rats show increased activity of acetylcholinesterase, the enzyme that degrades acetylcholine and decreased brain choline acetyltransferase activity, the enzyme that facilitates acetylcholine synthesis (Messier & Gagnon, 1996).

A strong link between impaired glucose regulation and cognitive decline is suggested by evidence showing that type 2 diabetes impairs cognitive function, that it can be reversed by improving glucose regulation, and that there are potential mechanisms that may explain the effects. Further evidence, presented next, indicates that diabetes may be an independent risk factor for the development of dementia and AD.

2.2.1.3 Diabetes, Alzheimer Disease, and dementia

Impaired glucose regulation and type 2 diabetes may contribute to the development of AD (Craft et al., 1992; Finch & Cohen, 1997). This suggestion stems from the observation that a disruption of glucose regulation and utilization is prevalent in these patients (Meneilly & Hill,

1993) and that any prolonged alteration in glucose metabolism or energy production can contribute to an irreversible loss of nerve cells (Sims et al., 1987). One hypothesis linking diabetes with the development of AD suggests that the chronic hyperglycaemia of diabetes leads to the glycosylation of proteins resulting in the excessive formation of advanced glycation end-products, which may increase oxidative stress resulting in cellular damage (Finch & Cohen, 1997; Ott et al., 1999). The presence of advanced glycation end-products in the amyloid plaques and neurofibrillary tangles of the brains of AD patients supports this hypothesis (Munch et al., 1998).

Type 2 diabetes increases the risk of vascular dementia, likely related to the finding that diabetes is an independent risk factor for stroke, which can damage vital brain regions (Tatemichi et al., 1993), whereas the link between diabetes and AD is only recently becoming evident (Ott et al., 1999). Although a number of case-control studies reported that diabetes was a risk factor for vascular and other dementias but not for AD (Landin et al., 1993; Mortel et al., 1993; Nielson et al., 1996), an association between insulin resistance, diabetes and AD was shown in two population-based prevalence studies (Ott et al., 1996; Kuusisto et al., 1997), independent of other factors including atherosclerosis, blood pressure, and antihypertensive drug treatment (Ott et al., 1996). Furthermore, more recent evidence from three longitudinal population-based studies has shown a strong increased risk of AD in patients with diabetes (Yoshitake et al., 1995; Leibson et al., 1997; Ott et al., 1999). For instance, in a recent study, over 6000 non-demented subjects over age 55 were followed for an average of 2 years (Ott et al., 1999). After assessing evidence of dementia and diabetes at baseline and follow-up, these investigators found that 126 patients became demented, and that type 2 diabetes almost doubled the risk of vascular dementia, other dementia, and AD, independent of age, sex, and other confounds.

There appears to be strong evidence that type 2 diabetes is an independent risk factor for vascular dementia and AD, possibly related to different mechanisms. Because of these relationships, another interesting question is whether or not cognitive function is impaired at earlier stages of glucose dysregulation, before the diagnosis of diabetes.

2.2.2 Glucose regulation and cognitive performance in the absence of diabetes

Studies have shown that poor glucose regulation is associated with poor cognitive performance in subjects who do not have diabetes. Two studies have reported that elderly individuals with impaired glucose tolerance performed worse on cognitive tests than those with normal glucose tolerance (Kalmijn et al., 1995; Vanhanen et al., 1998). Impaired glucose tolerance is generally considered a pre-diabetic phase that may increase the risk of developing diabetes (American Diabetes Association, 1997). In addition, non-diabetic subjects with poorer glucose regulation performed worse than those with better glucose regulation on several cognitive tasks (Hall et al., 1989; Manning et al., 1990; Craft et al., 1992; Parker & Benton, 1995; Messier et al., 1997). However, in these studies the severity of glucose regulation was unclear because fasting plasma glucose levels were not reported, and non-standard indices of glucose regulation were used. That is, the difference between peak blood glucose after a glucose-containing drink and fasting blood glucose was used as an index of glucose regulation. In addition, it is not clear if these subjects had normal or impaired fasting glucose concentrations or glucose tolerance, which would determine whether they were subjects who were at risk for developing diabetes. Two recent reports, published after the experiments in this thesis were completed, suggest that cognitive impairments may indeed be evident well before the diagnosis of impaired glucose tolerance is made (Messier et al., 1999; Donohoe & Benton, 2000). These authors found a relationship between poor glucose regulation and poor cognitive performance in healthy young adults who had normal fasting plasma glucose concentrations. No studies to date have reported the relationship between glucose regulation and cognitive performance in healthy elderly subjects with normal fasting plasma glucose concentrations.

The data presented suggest that cognitive performance may be impaired at very early stages of glucose dysregulation, even before the diagnosis of impaired fasting plasma glucose or impaired glucose tolerance. More research is needed in different age groups to clearly determine whether minor abnormalities in glucose regulation affect cognition in healthy individuals. Nevertheless, the current evidence suggests that there may be a continuum in cognitive impairment related to glucose regulation, such that as glucose regulation worsens throughout normal aging into clinical evidence of impaired glucose tolerance and then diabetes, the risk of cognitive decline increases and may lead to dementia or AD. Thus, the prevention of impairments in glucose regulation through healthy lifestyle habits may not only decrease the risk of developing diabetes, but may also decrease the risk of memory decline.

2.2.3 Glucose drinks and cognitive performance in healthy and cognitively impaired subjects

The influence of glucose injection or ingestion on various tests of cognitive function has been examined extensively in a range of rodent and human populations. Investigators have examined the independent effects of glucose administration on memory because of the potential importance of fluctuations in blood glucose concentration on the glucose supply to the central nervous system (CNS), and the observation that epinephrine improves memory in rodents with a concomitant elevation in peripheral glucose concentration (Korol & Gold, 1998). A wide range of these studies in rodents have shown enhanced memory performance following both peripheral (Gold, 1986; Messier & Destrade, 1988; Stone et al., 1990; Means & Fernandez, 1992; Kopf et al., 1993; Rodriguez et al., 1993) and central (Lee et al., 1988) injections of glucose. Studies examining the effects in healthy adults are summarized in **Table 2.1**.

Studies in humans have shown that a glucose-containing drink improves cognitive performance compared with non-energy saccharin or aspartame-sweetened placebo drinks in both healthy and cognitively impaired individuals (reviewed in (Rogers & Lloyd, 1994; Gold, 1995; Korol & Gold, 1998)). In most of these studies, a within-subjects design has been used whereby each subject is provided with 50 g of glucose on one day and a placebo on another day, each following an overnight fast. Subjects are then tested on a battery of standard cognitive tasks starting 15-20 min after ingestion. The most common result has been a beneficial effect of glucose on cognitive function within about an hour after ingestion, and the most robust effects have been observed in subjects who have minor or major memory deficits and minor deficits in glucose regulation. Some have suggested that a specific blood glucose concentration (8-10 mmol/L) is necessary for improved performance (Parsons & Gold, 1992; Manning et al., 1993; Benton et al., 1996), that the effects may be more pronounced on tasks of greater complexity (Holmes et al., 1986; Benton, 1990), that the effects are most robust on tests of declarative verbal memory (Manning et al., 1990; Craft et al., 1994; Manning et al., 1997; Foster et al., 1998), with improvements being observed on both memory storage and retrieval (Manning et al., 1998), and that the effects are more pronounced in men than in women (Craft et al., 1994).

Table 2.1 Effect of Glucose Consumption on Cognitive Performance in Healthy Adults

Reference	Subjects	Dietary restrictions before testing	Treatments	Control	Time testing began after treatment	Outcome/Task [1]
Gonder-Frederick et al. 1987	11 (4 male, 7 female) age = 67 y	overnight fast	50 g glucose	23.7 mg saccharin	15 min	+ paragraph recall + Wechsler memory scale
Hall et al. 1989	12 (sex not reported) age = 20 y	overnight fast	50 g glucose	23.7 mg saccharin	10 min	0 Wechsler memory scale
Benton 1990	20 male age = 20 y	subjects asked to not eat for 4 h	25 g glucose	aspartame	20 min	+ monitoring digits
	40 (20 male, 20 female) age = 21 y		25 g glucose	aspartame	20 min	0 eye-hand coordination
Manning et al. 1990	17 (5 male, 12 female) age = 73 y	overnight fast	50 g glucose	saccharin	10 min	+ word recall + paragraph recall 0 non-memory tasks (attention, motor speed, IQ, figure design, digits forward)
Azari 1991	18 male age = 21 y	overnight fast, breakfast given, no food for 4 h	30 g glucose + 350 mg aspartame or 100 g glucose	450 mg aspartame	30 min	0 recall and recognition of words
Manning et al. 1992	22 (8 male, 14 female) age = 67 y	overnight fast	50 g glucose	23.7 mg saccharin	24 h	+ paragraph recall
Parsons & Gold 1992	10 (5 male, 5 female) age = 68 y	overnight fast	50 g glucose or 25 g glucose + 24.9 mg saccharin or 10 g glucose + 38.8 saccharin	50.6 mg saccharin	5 min	+ paragraph recall (25 g glucose) 0 paragraph recall (10 g glucose) 0 paragraph recall (50 g glucose)

Craft et al. 1992	14 (sex not reported) age = 71 y	overnight fast	50 g glucose	23.7 mg saccharin	15 min	+ paragraph recall (subjects with good glucose regulation only) 0 pattern recall 0 paired associate recall
Benton & Owens 1993	153 (100 male, 53 female) age = 22 y	no dietary restrictions	50 g glucose	aspartame and acesulfame K	15 min	0 word recall (+ with increasing blood glucose) 0 spatial memory
	53 female age = 22 y		50 g glucose then 25 g glucose 45 and 75 min later	aspartame and acesulfame K at same 3 times	15 min (paragraph) 50 min (word list)	0 paragraph or word recall 0 word recall (+ with higher blood glucose values)
Benton et al. 1994	70 female age = 21 y	no dietary restrictions	50 g glucose then 25 g glucose 25 min later	aspartame and acesulfame K	20 min	0 word recall + recall of first and last words - rapid information processing test
	50 male age = 22 y		50 g glucose then 25 g glucose 25 min later	aspartame and acesulfame K	40 min	0 stroop task
Craft et al. 1994	27 young (14 male, 13 female) age = 21 y and 32 elderly (16 male, 16 female) age = 69 y	overnight fast	50 g dextrose	2 mL sodium saccharin sweetener	15 min	+ paragraph recall (older men with good glucose regulation and younger men with poor glucose regulation) - paragraph recall (older men with poor glucose regulation and younger men with good glucose regulation) 0 paragraph recall (women) - Stroop test 0 procedural memory 0 working memory 0 verbal fluency
Parker & Benton 1995	100 female age = 20 y	no dietary restrictions	50 g glucose then 25 g glucose 30 min later	aspartame and acesulfame K	20 min	+ dichotic listening task

Allen et al. 1996	28 (6 male, 22 female) age = 73 y	no dietary restrictions reported	50 g glucose	23.7 mg saccharin	15 min	+ Rey/Taylor delay + verbal fluency + figural fluency 0 Rey/Taylor copy 0 dichotic listening 0 grooved pegboard 0 Boston naming test 0 trails A 0 trails B 0 meier visual test
Manning et al. 1997	24 young (8 male, 16 female) age = 19 y and 23 elderly (8 male, 15 female) age = 67 y	overnight fast	50 g glucose	23.7 mg saccharin	15 min	+ word recall (elderly) 0 word recall (young) 0 priming (young and elderly) 0 recognition (young and elderly)
Messier et al. 1997	15 (8 male, 7 female) age = 62 y	overnight fast	50 g glucose + 4 mg saccharin	50.6 mg saccharin	0 min	0/- immediate, delayed paragraph recall depending on sex and glucoregulation + first 7 items of paragraph (males with good glucoregulation) - first 7 items of paragraph (males with poor glucoregulation) +/0 on several attention tests 0 several short-term memory tests +/-/0 list learning
Foster et al. 1998	female	overnight fast	25 g glucose	saccharin or water		+ long-term verbal recall 0 short-term verbal memory 0 long-term non-verbal memory
Manning et al. 1998	20 (6 male, 14 female) age = 67 y	overnight fast	50 g glucose	35 mg saccharin	0 min or 24 h after testing	+ paragraph recall on both memory storage and retrieval (storage stronger)
Messier et al. 1998	100 female age = 21 y	overnight fast	10, 100, 300, 500, 800, or 1000 mg	51 mg saccharin or water	0 min	+ word recall (first 5 words); 300 mg strongest

			glucose per kg body weight			+/- word recall (last 5 words) 0 word recall (middle 5 words)
Benton & Parker 1998	2 studies (27 and 78 young) age = 22/23 y	overnight fast	50 g glucose	2 g Sweetex (aspartame + saccharin)	20 min	+ word recall + memory while counting backwards 0 paragraph recall 0 abstract reasoning
Fucetola et al. 1999	20 young and old (7 male, 13 female) age = 41 y	overnight fast	25, 50 or 75 g glucose	64 mg saccharin	15 min	0 non-verbal recognition 0 immediate and delayed paragraph recall 0 spatial memory
Messier et al. 1999	36 (18 men, 18 women) age = 21 y	overnight fast	50 g glucose + 4 mg saccharin	50.6 mg saccharin	10 min	+ word recall (subjects with poor glucose regulation) 0 word recall (subjects with good glucose regulation)
Metzger 2000	34 (11 male, 23 female) age = 21 y	overnight fast	50 g glucose	23.7 mg saccharin	15 min	+ facial recognition (a nonverbal task)

[1] +, better performance after treatment; -, poorer performance after treatment; 0, no significant effect of glucose drink.

The evidence showing that the memory-enhancing effects of glucose are stronger in subjects with relatively poor baseline memories is based on studies examining populations with various baseline memory abilities. These studies have demonstrated pronounced memory improvements with glucose drinks in healthy elderly subjects (Gonder-Frederick et al., 1987; Manning et al., 1990; Craft et al., 1992; Manning et al., 1992; Parsons & Gold, 1992; Allen et al., 1996; Manning et al., 1997) and in patients with AD (Craft et al., 1992; Craft et al., 1993; Manning et al., 1993), Down's syndrome (Manning et al., 1998) and schizophrenia (Fucetola et al., 1999; Newcomer et al., 1999), and lesser improvements or no improvements in healthy young adults (Hall et al., 1989; Manning et al., 1997). In addition, numerous studies in animals showing that glucose attenuates memory impairments caused by various amnesic agents (Stone et al., 1988; Messier et al., 1990; Ragozzino & Gold, 1991; Stone et al., 1991; Ragozzino et al., 1992; Ahlers et al., 1993) and aging (Winocur, 1995) support the notion that the effects of glucose are more sensitive in individuals with pre-existing cognitive deficits.

Because some of the earlier studies in younger subjects found no effects of glucose ingestion on memory performance, it has been suggested that the glucose-enhancing effect may be limited to populations with pre-existing memory deficits or vulnerabilities to memory disorders (Azari, 1991). More recently, however, glucose has been shown to improve performance in younger subjects when tasks are of sufficient difficulty, suggesting that the failure to observe improvements in young subjects in some studies may be partly related to the inclusion of less complex tests. That is, younger subjects may have performed so well at baseline that it was difficult to observe any significant improvements. For instance, in one study, glucose ingestion improved memory in 20 year-old subjects when they were tested on a longer version of the paragraph recall test of the Wechsler Memory Scale than has commonly been used in glucose studies (Korol et al., 1995). Others have found improvements in young adults on some verbal and nonverbal memory tasks but not on others (Benton et al., 1994; Benton & Parker, 1998; Messier et al., 1998; Metzger, 2000). Because of the mixed findings, the prevailing view is that the effects of glucose ingestion are most robust in subjects with pre-existing deficits, but that under certain circumstances, improvements can be seen in healthy young subjects, or at least in a subgroup of these subjects.

The greater response in subjects with poorer baseline cognitive performance may be related to the fact that they simply have the most room for improvement, but it may also be

related to glucose regulation. Elderly subjects and patients with AD, who show the most pronounced improvements with glucose, have poorer glucose regulation than healthy young subjects (Meneilly & Hill, 1993). Importantly, these subjects do not have diabetes, but have a relative impairment in glucose regulation. Furthermore, the benefits in healthy young subjects may only occur in those with relatively poor glucose regulation. Indeed, studies in young adults without diabetes have shown that only those with the poorest glucose regulation show memory improvements following glucose ingestion (Craft et al., 1994; Messier et al., 1999), or show the most robust improvements (Korol et al., 1995). In these studies, glucose regulation was defined as the difference between baseline blood glucose concentration and the concentration 60 min after glucose ingestion; a greater difference represented poorer regulation. Finally, support for this hypothesis comes from studies in rodents showing that the cognitive enhancing effects of glucose injection are most robust or only evident in animals with poorer glucose regulation (Winocur, 1995; Messier & Gagnon, 2000).

Further evidence supports the notion that glucose regulation is an important factor in determining whether or not individuals are responsive to the cognitive-enhancing effects of glucose (Craft et al., 1994). In this study, young and old healthy subjects of both genders were each divided into those with good and those with poor glucose regulation. Healthy elderly male subjects with good glucose regulation and young male subjects with poor regulation were the only groups that showed enhanced memory following a glucose drink. By contrast, glucose did not improve memory in the elderly subjects with poor glucose regulation, in the young subjects with good glucose regulation, or in any group of female subjects. Importantly, the young male subjects designated as "poor glucose regulators" had blood glucose concentrations that were similar to the elderly subjects designated as "good glucose regulators". Thus, only men with a specific range of glucose regulation were sensitive to the beneficial effects of a glucose load, regardless of age. In other words, glucose did not improve memory if glucose regulation was very good (i.e., young subjects with good glucose regulation) or very poor (i.e., old subjects with poor glucose regulation), but did improve memory if glucose regulation was in the middle (i.e., moderately impaired). Another study showing that only elderly subjects with better glucose regulation showed improvements on a memory task supports these findings (Messier et al., 1997). Finally, Craft et al. (Craft et al., 1994) suggested that the finding that the effects of

glucose ingestion were limited to men may be related to differences in glucose regulation between the genders.

The cognitive-enhancing effects of glucose appear to be strongest on tests of declarative memory, but can enhance performance on other cognitive tasks as well. Declarative memory can be defined as memory that is accessible to conscious awareness; a memory that can be declared or stated (Squire, 1987). This memory includes episodic memory (specific time and place events) and semantic memory (facts and general information) and is usually expressed on tests of recall or recognition. Word list recall and paragraph recall/logical memory have been most commonly used in the glucose studies. This type of memory is believed to be controlled by the hippocampus, surrounding medial temporal lobe, and related structures (Squire & Zola, 1996). Several authors have found improvements in declarative memory following the consumption of a glucose drink (Gonder-Frederick et al., 1987; Manning et al., 1990; Craft et al., 1992; Manning et al., 1992; Parsons & Gold, 1992; Craft et al., 1994; Manning et al., 1997), and no significant improvements or much weaker effects on tests mediated by other brain regions including working memory (Hall et al., 1989; Craft et al., 1994; Korol et al., 1995), procedural memory (Craft et al., 1994; Manning et al., 1997), response inhibition (Benton et al., 1994; Craft et al., 1994), and attention (Manning et al., 1990). Working memory, which can be defined as temporary memory necessary for the completion of tasks on which one is currently working (Pinel, 1993), depends on the prefrontal lobe (Goldman-Rakic, 1992); procedural memory, which is revealed by improved performance without conscious recall or recognition (Squire, 1987), depends on the striatum (Squire & Zola, 1996); response inhibition depends on the frontal lobe; and attention is mediated by a neural network including the parietal and frontal lobes (Banich, 1997). These data are consistent with animal studies that have shown that glucose enhances performance on tests mediated by hippocampal function but not on tests mediated by other brain regions including the frontal lobes (Winocur, 1995; Winocur & Gagnon, 1998; Greenwood & Winocur, 2001). By contrast, some authors have observed limited improvements on specific components of tests that examine working memory and attention (reviewed in (Korol & Gold, 1998)). Thus, the evidence to date indicates that the medial temporal lobe, including the hippocampus and related structures, is most sensitive to the effects of glucose on cognitive function, but glucose may enhance performance on other tasks as well, albeit to a lesser extent.

Several hypotheses exist to explain the enhanced cognitive performance that occurs following glucose ingestion (reviewed in (Messier & Gagnon, 1996)), but these are not necessarily mutually exclusive. One common hypothesis is that peripheral increases in blood glucose concentration may impact on the uptake and utilization of glucose by the brain, and interact with neurotransmitters to improve cognitive function (Gold & Stone, 1988; Wenk, 1989; Messier & Gagnon, 1996). For instance, glucose injections may increase acetylcholine synthesis via a transformation of glucose into the acetylcholine precursor, acetylcoenzyme A (Messier et al., 1990), or by influencing opiate inhibition of acetylcholine turnover in the hippocampus (Stone et al., 1991). Indeed, direct evidence has shown that i.p. injections of glucose in rats increase acetylcholine synthesis, via increased availability of acetylcoenzyme A, during conditions of increased neuronal activity (Durkin et al., 1992). Other studies in rodents support this hypothesis (Ragozzino et al., 1996; Ragozzino et al., 1998).

Recent evidence in rats supports the hypothesis that the effects of glucose act centrally and provides insight into the potential reasons for observing more robust effects in the elderly and on declarative memory tasks (McNay & Gold, 2001; McNay et al., 2001). These authors first showed that extracellular glucose concentration is greater in the hippocampus, which is involved in declarative memory tests, than in the striatum, which is not involved in such tests, under resting conditions (McNay et al., 2001). This was the first direct evidence that these concentrations differ in different brain regions and suggest that there could be a greater requirement for glucose in the hippocampus. They also found that hippocampal extracellular glucose concentrations decreased in the hippocampus, but not in the striatum, while rats were working on a spatial memory task, suggesting that the hippocampus is involved in completing this task as it requires an increased capacity of glucose during the test. However, these results are limited by the fact that only two brain regions were examined. In a second series of experiments, these decreases in hippocampal extracellular glucose concentrations during memory testing were shown to be greater in older than in younger rats, suggesting that the poorer memory performance of the older animals may be partly explained by the decreased ability of these animals to supply a sufficient amount of glucose during conditions of cognitive demand (McNay & Gold, 2001). Finally, when glucose was peripherally injected in young and old rats the decrease in hippocampal extracellular concentrations was completely attenuated and memory performance was improved in both groups of animals. These authors noted that it was

important to use a task of sufficient difficulty for glucose to be able to improve performance in the younger animals. In summary, these studies suggest that one of the reasons for observing greater enhancements of performance following glucose ingestion in older subjects and on declarative memory tasks might be that more glucose is used by the hippocampus than other brain regions while completing these tasks, especially in older subjects, and that their normal functioning is insufficient to supply enough glucose to function at an optimal level. Thus, the ingestion of glucose may make up for this deficit in the ability of the hippocampus to supply a sufficient amount of endogenous glucose during the completion of declarative memory tasks.

A second hypothesis suggests that hyperglycaemia-induced insulin elevations may be involved in the mechanism that mediates the effects of glucose ingestion on cognitive performance (Craft et al., 1996; Craft et al., 1999). These researchers found that raising insulin concentrations improved memory in subjects with AD even when glucose concentrations were kept at baseline (Craft et al., 1996), and that increases in glucose concentration failed to improve memory when the endogenous insulin response was suppressed (Craft et al., 1999). Although the mechanism of action of insulin has not been elucidated, recent evidence has shown that changes in peripheral insulin are reflected centrally and there are a number of ways in which insulin in the CNS could affect memory.

Although it was previously believed that the brain was not sensitive to insulin, this may be incorrect (reviewed in (Park et al., 2000)). Indeed, peripheral insulin concentrations are reflected in the cerebrospinal fluid (CSF) in both normal and AD subjects (reviewed in (Plata-Salaman, 1991; Wozniak et al., 1993)) and both plasma and CSF insulin are elevated in AD patients (Fujisawa et al., 1991). It is not clear if insulin is transported from peripheral tissues to the CNS or if peripheral concentrations influence CSF-synthesized insulin (Plata-Salaman, 1991), however, there is some evidence that insulin crosses the blood-brain barrier (Banks et al., 1997). The presence of insulin receptors throughout the CNS supports the notion that insulin can act centrally; the highest concentrations of insulin receptors are in the cerebral cortex, hypothalamus, olfactory bulb, cerebellum, and hippocampus (Park et al., 2000). Thus, the presence of insulin receptors in the hippocampus raises the possibility that insulin could affect memory by influencing hippocampal activity. Indeed, increases in plasma insulin have been shown to increase insulin binding in the hippocampus (Craft et al., 1999) and the observation that the insulin-sensitive glucose transporter, GLUT 4, is present in the hippocampus and other

brain regions (Livingstone et al., 1995) provides a direct way in which insulin could affect brain glucose uptake and utilization. Furthermore, insulin can increase the firing rates of hippocampal neurons (Park et al., 2000). Finally, these authors have speculated that insulin could also act by stimulating CNS glucose uptake leading to an increase in acetylcholine synthesis.

A third hypothesis is that the site of action of glucose may be in the liver, which may in turn send a neural signal to the CNS. The fact that vagotomy and celiac ganglion lesion decreases the memory-enhancing effects of glucose (White, 1991) and peripherally-injected drugs (Flood et al., 1987), and that fructose, which does not cross the blood-brain barrier, enhances memory (Messier & White, 1987; Rodriguez et al., 1994), supports the argument that peripheral effects may be important for memory enhancement. The peripheral action of glucose may involve a neural signal produced when glucose is carried into cells by glucose transporters (Messier & Gagnon, 1996).

Finally, the effects of glucose ingestion on memory may be related to a general effect of energy ingestion on memory performance rather than a specific effect of glucose *per se*. That is, the effect of any energy ingestion leads to the release of a number of gut peptides, which leads to a stimulation of the vagus nerve, which could affect cognition. Studies showing that cholecystokinin (CCK) and other gut peptides improve memory, likely via stimulation of the vagus nerve (Morley et al., 1994), and that direct electrical stimulation of the vagus in humans improves memory (Clark et al., 1999) support this hypothesis. Research examining the specific effects of different macronutrients that do not affect blood glucose concentration are required to test this hypothesis.

2.2.4 Meals and cognitive performance

Very little research has been conducted examining the influence of pure macronutrient loads, other than glucose, on cognitive performance, however, several studies have examined the acute effects of mixed meals on cognitive performance (see **Table 2.2**). In addition, the influence of raising blood glucose concentration with carbohydrate-rich foods, other than pure glucose, on cognitive performance has received little attention. Thus, it is not known if glucose has a special effect on cognitive function or if any carbohydrate or energy source has a similar effect.

Table 2.2 Effect of Meals on Cognition in Healthy Subjects

Reference	Subjects	Dietary restrictions before testing	Treatments	Control	Time testing began after treatment	Outcome/Task
Smith et al. 1988	11 (5 male, 6 female) x = 27 yrs	not reported	lunch: high starch (55% of energy) or high sucrose (55% of energy) - all diets contained 1/3 of individual daily energy requirements	high protein lunch (55% of energy)	~ 30 min	- peripheral targets on search test
Michaud et al. 1991	319 (150 male, 169 female) x = 16 yrs	not reported	subjects instructed to consume more energy at breakfast than they had 2 weeks earlier (resulted in 63% increase in energy intake)	usual breakfast	3-4 h	+ short-term memory - concentration (not due to changes in blood glucose)
Benton and Sargent 1992	33 (17 male, 16 female) x = 21 yrs	overnight fast	breakfast (1370 kJ drink: 18.5 g protein, 37.7 g carbohydrate, 12.2 g fat)	no breakfast	2 h	+ spatial memory (+ correlation with high blood glucose) + immediate recall
Smith et al. 1992	48 university students (24 male, 24 female)	not reported	breakfast (eggs, bacon, toast, margarine) and lunch (tomato soup, bread, chicken, potato, peas, carrots)	no breakfast and same lunch	~1.5 h after breakfast and ~1 h after lunch	0 free recall + recognition memory (after breakfast) - logical reasoning (after breakfast) - semantic memory (after lunch) 0 sustained attention
Lloyd et al. 1994	18 (3 male, 15 female) x = 27 yrs	did not eat for 3 h	lunch: low fat/high carbohydrate (29%/54% of energy) or medium-fat/medium carbohydrate (45%/42% of energy)	high fat/low carbohydrate lunch (62%/ 24% of energy)	30 min	0 working memory 0 free recall + reaction time (medium fat/ carbohydrate meal compared to 2 other meals; similar to

						habitual lunch composition)
Smith et al. 1994	48 university students (24 male, 24 female)	overnight fast (8 h)	cooked breakfast (1.89 MJ; eggs, milk, bacon, toast, margarine) or cereal/toast breakfast (1.89 MJ; cereal, milk, sugar, margarine)	no breakfast	1 h	0 three sustained attention tasks
	48 different university students (24 male, 24 female)		same cooked breakfast	no breakfast	1 h	+ free recall + recognition 0 semantic memory - working memory
Green et al. 1995	21 female (aged 18 to 25)	not reported	ate normally for 24 hours, or missed one meal, or missed two meals	24 hour food deprivation	after 24 h feeding condition	0 vigilance task 0 reaction time 0 focused attention 0 free recall
Lloyd et al. 1996	16 (2 male, 14 female) x = 26 yrs	overnight fast (10.5 h)	breakfast: low fat/high carbohydrate (27%/62% of energy) or medium-fat/medium carbohydrate (44%/47% of energy) or high fat/low carbohydrate (56%/34% of energy)	no breakfast	30 min	0 working memory 0 free recall 0 reaction time
Benton & Parker 1998	2 studies (40 and 91 young) age = 22/23 y	overnight fast	breakfast: 1049 kJ (43 g CHO, 7 g protein, 7 g fat)	placebo drink (2 g Sweetex: aspartame + saccharin)	20 min	+ word list recall + memory while counting backwards + story recall 0 abstract reasoning

+, better performance after treatment; -, poorer performance after treatment; 0, no significant effect of treatment

In contrast to the glucose-drink studies, which have shown the greatest benefit in elderly and cognitively impaired subjects, it appears that the influence of meals on cognitive function has only been examined in children and healthy young adults. Furthermore, cognitive testing has generally begun 30 min to 4 h following meal consumption, whereas cognitive testing began 15-20 min following glucose-drink ingestion in almost every published study. In evaluating the following discussion of meals and cognitive function, these differences between meal and glucose-drink studies should be considered.

Studies with children show that those who consume breakfast perform better on cognitive tests than those who do not eat breakfast (reviewed in (Kanarek, 1997)). By contrast, the evidence from studies of adult subjects is much weaker. Although some data demonstrate that subjects who consume breakfast versus no breakfast (Benton & Sargent, 1992; Smith et al., 1994; Benton & Parker, 1998) or a larger than normal breakfast (Michaud et al., 1991) perform better on tests of memory, others have shown no effect of missing any meal on cognitive performance (Rogers & Lloyd, 1994; Green et al., 1995; Lloyd et al., 1996).

The improvements in performance may be related to a meal-induced increase in blood glucose (i.e., similar to the effects following a glucose drink) that could impact on brain glucose availability (Benton & Sargent, 1992; Smith et al., 1994). One study showing that a mixed macronutrient breakfast and a glucose drink had comparable effects on memory performance supports the notion that the cognitive-enhancing effects of breakfast may be related to the increase in blood glucose (Benton & Parker, 1998).

A performance impairment known as the "post-lunch dip" has been a common finding in studies examining the effects of lunch on cognition (Rogers & Lloyd, 1994). It has been suggested that these impairments may be produced by the carbohydrate content of the lunch (Smith et al., 1988) or by a simple endogenous change in daily rhythm (Kanarek, 1997). Others have suggested that macronutrient composition is not important in mediating cognitive performance (Lloyd et al., 1994; Lloyd et al., 1996). These investigators found that the meal that most closely matched the macronutrient composition that was normally consumed by the subjects (i.e., low-fat/high-carbohydrate breakfast and medium-fat/medium carbohydrate lunch) resulted in the best mood and cognitive performance scores. Thus, these authors have suggested that similarity to habitual macronutrient composition may be more important in mediating cognitive performance than actual macronutrient composition. These results are supported by

studies that show that deviating from normal breakfast habits (e.g., breakfast eaters versus non-eaters (Richards, 1972, cited in (Lloyd et al., 1996)) or lunch habits (e.g., size of meal (Craig & Richardson, 1989)) leads to poorer cognitive performance.

Some recent studies in healthy young adults, conducted after or during the experiments conducted in this thesis, have examined the effects of pure macronutrients and different carbohydrates on cognitive performance with mixed results (Catherine, 2000; Woodend, 2000; Tecimer, 2001). In these studies from the same laboratory, sucrose improved immediate recall of a word list 15 min after ingestion compared with amylose, amylopectin, polycose, glucose-fructose, and control in one set of experiments (Catherine, 2000), but failed to improve performance in other studies with similar designs (Woodend, 2000; Tecimer, 2001). The authors suggested that the effects of sucrose may only occur at an optimal dose (i.e., 75 g) and may be related to the glucose:fructose ratio rather than to blood glucose concentration, but the failure to reproduce the findings consistently makes the interpretation unclear. In experiments comparing macronutrients, safflower oil improved performance on delayed word list recall compared with an isoenergetic sucrose preload, but neither treatment differed from a water control (Woodend, 2000). Soy protein weakly improved memory compared with whey and egg protein on one component of a word list test, but did not differ from sucrose or control (Tecimer, 2001). Thus, these data suggest that the macronutrients may have different effects on memory in young adults, but more research examining specific doses and sources are required to clearly determine the acute effects of each macronutrient.

In summary, no generalizations about the positive or negative interactions between specific macronutrients and cognitive function can be made at this time (Kanarek, 1997; Bellisle et al., 1998). The reason for this may be methodological. First, although the impact of relatively high protein, carbohydrate and fat meals on cognition has been examined, all meals, other than the studies just mentioned, have contained some carbohydrate. Thus, because of the known effects of glucose on cognition, it is impossible to determine if the effects of mixed macronutrient meals on performance are related to a carbohydrate-induced rise in blood glucose, to another macronutrient, or to energy intake alone. Second, as noted, testing has generally been conducted 30 min to 4 h after ingestion even though the most robust effects of glucose occur 15-20 min post-ingestion. Finally, testing has generally been limited to children and young adults, possibly concealing potential beneficial effects of macronutrients in individuals with poorer

baseline memory skills, such as the elderly. More research is needed to examine the influence of specific macronutrients on cognitive function, in both acute and chronic situations, at different time points, and in subjects in different age groups.

2.2.5 Summary

The data presented suggest that deficits in glucose regulation are associated with cognitive impairments, even in individuals without diabetes. More extensive deficits in glucose regulation, as is observed in diabetes, appear to lead to greater deficits in cognitive function, and may increase the risk of developing dementia and AD. The minimum impairment in glucose regulation needed to observe a deficit is not clear at this time.

Glucose ingestion improves memory performance compared with a non-energy placebo in individuals with memory deficits and with relatively poor glucose regulation, such as the healthy elderly and those with AD. Beneficial effects of glucose have also been shown in some studies of healthy young adults, but the effects in this age group may be limited to those with relatively poor glucose regulation or poor baseline memories. Several studies have also shown an improvement with the consumption of a mixed macronutrient meal, but it is not clear if these findings are related to the intake of a specific macronutrient, to a rise in blood glucose concentration, or to energy ingestion in general. More research is needed to determine the effects of carbohydrate foods and other macronutrients on cognitive performance, particularly in groups that are vulnerable to cognitive deficits such as the elderly.

2.3 Regulation of Appetite and Food Intake

2.3.1 Overview of appetite and food intake regulation

The prevalence of overweight and obesity in adults (World Health Organization, 1997) and the anorexia of aging, or the decrease in energy intake in the elderly, increase the risk of morbidity and mortality (Morley, 1997). Thus, it is important to understand the regulation of appetite and food intake throughout the lifespan.

A number of factors involved in appetite regulation in humans, including social, psychological, medical, and physiologic factors, may change throughout aging. Although non-physiologic factors, such as socio-economic status, functional impairment, dementia, depression, alcoholism, and drug use can have a significant impact on food intake behaviour

(reviewed in (Morley, 1997)), the focus of this review and the research presented in this thesis will be on the physiologic factors, particularly in the healthy elderly. Physiologic factors that affect appetite include signals arising from the perception, ingestion, digestion, absorption, and metabolism of food and specific nutrients (Anderson, 1994). These processes contribute to a complex interaction of peripheral and central signals that lead to the initiation, maintenance, and termination of food intake.

Before presenting the potential changes in appetite regulation that can occur throughout aging, it is useful to give a brief overview of food intake regulation. At the most basic level, when there is a physiologic need for food, sensations of hunger interact with olfaction, taste, vision, and hearing to drive an organism to seek food and initiate ingestion until the requirement for food is satisfied (Anderson, 1996; Morley & Thomas, 1999). This requirement for food may be driven by an energy deficit, but may also be driven by more nutrient-specific requirements. That is, although energy requirements may override other nutritional needs, animals and humans may also be driven to satisfy a specific need for protein, carbohydrate, fat, or for specific micronutrients (Anderson et al., 1992). Animal studies have suggested that specific mechanisms may be involved in regulating such macronutrient-specific appetites (reviewed in (Anderson, 1996)). In general, the regulation of appetite, or the duration of satiety and the interval to the next ingestion of food, depends on a complex peripheral satiety system and a central feeding system (Morley, 1987).

Both preabsorptive and postabsorptive peripheral signals are involved in communicating satiety to the CNS. The presence of food in the gastrointestinal tract, which causes distension in the stomach and duodenum, acts as a potent preabsorptive satiety signal, likely communicating with the brain via the vagus nerve (Morley, 1987). Other preabsorptive signals that increase satiety, also by communicating via the vagus nerve, include a slower rate of gastric emptying (Shafer et al., 1987), signals to the brain from chemoreceptors in the wall of the small intestine that are activated by the products of macronutrient digestion (Read et al., 1994), and the release of numerous gastrointestinal peptide hormones (reviewed in (Stubbs, 1999)). The peptides that influence satiety include, but are not limited to, CCK, which acts by stimulating the ascending vagal fibres (Morley et al., 1994), pancreatic glucagon, which exerts its effects on the liver, and the bombesin-like peptides, which may act in part by stimulating CCK (Bray, 1992; Stubbs, 1999). Postabsorptive signals, which arise after digested nutrients have been absorbed, include

signals initiated by entry into the portal vein of the liver, or by fluctuations of nutrient concentrations in plasma or the brain (reviewed in (Anderson, 1994)). Information about peripheral glucose and fatty acid metabolism that is relayed from the liver to the brain via the vagus nerve, and central monitoring of circulating glucose, insulin, and amino acids can impact the control of feeding (Anderson, 1994). For instance, a transient increase in peripheral insulin, followed by a decline in peripheral blood glucose, signals meal initiation in both rats and humans (Campfield & Smith, 1990).

The role of the central feeding system is to interpret and integrate the peripheral signals and to respond by seeking and ingesting food to maintain adequate levels of energy and other nutrients to carry out daily activities. The hypothalamus, which is part of the limbic system, is recognized as the brain region that acts as the major integrator of peripheral signals that reflect feeding status and energy balance (Anderson, 1994). The major areas within the hypothalamus that are involved in food intake regulation include the lateral hypothalamus, ventromedial nucleus, and paraventricular nucleus. These regions are involved in regulating energy intake and intake of specific macronutrients. It is believed that several neurotransmitters and centrally acting peptides are involved in regulating energy and macronutrient intake including an opioid-dopamine system that may be primarily responsible for the ingestion of high-fat foods, a neuropeptide-Y (NPY)-norepinephrine system that may be responsible for carbohydrate intake (Morley et al., 1994), and a serotonin-mediated system that may be responsible for balancing protein and carbohydrate intake (Anderson, 1994).

2.3.2 Appetite and food intake regulation in the elderly

Various components of appetite regulation are disrupted throughout aging, which could paradoxically contribute to both the substantial increase in body fat that occurs in middle age (Shimokata et al., 1989), and to the weight loss that becomes increasingly common after age 65 (Fischer & Johnson, 1990). The factors involved in the anorexia of aging, or the decrease in energy, remain unclear, however, they potentially include declines in olfaction and taste sensation, loss of teeth, increased use of medications, depression, and social isolation (reviewed in (Morley, 1997; Hetherington, 1998; Roberts, 2000)). In addition, more recent evidence suggests that physiologic impairments may also be involved, such that food intake may be

primarily influenced by external, rather than physiologic factors, in the elderly (De Castro, 1993).

The regulation of food intake has been shown to be impaired in the elderly in comparison to younger individuals over both the short and long term. In a short-term study, young men (18-35 y) showed more accurate food intake regulation 30 min after a preload meal than elderly men (60-84 y) (Rolls et al., 1995). In this study, both groups of subjects first consumed yoghurt preloads, that varied in energy, carbohydrate, and fat content, and then self-selected a lunch 30 min later. The young subjects' total consumption (preload + lunch) was within 10% of their baseline intake, whereas the older subjects overate by 10-30%. Importantly, the macronutrient composition of the preloads did not influence the satiety of the young or old subjects. In support of these findings, another preload study found that healthy middle-aged and elderly subjects compensated less accurately for the preloads when they were provided with an ad libitum meal 60 min later, than younger subjects (Keene et al., 1998). In a longer-term study, elderly men and women experienced less frequent hunger than young subjects over a 6-week span in which energy intake and body weight were substantially reduced (Moriguti et al., 2000). Finally, in another long-term study, Roberts (Roberts, 2000) found that younger men more accurately returned to their original weight after losing or gaining weight than older men. In this study, energy intake was substantially increased or decreased for a 21-day period, followed by 10 days of ad libitum consumption. During the overfeeding and underfeeding periods, both groups gained or lost similar amounts of weight, but after the 10-day ad libitum period the young men returned to their original weights by consuming the necessary amount of energy, whereas the older subjects tended to remain at their new weights. Taken together, it appears that appetite regulation is significantly blunted in the elderly, however the elderly are still somewhat sensitive to post-ingestion physiologic appetite signals.

Although appetite regulation may be blunted in the elderly, the differences between young and old adults may be less pronounced when the time period between preload ingestion and lunch intake is increased (Zandstra et al., 2000). These authors showed that the ability to regulate food intake 90 min post-ingestion did not differ between older (61-86 y) and younger (18-26 y) subjects. They found that energy compensation at lunch for four different preloads ranged from 15 to 44% in young adults and from 17 to 23% in the elderly, but these differences were not significant. One explanation for these data is that differences in the time interval

between preload consumption and lunch presentation between this study (90 min) and others (30-60 min) (Rolls et al., 1995; Keene et al., 1998) explains the fact that compensation did not differ between the age groups. That is, it is possible that physiologic effects on appetite are stronger in younger than in older subjects soon after preload consumption, but that the differences are less pronounced after a longer period has passed. Although the reason for these differences cannot be determined from these studies, they may be related to the delayed gastric emptying that is observed in aging (Clarkston et al., 1997). Another explanation for these data is that the study might have been underpowered. That is, if more subjects had been tested the greater compensation observed in the younger subjects would have been significantly different from the compensation observed in the older subjects. Again, it appears that there are clearly some differences in appetite regulation between young and old subjects, but the magnitude and specific nature of the differences is not yet defined.

In addition to behavioural and social changes that occur in aging (Morley, 1997), there are several physiological changes that could contribute to age-related deficits in appetite regulation. The delayed gastric emptying that is observed in the elderly (Clarkston et al., 1997) could contribute to both a delayed satiety response and an ultimate increase in satiety because of increased gastric distension. Other physiologic changes that occur in aging that could significantly influence food intake regulation include increases in CCK, insulin, and amylin concentrations, a decreased ability of opioids to influence food intake, a decline in NPY concentrations in the hypothalamus, and an impaired ability to detect hypoglycaemia (reviewed in (Morley, 1997; Morley & Thomas, 1999; Roberts, 2000)). As mentioned, CCK, insulin, and amylin can act as satiety signals, the opioids and NPY are involved in the feeding drive, and declines in circulating glucose can initiate feeding.

The degree to which appetite responses are functional in aging may be important for regulating body weight because adaptive thermogenesis associated with excess energy intake appears to be blunted (Roberts et al., 1996). Thus, unlike younger adults, seniors are likely more dependent upon maintaining body weight through adjustments in intake because thermogenesis cannot override the adverse effects of over-consumption.

Another interesting area that has not been extensively investigated in the elderly is the importance of restrained eating. Restrained eating can generally be defined as concern about food intake and body weight (Herman & Polivy, 1980), and is an important characteristic that

may significantly impair an individual's ability to regulate food intake based on physiological signals. Several studies have shown that lean, unrestrained young adults generally have a better ability to regulate food intake than obese adults (Herman & Polivy, 1980; Rolls et al., 1994) and that restrained eaters may be more sensitive to external cues than unrestrained eaters (Fedoroff et al., 1997). Anecdotally, it may be expected that younger adults would be restrained eaters for reasons such as body image, whereas elderly individuals may be restrained because of concerns about their health status. Whether or not restrained eating, and these different reasons are important in terms of regulating food intake in the elderly has not been systematically tested, but may be important to consider.

In summary, several experiments suggest that appetite and food intake regulation may be impaired throughout normal aging, which could contribute to both the increase in body weight that occurs throughout middle age as well as the anorexia that occurs in advanced aging. The specific mechanisms that would be expected to contribute to the behaviours that would lead to these divergent conditions are not known at this time. Although most short-term research suggests that the elderly overeat, which could lead to increases in body weight, more long-term research and more research in both middle and advanced age are needed to determine if these physiologic disturbances could also contribute to the anorectic effects of aging. More work is needed to determine the extent and nature of the deficits in energy and macronutrient regulation in elderly subjects over the short and long term, and to determine the importance of both environmental and physiological factors.

2.3.3 Effects of protein, carbohydrate, and fat on appetite and food intake regulation

As discussed, when energy needs are increased, the physiological response of organisms is to consume more food. Because energy is provided by the three macronutrients, protein, carbohydrate, and fat, each of these has been investigated for a specific role in appetite regulation. It has been suggested that the macronutrients may influence appetite differently, such that protein increases satiety more than carbohydrate, which increases satiety more than fat (Stubbs, 1995). The evidence for this hypothesis and potential mechanisms will be discussed. The fourth macronutrient, alcohol, also provides energy and may influence appetite (Poppitt et al., 1998), but its effects are beyond the scope of this discussion. Finally, not only do the

macronutrients differentially affect food intake, but different types of each macronutrient influence food intake. The evidence for this will be discussed in the next section.

It is relevant to this discussion that most of the work examining the effects of macronutrients on appetite and food intake regulation has been conducted in young adults, however, because of the potential for impairments in regulation in the elderly, it is important for future studies to address whether macronutrient-specific effects on satiety are similar or different in the elderly.

For the purpose of this review, it is important to distinguish between the terms satiety and satiation. Satiety has been defined as the ability of a food to inhibit hunger and reduce food intake at a subsequent meal, whereas satiation refers to the ability of a food to shorten the duration of a meal (Blundell, 1979). The present discussion and research presented here concerns satiety, but satiation is a closely related concept.

2.3.3.1 Effects of dietary macronutrient ingestion on satiety and food intake in young adults

Although protein, carbohydrate, and fat all provide energy, each one has properties that lead to different signals to the CNS, and ultimately have different effects on hunger and satiety. Indeed, controlled studies in rodents have shown that protein preloads given by gavage induce higher satiety than the other macronutrients, and that carbohydrate induces higher satiety than fat (reviewed in (Anderson, 1996)). Numerous researchers have attempted to determine whether these findings are also evident in humans. Overall, the totality of the data support the notion that protein has a stronger physiologic effect on satiety than the other macronutrients, however, the data are not as robust as in animals. The most obvious explanation for the less robust effects in humans, as presented below, is that they are due to factors other than the physiologic effects of the macronutrients and to inconsistencies in the methodologies used across studies. That is, the observed physiologic effects of each macronutrient on subsequent food intake in humans may be minimized because of a number of confounding variables including social and environmental influences as well as differences in food characteristics, such as taste, appearance, and energy density.

Epidemiologic and metabolic studies have been used to support the argument that protein has the strongest effect on satiety and fat the weakest. For instance, under free-living conditions high fat intake is positively associated with being overweight, and high carbohydrate

intake is negatively associated with being overweight (reviewed in (Hill & Prentice, 1995; Lissner & Heitmann, 1995)). Metabolic feeding studies have suggested that fat may increase satiety less than carbohydrate because when individuals are allowed to consume an ad libitum diet over several days, there is over-consumption of energy on a high-fat diet compared with a high-carbohydrate diet (Lissner et al., 1987; Stubbs et al., 1995; Tremblay et al., 1995). Despite these findings, these studies do not clearly indicate that the macronutrients are the specific factors that mediate food intake because in addition to the differences in macronutrient content, these diets also vary in total energy and energy density, which could also affect satiety.

To eliminate energy as a confounding variable others have examined the effects of the macronutrients on appetite by comparing two or three diets containing high concentrations of protein, carbohydrate, or fat of equal energy on subsequent satiety. One accepted method of assessing the short-term effects of food on satiety is usually referred to as the preload paradigm. This method involves providing subjects with preload meals, such as high-carbohydrate and high-fat meals, on separate days, preferably containing the same amount of total energy and energy density, and assessing subsequent subjective ratings of appetite, such as hunger and fullness, over the next 30 min to several hours (Rolls & Hammer, 1995). Visual analogue scales (VASs), which ask subjects to rate their feelings on a word-anchored scale, are commonly used to assess subjective satiety and hunger (Rogers et al., 1988; Stewart et al., 1997). To get a more accurate picture of the effects of preloads on satiety, many studies also provide subjects with an ad libitum test meal at the end of the post-preload period and measure the amount consumed; the more consumed, the lesser the effect of the preload on satiety. It is noteworthy that because of issues such as palatability and the ability to blind subjects to the content of the preloads, it is often quite difficult to keep the preloads isoenergetic and of equal energy density. Conversely, when energy and energy density are controlled it is difficult to control palatability and to blind subjects to the content of the preloads. Failing to control these factors and others can have a profound effect on results because high volume (Black et al., 1993; Rolls et al., 2000) and weight (De Graaf & Hulshof, 1996), low energy density (Drewnowski, 1998; Rolls, 2000), and low palatability (Holt et al., 1995; Drewnowski, 1998) are associated with higher satiety and decreased food intake, independently of macronutrient composition.

Using this preload paradigm, some authors have shown carbohydrate to increase satiety or decrease food intake more than fat (Rolls et al., 1994; Stubbs et al., 1996; Stubbs et al., 1999),

whereas others have found no difference between these macronutrients (De Graaf et al., 1992; Crovetti et al., 1998; Poppitt et al., 1998; Rolls & Bell, 1999; Marmonier et al., 2000; Rolls, 2000). However, it has been suggested that this discrepancy may be due to differences in energy density. That is, when energy content, energy density, and palatability of the preloads are held constant, fat and carbohydrate appear to have similar effects on subsequent satiety and energy intake (reviewed in (Rolls & Hammer, 1995; Rolls & Bell, 1999; Rolls, 2000)).

Others have used the preload paradigm to examine the effects of protein on satiety. In contrast to the data suggesting that carbohydrate and fat may or may not differ in their effects on satiety, there is a strong consensus that protein induces higher satiety and decreases subsequent intake more than carbohydrate or fat. Indeed, numerous studies have shown that a high protein meal induces higher subjective satiety or decreases subsequent food intake more than high carbohydrate or fat foods (Booth, 1970; Rolls et al., 1988; Teff et al., 1989; Barkeling et al., 1990; Holt et al., 1995; Stubbs et al., 1996; Vandewater & Vickers, 1996; Porrini et al., 1997; Crovetti et al., 1998; Poppitt et al., 1998; Latner & Schwartz, 1999; Stubbs et al., 1999; Marmonier et al., 2000). By contrast, very few studies have failed to observe greater satiety after protein ingestion (Geliebter, 1979; De Graaf et al., 1992).

One limitation of many of the earlier studies in humans is that only two macronutrients were compared within the same study. To further understand whether each macronutrient exerts different effects on satiety it is important to examine some recent studies that have compared all three macronutrients within the same study (De Graaf et al., 1992; Stubbs et al., 1996; Crovetti et al., 1998; Poppitt et al., 1998; Stubbs et al., 1999; Marmonier et al., 2000). Although five of these six studies found protein to increase subjective satiety more than carbohydrate and/or fat (Stubbs et al., 1996; Crovetti et al., 1998; Poppitt et al., 1998; Stubbs et al., 1999; Marmonier et al., 2000), only two found protein to actually decrease subsequent food intake more than the other macronutrients (Poppitt et al., 1998; Stubbs et al., 1999); the other studies found no differences in food intake after the macronutrient preloads. Various protocols were used in these studies, which may help explain the discrepancies, but in general, it appears that high-protein preloads increase subjective satiety consistently, but that this increased feeling of satiety does not always lead to a significant reduction in food intake.

In three of these studies, the preloads were designed to contain a high amount of each macronutrient, but at substantially varying percentages (De Graaf et al., 1992; Poppitt et al.,

1998; Marmonier et al., 2000). These studies showed protein to either increase subjective satiety and decrease energy intake (Poppitt et al., 1998), increase subjective satiety, but not affect energy intake (Marmonier et al., 2000), or not affect subjective satiety or energy intake (De Graaf et al., 1992). In the study by Poppitt et al. (Poppitt et al., 1998) lean women, aged 20-60 y consumed a baseline meal and high protein, carbohydrate, and fat meals containing 59%, 68%, and 69% of energy as the highest macronutrient, respectively, within a range of energy densities (2.4-3.1 kJ/g). The high-protein meal led to less subjective hunger and less energy intake 90 min later than the other high macronutrient preloads. In another study (Marmonier et al., 2000), healthy lean young men consumed a baseline lunch and one of three post-lunch snacks on three days containing 77% of energy as protein, 84% as carbohydrate, or 58% as fat. Importantly, energy density differed among the preloads (range of 5.0-15.1 kJ/g). They found that the high protein snack delayed the request for dinner longer than the high carbohydrate snack, which delayed the request longer than the high fat snack, but the macronutrient content of the snacks did not affect energy intake at dinner, which was consumed about 2 h after the snacks. The fact that both studies showed higher subjective satiety after protein ingestion, but that this only led to an actual decrease in intake in the first study may be related to the different compositions of the diets or to the fact that there was a longer lag between preload and subsequent energy intake in the second study (i.e., 2 h) than in the first (i.e., 90 min). Finally, in the study by De Graaf et al. (De Graaf et al., 1992) lean young women consumed isoenergetic and isoenergetically dense liquid preloads at three energy levels (400-1700 kJ) containing 77%, 99%, or 92% of energy as protein, carbohydrate, and fat respectively. No differences in subjective appetite ratings were observed over the next 3.5 h at any energy level, and no differences in energy intake were observed throughout the remainder of the day. Again, the very long lag between the preloads and energy intake or the different macronutrient compositions used in this study could explain the reason for a lack of effect on intake. One limitation of all three of these studies is that the varying percentages of macronutrients in each preload make the interpretation of the effects of each macronutrient difficult (Latner & Schwartz, 1999).

In contrast to the method of the three noted studies, three other studies attempted to keep the level of each macronutrient the same, while keeping energy content and density constant (Stubbs et al., 1996; Crovetti et al., 1998; Stubbs et al., 1999). All three studies showed the high-protein preload to induce higher subjective satiety than the other high-macronutrient

preloads, but only one found protein to decrease energy intake at the next meal (Stubbs et al., 1999). This latter study was specifically designed to control for several potential confounding variables. In one study in young men, subjective satiety was lowest during the 5 h between breakfast and lunch after a high-fat breakfast than after isoenergetically dense high-protein and high-carbohydrate breakfasts, which each comprised 57-61% of energy as the main macronutrient, and subjective satiety was greatest after the protein breakfast over 24 h (Stubbs et al., 1996). However, the effects of the breakfasts on energy intake at lunch and throughout the day were not affected by the macronutrient composition of the breakfasts. In another study in lean young women, the three macronutrient preloads comprised 68-70% of energy as each macronutrient, and total energy and energy density were similar among the preloads (Crovetti et al., 1998). These authors found that protein increased satiety sensations more than the other macronutrients over the next 7 h, however energy intake was the same after 7 h. Finally, in a study in lean young men, subjects consumed isoenergetic and isoenergetically dense high-protein, high-carbohydrate or high-fat breakfasts, each containing 60% of energy as the main macronutrient (Stubbs et al., 1999). Furthermore, the remaining 40% of energy contained equal proportions of the other two macronutrients. Subjective satiety was greatest 4 h after the protein breakfast, and energy intake at lunch was greater after the high-fat breakfast than after the other two breakfasts. However, post-breakfast ad libitum energy intake did not differ among the macronutrient preloads over the whole day. Taken together, these data suggest that high protein ingestion induces higher subjective satiety than carbohydrate or fat over a wide range of energy levels, and over a variable time span, however, it may only decrease energy intake under more specific circumstances. These circumstances cannot be defined at this time but likely relate to total energy and energy density of the preloads, the time between the preloads and the test foods and possibly the composition and taste of the test foods. Importantly, although the paradigm used in these studies (i.e., the percent of energy from the highest macronutrient is equal across all preloads) eliminates the variability in the percent of energy derived from each macronutrient, the remaining 30% or 40% of energy could affect satiety. Thus, the reason for the various effects of each macronutrient on satiety may be related to the influence of the other macronutrients on satiety. For instance, the minimal effects of fat or carbohydrate on subjective satiety may be partly related to the non-fat or non-carbohydrate components of the high-fat and carbohydrate preloads. To avoid these confounds, the effects of pure macronutrients must also

be examined.

2.3.3.2 Effects of pure macronutrients on satiety and food intake

As discussed, numerous studies have examined the effects of the macronutrients on satiety by comparing meals high in each macronutrient, however, the clearest way to determine whether or not macronutrients differ in their effects on satiety on a per joule basis is to examine the effects of pure macronutrients on satiety (Rolls & Hammer, 1995). To date, only one study has tested the effects of the ingestion of all three pure macronutrients on satiety in humans (Geliebter, 1979), partly due to the difficulty in developing palatable preloads that will not cause nausea (Rolls & Hammer, 1995). Geliebter (Geliebter, 1979) did not find any differences among the effects of isoenergetic pure protein (egg albumin), carbohydrate (cornstarch), and fat (corn oil) preload drinks (450 mL and 1184 kJ) on subjective appetite and liquid meal food intake 70 min later, in healthy young men, aged 21-36 y.

In contrast to this study, two recent studies showed that pure macronutrients differentially affect subsequent food intake (Tecimer, 2001; Woodend & Anderson, 2001). A carbohydrate preload (sucrose) suppressed food intake 60 min after ingestion compared with an isoenergetic (1254 kJ), equal volume (300 mL) fat preload (safflower oil) and a non-energy control (Woodend & Anderson, 2001). Whey and soy protein, but not egg protein or sucrose, suppressed food intake compared with a control 60 min after ingestion (Tecimer, 2001). Thus, based on these limited data, it appears that some proteins induce higher satiety than some carbohydrates, which induce higher satiety than some fats, but the results are inconsistent. The mixed data indicate that differences in macronutrient source or minor differences in energy, volume, and the time between preload and food intake can affect food intake. More studies using different macronutrient sources, doses, and time periods between preload and food intake are required to determine the generality of these findings.

Although few studies have investigated the effects of orally ingesting pure macronutrients on subsequent satiety and energy intake, others have examined the effects of providing pure macronutrients using direct gastric or intestinal infusions. Such infusions of nutrients allows a clearer investigation of the physiologic effects of specific areas of the gastrointestinal tract on appetite regulation because orosensory cues are absent, however, the generality of the findings must be made with caution because of the importance of the entire

feeding system in a normal oral feeding situation (French, 1999). Although the effects of infusing carbohydrate and fat have been studied in humans by a few investigators, the effects of infusing purified protein solutions have not been reported because it is very difficult to infuse an energy dense solution without it solidifying (French, 1999).

The results are mixed among the few studies that have infused carbohydrate and fat intragastrically or intraintestinally in healthy young adults. Although three studies have found no difference in the effects of intragastric infusions of pure fat and carbohydrate preloads on subsequent satiety and food intake (Shide et al., 1995; Cecil et al., 1998a; Cecil et al., 1999), two recent studies have actually found fat to induce higher satiety than carbohydrate when given directly into the small intestine (Cook et al., 1997; Andrews et al., 1998). The differing effects of each part of the gastrointestinal tract on food intake regulation may help explain these results. It has been shown that a food given orally has a greater impact on subsequent satiety than the same food given intragastrically, which has a greater impact than when it is given intraduodenally, suggesting that orosensory, gastric, and intestinal factors interact to elicit the greatest expression of fullness and suppression of hunger (Cecil et al., 1998b). Moreover, the influence of energy intake on gastric distension appears to override the direct effects of intestinal chemostimulation (Cecil et al., 1998b). Thus, the appetite suppressing effect of fat, compared with carbohydrate, that is observed when both are administered intraintestinally may not be strong enough to actually suppress food intake in a normal feeding situation. Consequently, the reports that carbohydrate increases satiety more than fat may be more related to differences in energy, energy density, or palatability, than to a true difference in their physiologic effects on appetite on a per joule basis (Rolls, 2000).

Taken together, the majority of the evidence obtained from a wide range of epidemiologic, metabolic, oral feeding, and infusion studies suggest that in young adults protein increases subsequent satiety and decreases food intake more than carbohydrate or fat, but results vary depending on the source of each macronutrient. When energy, energy density, and palatability are controlled, the strongest evidence suggests that there may be no detectable difference in the effects of carbohydrate compared with fat on appetite and food intake regulation, but the evidence remains mixed. Due to the minimum number of studies that have examined the ingestion of pure macronutrients on appetite and food intake regulation, while controlling confounding variables, more studies are needed to clearly determine the specific

physiologic effects of each macronutrient. In addition, as mentioned earlier, the specific effects of each macronutrient on satiety and food intake has not been described extensively in the elderly.

2.3.3.3 Mechanisms explaining the macronutrient effects on satiety and food intake

Although the reasons for an increased effect of protein on satiety are not totally clear, there are several mechanisms that may explain its effects (reviewed in (Stubbs, 1999)). For instance, the ingestion of protein is a potent stimulator of CCK, glucagon, and insulin release, which could increase satiety. The small intestine is also lined with amino-acid receptors, which could detect protein ingestion, and post-absorptive fluctuations in plasma amino acids may also exert an effect. Others have suggested that the effect of protein on satiety is related to the ability of the body to increase protein oxidation in response to an increase in protein intake (Stubbs et al., 1996), or to the greater thermic effect of protein compared with carbohydrate and fat (Crovetti et al., 1998).

The review by Stubbs (Stubbs, 1999) describes numerous effects of each macronutrient on various sites throughout the body that indicate that each macronutrient causes different appetite signals to arise which can account for the effects of each macronutrient on satiety. That is, each macronutrient exerts different signals throughout the body, which include gustatory signals, and signals related to the stomach, small intestine, large intestine, portal circulation and liver, peripheral circulation, cellular metabolism, tissue stores, neuroendocrine factors, and potentially nutrient genotype interactions (Stubbs, 1999). The potential mechanisms are too exhaustive to be repeated here, but the important point is that each macronutrient exerts different peripheral and central effects throughout ingestion, digestion, absorption, and metabolism such that the CNS can detect which macronutrients are being consumed and respond by consuming more or less food, or specific macro- or micronutrients depending on the needs of the body. Of course, in the human context, social and other environmental cues sometimes override these physiologic mechanisms.

2.3.4 Effect of type of macronutrient on appetite and food intake regulation

Several investigators have examined the effects of different sources of each macronutrient on appetite. That is, the question of whether all carbohydrates, or all fats, or all

proteins are the same has been investigated, and the data generally suggest that they are not the same. Such differences among types of macronutrients may partly explain some of the discrepancies observed in previous studies that compared different macronutrients. For instance, a study comparing a high long-chain saturated fatty acid-preload with a glucose preload may differ greatly from a study comparing a highly monounsaturated fat load with a starch load, but both studies may have reported the general effects of high-carbohydrate compared with high-fat preloads on appetite.

In humans, only a few studies have examined the effects of different proteins on appetite regulation, but suggest that different sources have different effects within 3 h after ingestion. Three studies have indicated that different proteins affect satiety differently (Uhe et al., 1992; Turnbull et al., 1993; Tecimer, 2001), and one found no differences among protein sources (Lang et al., 1998). In one study fish increased subjective satiety more than beef or chicken 3 h later in lean young men (Uhe et al., 1992), and in another study, mycoprotein, a food high in protein and dietary fibre, increased satiety and decreased food intake more than chicken 3 h later in young women (Turnbull et al., 1993). These studies were limited by the mixed macronutrient content of the meals and differences in fibre content, but a recent study examining pure protein sources supports the notion that different sources have different effects on satiety (Tecimer, 2001). As mentioned earlier, this study found that pure whey and soy protein, but not egg protein, suppressed food intake compared with a control 60 min after ingestion. By contrast, Lang et al. (Lang et al., 1998) found no differences in the effects of egg albumin, casein, gelatin, soy protein, pea protein, or wheat gluten in mixed macronutrient meals on satiety or food intake 8 h later in young men. The lack of a difference in the effects may have been due to the mixed macronutrient content of the preloads or to the prolonged period after preload ingestion. Thus, protein sources appear to have different effects on satiety, particularly when pure protein sources are used and within 1 to 3 h after ingestion.

The specific mechanism mediating the effects of different protein sources on appetite are not known. However, potential mechanisms include the varying effects of protein quality on digestion, gastric emptying, the rate of amino acid absorption and oxidation, plasma and brain amino acid concentrations and patterns, and insulin and glucagon release (Lang et al., 1998).

Different effects of fat sources on appetite have also been observed (reviewed in (French, 1999)). Few studies have been conducted in humans, but they suggest that chain length (Van

Wymelbeke et al., 1998) and saturation (French, 1999; Lawton et al., 2000) of the fatty acids affects satiety in healthy young adults. Factors influencing the effects of fats include the fact that the melting point, ease of emulsification, and therefore ease of digestion and absorption differ greatly depending on degree of saturation (French, 1999). In addition, chemical signals in the upper small intestine, including CCK, are sensitive to the composition of long-chain fatty acids.

The most work examining different sources of a macronutrient on appetite regulation has been conducted with carbohydrates. Because carbohydrates are the major energy source in our diets, and because the brain requires glucose, it is logical that carbohydrates and the glycaemic response to their ingestion could have an impact on appetite (FAO/WHO, 1998). Various carbohydrates have been shown to affect food intake behaviour, including different types of sugars, starch, and fibre (reviewed in (FAO/WHO, 1998)). For instance, the ingestion of a fructose preload leads to decreased food intake compared with a glucose preload, possibly due to differences in gastric emptying, the more gradual effect of fructose on the rate of glucose uptake and utilization, or to the greater increase in glycogen content from fructose ((Anderson, 1995; FAO/WHO, 1998)). The two digestible starches, amylose and amylopectin, also differentially affect satiety. In general, amylose has been shown to induce higher satiety than amylopectin, possibly due to the slower rate of absorption of amylose (FAO/WHO, 1998). Finally, dietary fibre has been shown to increase satiety, which may be attributable to its incomplete absorption or to its effects on decreasing energy density and palatability, prolonging chewing time, increasing gastric distension, delaying gastric emptying and intestinal transit, and affecting gastrointestinal hormones (FAO/WHO, 1998; Burton-Freeman, 2000).

The notion that the effect of foods on glucose metabolism impacts food intake originates with the glucostatic theory (Mayer, 1953). This theory suggests that hunger will increase when glucose utilization is low and satiety will increase when glucose utilization is high. Some support for this theory has been reported by researchers who have observed meal initiation in response to an increase in insulin and a drop in blood glucose concentration in both rats and humans (Campfield & Smith, 1990). Although this theory clearly does not completely explain the effects of carbohydrates on food intake regulation, it suggests that changes in blood glucose may be important.

The effect of carbohydrate ingestion on postprandial blood glucose concentration can be described by the glycaemic index (GI) (Jenkins et al., 1981). GI can be defined as the effect of ingesting a specific amount of carbohydrate on subsequent blood glucose concentration relative to the same amount of carbohydrate ingested as glucose or as another standard food, such as white bread. A food with a low GI gradually raises blood glucose whereas a food with a high GI more rapidly increases blood glucose. In general, lower GI values are related to slower rates of digestion and absorption than high GI values (Wolever, 1990).

It has been hypothesized that a low GI food may result in more prolonged satiety than a high GI food. Low GI foods could increase satiety partly because of their influence on plasma glucose, insulin, and CCK concentrations (Holt et al., 1992). Although a recent review noted that 15 studies have supported this hypothesis by showing that lower GI foods lead to increased satiety, delayed return to hunger, or decreased ad libitum intake compared with higher GI foods (Ludwig, 2000), many of these studies have not been well controlled for potential confounding variables such as energy density, fibre, and protein content (FAO/WHO, 1998). Moreover, at least five studies have not supported the hypothesis that low GI foods increase satiety more than high GI foods (Krishnamachar & Mickelson, 1987; Barkeling et al., 1995; Holt et al., 1996; Stewart et al., 1997; Anderson et al., 2001). In fact, a recent study comparing isoenergetic, equal volume liquid preloads of pure carbohydrates varying in GI showed that food intake was actually lower 60 min after ingestion of the high GI loads (glucose, polycose and sucrose) than of the low GI loads (amylose, amylopectin and a fructose-glucose mixture) (Anderson et al., 2001). Thus, the effects of foods varying in GI on appetite remain unclear (FAO/WHO, 1998; Roberts, 2000).

As with most of the studies examining the effects of macronutrients on food intake behaviour, the research studying the effects of foods varying in GI on satiety have generally been conducted in healthy young subjects because they would be expected to respond more physiologically to foods than older subjects (FAO/WHO, 1998). Nevertheless, it is important to study the effects of foods varying in GI on satiety in the elderly because the incidence of insulin resistance and diabetes is high in this age group (Harris et al., 1987), and consuming low GI foods has been used as part of the treatment for these conditions (Wolever, 2000).

2.4 Link between nutrition, cognition, and appetite in the elderly

The previous discussion describes the effects of macronutrients on cognitive performance and on appetite and food intake regulation. There are potentially some common mechanisms that may link these behaviours. As discussed, both cognition and appetite regulation appear to be disrupted with aging. In addition, the vagus nerve, various gut peptides such as CCK, and neurotransmitters such as serotonin have been shown to be involved in mediating both appetite regulation and cognition. Thus, the disruption in any of these variables will likely disrupt both appetite regulation and cognitive function. One example of the link among these variables is the relationship between fatty acid consumption, appetite regulation, and memory in rodents. It has been demonstrated that saturated fatty acids are the important component of fat involved in mediating both macronutrient selection and memory performance in rats fed various high fat diets (reviewed in (Kaplan & Greenwood, 1998)). Furthermore, the potential impairment of glucose regulation caused by saturated fatty acid intake could explain both the memory deficits and the feeding behaviour (Kaplan & Greenwood, 1998). Thus, research examining the effects of nutrition on appetite regulation may be helpful for understanding cognitive function, and the reverse may also be true.

2.5 Introduction to experimental work

Two studies were conducted for this thesis. In the first experiment, the effects of glucose regulation and the ingestion of carbohydrates varying in GI on cognitive performance, appetite regulation and food intake were examined in healthy elderly subjects. In the second experiment, the effects of the ingestion of equal energy and volume drinks of pure protein (whey), carbohydrate (glucose), and fat (safflower oil) on the same parameters were examined in healthy elderly subjects. The results of these studies are discussed in Chapters 4 to 7. Each chapter has been presented as an intact manuscript. The effects of carbohydrates on cognition are presented in Chapter 4, the effects of macronutrients on cognition are presented in Chapter 5, the effects of carbohydrates on appetite regulation are presented in Chapter 6 and the effects of macronutrients on appetite regulation are presented in Chapter 7.

CHAPTER 3

HYPOTHESES AND OBJECTIVES

3. HYPOTHESES AND OBJECTIVES

3.1 Hypothesis 1

Energy ingestion from carbohydrates (glucose, potato, and barley), protein (whey), and fat (safflower oil) enhances cognitive performance compared with a placebo in healthy elderly persons.

3.2 Objectives 1

1. To determine the relationship between glucose regulation and baseline cognitive performance in healthy elderly persons with normal fasting plasma glucose concentrations.
2. To determine the effects of carbohydrates varying in GI (glucose, potato, and barley) on cognitive performance.
3. To determine the effects of isoenergetic, equal volume drinks of pure protein (whey), carbohydrate (glucose), and fat (safflower oil) on cognitive performance.

3.3. Hypothesis 2

Carbohydrates (glucose, potato, and barley) influence satiety differently from each other, and protein (whey) induces higher satiety than carbohydrate (glucose) or fat (safflower oil) in healthy elderly persons.

3.4 Objectives 2

1. To determine the effects of carbohydrates varying in GI (glucose, potato, and barley) on subjective appetite and food intake.
2. To determine the effects of isoenergetic, equal volume drinks of pure protein (whey), carbohydrate (glucose), and fat (safflower oil) on subjective appetite and food intake.

CHAPTER 4

COGNITIVE PERFORMANCE IS ASSOCIATED WITH GLUCOSE REGULATION IN HEALTHY ELDERLY PERSONS AND CAN BE ENHANCED WITH GLUCOSE AND DIETARY CARBOHYDRATES

These data have been reported in a published document:

Kaplan, R.J, Greenwood, C.E., Winocur, G. & Wolever, T.M.S. (2000). Cognitive performance is associated with glucose regulation in healthy elderly persons and can be enhanced with glucose and dietary carbohydrates. *American Journal of Clinical Nutrition.* 72: 825-836.

4. Cognitive performance is associated with glucose regulation in healthy elderly persons and can be enhanced with glucose and dietary carbohydrates

4.1 Introduction

The proportion of North Americans with cognitive impairments is increasing as the population ages. It is important to understand environmental factors, such as nutrition, that may help prevent or reduce such deficits (Greenwood & Winocur, 1999). However, research examining the role of specific macronutrients on cognitive function in adults is inconclusive (Kanarek, 1997; Bellisle et al., 1998).

Several lines of evidence suggest that impaired glucose regulation is associated with impaired cognition and that improved regulation leads to cognitive improvements. Elderly subjects with type 2 diabetes generally perform worse on memory tests than do age-matched control subjects (Strachan et al., 1997), and nondiabetic subjects with poor glucose regulation perform worse than do those with better regulation (Hall et al., 1989; Manning et al., 1990; Craft et al., 1992; Kalmijn et al., 1995; Parker & Benton, 1995; Messier et al., 1997; Vanhanen et al., 1998; Messier et al., 1999). Moreover, diabetes-associated cognitive deficits can be enhanced by improving glucose regulation with oral hypoglycemic agents (Gradman et al., 1993; Meneilly et al., 1993).

A wide range of studies have shown that a glucose drink enhances cognitive performance compared with a placebo drink in healthy subjects and in subjects with memory deficits or poor glucose regulation (Korol & Gold, 1998). Performance is improved more consistently in healthy elderly subjects (Hall et al., 1989; Manning et al., 1997) and in patients with Alzheimer disease (Craft et al., 1992; Craft et al., 1993; Manning et al., 1993), who usually have poor memories and glucose regulation (Meneilly & Hill, 1993), than in healthy young subjects. Generally, testing begins 15–20 min after ingestion of the glucose (usually 50 g), and it has been suggested that a specific range of blood glucose concentration (8–10 mmol/L) is optimal for improved memory (Parsons & Gold, 1992; Manning et al., 1993; Benton et al., 1996). The benefits appear to be most robust on tests of declarative long-term verbal memory (conscious recollections about facts or events) (Manning et al., 1990; Craft et al., 1994; Manning et al., 1997; Foster et al., 1998), which is mediated by the medial temporal lobe, including the hippocampus and related structures (Squire & Zola, 1996).

In contrast with the glucose-drink studies, research on the effect of meals on cognitive function in healthy adults has yielded few conclusions about the effects of specific macronutrients on performance (Kanarek, 1997; Bellisle et al., 1998). The lack of consistent findings may be because these studies used very different paradigms, with testing usually beginning 30 min to 4 h after meal ingestion; additionally, only young adults were investigated.

Although the influence of glucose drinks on memory has been examined extensively, the influence of raising blood glucose concentration with common carbohydrate foods on cognitive performance has not been tested systematically. Thus, it is not known whether glucose has a special effect on cognitive function or whether any carbohydrate or energy source has a similar effect.

The purpose of the present study was to determine whether measures of glucose regulation were associated with cognition in healthy elderly persons with normal fasting plasma glucose [<6.1 mmol/L (American Diabetes Association, 1997)] and to determine the influence of glucose and common carbohydrate foods on memory and nonmemory cognitive performance in these subjects. The importance of the time point after ingestion on performance was also examined.

4.2 Subjects and methods

4.2.1 Subjects

Ten male and 10 female community-dwelling subjects aged 60–82 y were contacted through a database of previously recruited subjects at the Memory Laboratory of the University of Toronto. Subjects participated voluntarily; compensation was provided for travel. All procedures were approved by the Baycrest Centre for Geriatric Care and the University of Toronto ethics committees. Only subjects who spoke English as their native language were selected. Level of education ranged from 8 to 12 y. Evidence of diabetes [fasting plasma glucose ≥ 7.0 mmol/L (American Diabetes Association, 1997)] or cognitive decline [below age- and education-adjusted lower quartile on the Mini-Mental State Examination (MMSE; (Folstein et al., 1975; Spreen & Strauss, 1998)] were used as exclusion criteria. However, all subjects recruited met these criteria; no subjects were excluded.

4.2.2 Procedure

A repeated-measures crossover design was used such that each subject served as his or her own control and participated in all of the 4 sessions. After an overnight (10–12 h) fast during which only water was permitted, the subjects arrived at the testing centre at 0830 on the first day to complete a 30-min screening and at 0900 on the remaining 3 d. Each subject was tested individually with one test food or drink on 4 separate mornings, each separated by ≈1 wk and ≥2 d to minimize potential carryover effects. The 4 test foods and drinks were placebo or 1 of 3 treatments containing 50 g of available carbohydrate (carbohydrate minus dietary fibre): a glucose drink, instant mashed potatoes, or barley. The subjects were blinded to the placebo and glucose treatments. The order of the 4 sessions was counterbalanced such that each treatment was tested equally often on each day.

The glucose and placebo drinks were tested in an attempt to repeat the previously reported findings that 50 g glucose enhances declarative memory relative to placebo in healthy elderly subjects (Gonder-Frederick et al., 1987; Manning et al., 1990; Craft et al., 1992; Manning et al., 1992; Parsons & Gold, 1992; Craft et al., 1994; Manning et al., 1997). Mashed potatoes [high glycemic index (GI)] and barley (low GI) also containing 50 g carbohydrate were used to specifically test high-carbohydrate foods with very different effects on blood glucose (Jenkins et al., 1994).

The format for each of the 4 sessions was identical except for the first session, in which subject screening took place before testing. During screening, the subjects were administered the MMSE, fasting plasma glucose was measured for evidence of diabetes, and height and weight were measured. The subjects were also administered sample versions of each cognitive test to decrease the risk of practice effects once the subjects had experience with the test.

At 0900, blood was collected by finger prick and analyzed for fasting serum insulin at a later date. One additional drop was collected for measurement of fasting plasma glucose. After blood collection, the test food or drink was given to the subject, who was asked to try to consume the entire amount within 5 min (drinks) or 10 min (foods). Plasma glucose was measured for each subject at 15, 60, and 105 min after the start of consumption of the test food or drink. Immediately after blood collection at 15 min, the subjects were tested on 3 verbal memory tasks (immediate word list recall and immediate and 20-min delayed paragraph recall). These memory tests were used because in previous studies glucose was shown to consistently enhance performance on similar tests in healthy elderly subjects (Gonder-Frederick et al., 1987;

Manning et al., 1990; Manning et al., 1992; Parsons & Gold, 1992; Craft et al., 1994; Manning et al., 1997). The subjects were first tested on immediate recall of an audiotaped narrative word list. Immediately after this test, immediate recall of an audiotaped narrative paragraph was tested. After a 20-min delay during which they were distracted with nonverbal tasks, the subjects were tested for recall of the same paragraph. The delay period was filled with a version of a visuomotor task [Trail Making Test (or Trails) Part B Adult Form (Reitan & Wolfson, 1985)], which is known to test general brain functions, and an attention task requiring the subjects to attend to specific aspects of a television program.

After blood collection at 60 min, the subjects were tested with alternative versions of the same tests. Thus, each subject was tested on all 3 declarative memory tasks, Trails, and the attention task both 15 min and 60 min after consumption of the test food or drink. At 105 min, the subjects were given the immediate word list recall test and Trails only (no paragraph recall or attention task). Eight different versions of the paragraph recall and attention tests and 12 versions of the word list and Trails tests were required. The order of administration of the 8 different versions of each test was counterbalanced across the 15- and 60-min time points such that each test version was paired equally often with each food or drink. The 4 additional versions of the word list test and Trails were counterbalanced with test food or drink at the 105-min time point only.

4.2.3 Dietary treatments

The 4 dietary treatments were 1) placebo: 300 mL lemon beverage (290 mL water and 10 mL lemon juice) sweetened with 23.7 mg sodium saccharin (Hermesetas Original; JL Freeman Inc, Boucherville, Canada), 2) glucose: 300 mL lemon beverage (270 mL water and 10 mL lemon juice) containing 50 g glucose (Dextrose monohydrate; Bio-Health, Dawson Traders Ltd, Toronto), 3) potato: 50 g of available carbohydrate from instant mashed potatoes (Carnation Mashed potatoes; Carnation Foods Company Ltd, Carberry, Canada), and 4) barley: 50 g of available carbohydrate from pearled barley (McNair pearl barley; McNair Products Co Ltd, Montreal). The placebo and glucose drinks were matched for sweetness in order to blind subjects to the treatment (Manning et al., 1997). The instant mashed potatoes were prepared by using the package directions, but instead of adding water, milk, butter or margarine, and salt, only water was added (equivalent to recommended amount of water plus milk); 61 g potato

flakes was added to 240 mL water and heated at full power in a microwave oven for 1.5 min. Barley was prepared by adding 60 g barley to 420 mL boiling water; the barley was cooked until all of the water was absorbed (≈30 min).

The subjects were given 2.5 g butter (Gay Lea unsalted; Gay Lea Foods, Weston, Canada) and salt and pepper as desired, and 120 mL water (President's Choice Natural Spring Water; Sunfresh Ltd, Toronto) to drink with the barley and potatoes to improve palatability and compliance. Not including the drinking water, the weight (in g), volume (in mL), protein (in g), carbohydrate (in g), fat (in g), energy [in kJ; based on previous analysis (Wolever & Bolognesi, 1996)], and GI values [white bread = 100 (Foster-Powell & Miller, 1995)] for the 4 dietary treatments were as follows: placebo (300, 300, 0, 0, 0, 0, and 0), glucose (330, 300, 0, 50, 0, 837, and 142), potato (312, 325, 4, 50, 2, 979, and 118), and barley (196, 200, 5, 50, 2, 996, and 36).

4.2.4 Cognitive tests

4.2.4.1 Memory tests

Word list recall is a test of short-term verbal declarative memory that is demonstrated by the recall of material immediately after it is presented and is of limited capacity; the information can be held for up to several minutes but will be lost or replaced by new information unless it is sustained by rehearsal (Butters et al., 1995). A variation of the Rey Auditory-Verbal Learning Test (Spreen & Strauss, 1998) was developed. Twelve different word lists were constructed to be similar in difficulty. Twelve unrelated, but familiar, 2-syllable nouns made up each list. Word frequency (Francis & Kucera, 1982) was similar in each version to make the lists of similar difficulty. Each word list was recorded on audiotape; words were spoken at a rate of ≈1/s. The subjects listened to the same list 3 times in succession and were asked to immediately recall as many words as possible (including words already repeated), in any order, after each administration. Recalls were tape recorded to improve scoring accuracy. The number of words recalled was scored for each of the 3 administrations. Differences from the first to the second to the third presentations of the list represent learning (Spreen & Strauss, 1998).

For paragraph recall, immediate and delayed (20 min) recall were examined to differentiate between short (immediate) and long-term (delayed) memory functions. A total score (immediate + delayed) was also used to assess overall paragraph recall performance.

Long-term memory can be defined as the "ability to recall information after an interval during which attention is focused away from the target information" (Butters et al., 1995). Eight paragraphs of comparable difficulty, length, and context, similar to the Logical Memory subtest of the Wechsler Memory Scale—Revised [WMS-R (Wechsler, 1987)], were used. Each paragraph contained 25 ideas or scoring units. The 2 original paragraphs from the WMS-R, 1 paragraph from the new WMS-III (Wechsler, 1997), and 3 additional paragraphs (Morris revision) that were developed to be similar to these 3 paragraphs (Morris et al., 1997), were used. Finally, we developed 2 paragraphs (Kaplan revision) to be similar to the 2 original WMS-R paragraphs. These paragraphs were developed on the basis of Morris' criteria (Morris et al., 1997) to be similar in number of sentences, words per sentence, total words, total syllables, syllables per sentence, syllables per word, and textual readability [evaluated by the Flesch-Kincaid Index, the Flesch Index, and the Fog Index (Schuyler, 1982)]. Specific scoring criteria were used for each paragraph.

For each test, the subjects listened to one paragraph on audiotape. Immediately after hearing the paragraph, the subjects were asked to recall as much of the story as they could in words that were as close as possible to the original words (short-term memory). After a 20-min delay period, the subjects were again asked to recall as much as they could from the paragraph (long-term memory). The subjects' answers were recorded on audiotape to improve scoring accuracy. The subjects were distracted with nonverbal stimuli (visuomotor and attention tasks) during the delay period to discourage them from rehearsing.

4.2.4.2 Visuomotor test

Twelve alternative versions of the standard Trails Part B Adult Form (Reitan & Wolfson, 1985) were used (original plus 11 new versions). This test measures speed for visual search, attention, mental flexibility, and motor function (Spreen & Strauss, 1998) and is a sound measure of general brain functions. Subjects are required to connect 25 encircled numbers and letters, somewhat randomly arranged on a page, in proper order (1 then A, then 2 then B, and so on) as quickly as they can. Time to complete the test was used as a measure of performance (shorter times represent better scores). The subjects were corrected by the experimenter when mistakes were made, but the timer was not stopped during this time.

4.2.4.3 Attention test

The principal purpose of the attention test was to provide subjects with distracting stimuli during the delay period after immediate paragraph recall such that subjects would have difficulty rehearsing. However, this task was also used as an attention task. The subjects watched 1 of 4 episodes of a popular situation comedy on videotape. A different episode was viewed during each of the 4 sessions. The subjects watched the first 10 min of each episode during the first delay period and the last 10 min during the second delay period. While watching the television program, the subjects were asked to keep track (by marking on a page) of the number of times each of the main characters' names or specific words were spoken and the number of times doors opened and closed. The percentage correct over the entire 20-min episode was used as the score on this task.

4.2.5 Blood glucose and insulin analyses

Blood was collected by finger prick into an empty vial (Eppendorf Scientific, Inc, Westbury, NJ) with a Penlet II Automatic Blood Sampler lancet device (Lifescan Canada Ltd, Mississauga, Canada). One drop of blood was used to measure plasma glucose with use of a blood glucose meter (One Touch Basic Meter; Lifescan Canada Ltd). This meter has been reported to be accurate to within 15% of laboratory results 96% of the time (One Touch Basic Test Strip package insert, 1997).

Approximately 5 drops of fasting blood were collected into an empty 1.5-mL vial (Eppendorf Scientific Inc) for serum insulin analysis. The blood was left at room temperature to clot. After each session, the vials were spun on a Beckman Microfuge II (Beckman Instruments Inc, Brea, CA) at 9000 X *g* for 10 min at room temperature and the serum was removed and frozen at -70°C until analyzed. Samples were analyzed in duplicate at the Banting and Best Diabetes Core Laboratory, University of Toronto, by using radioimmunoassay (Pharmacia Insulin RIA, Dorval, Canada). At the time of analysis, serum samples from all 4 sessions for each subject were pooled to get one estimate of fasting serum insulin for each subject. The main reason for using this method of collection was to improve compliance. The insulin assay required ≈250 μL serum for accurate duplicate analysis. We believed that using an additional, more invasive blood collection technique (i.e., collection of venous blood) may have deterred subjects from participating.

Homeostasis model assessment (HOMA) was used to estimate β-cell function and insulin resistance from fasting plasma glucose (average of all 4 sessions) and insulin concentrations (Matthews et al., 1985). This model was highly correlated with estimates from other standard techniques (Matthews et al., 1985). One-hundred-percent β-cell function and an insulin resistance value of 1.00 represent normal values for a normal-weight healthy person aged ≤ 35 y; lower β-cell and higher insulin resistance values represent relative impairments. Incremental area under the glucose response curve (gAUC) was determined from the plasma glucose values obtained after the consumption of the glucose drink.

4.2.6 Statistical analyses

Statistical analyses were conducted by using SAS 6.12 (SAS Institute Inc, Cary, NC). Repeated-measures analysis of variance (ANOVA) was used to determine the influence of food, time, delay (paragraph recall), repeat (3 presentations of word lists), and sex and their interactions on performance for each test. Simple contrasts were used to determine the effect of each food or drink compared with placebo. Contrasts comparing successive means were used for predicted outcomes (food effects on plasma glucose and Trails performance over time). To assess the relation between baseline cognitive performance and glucose regulation, linear and multiple regression analyses were conducted using performance under the placebo condition as the response variable and β-cell function, insulin resistance, gAUC (Wolever et al., 1991), and body mass index (BMI; in kg/m^2) as predictor variables. To assess the effect of each carbohydrate on cognitive performance, linear and multiple regression analyses were conducted using performance for each dietary treatment compared with placebo (improvement with food) as the response variable and overall placebo score (baseline performance), β-cell function, insulin resistance, and gAUC as predictor variables. Because of the potential for multicollinearity among response variables, the best model was predicted by determining the subset of response variables that had the highest adjusted R^2 value (Streiner, 1994). An analysis of the risk of regression to the mean (Trochim, 1999) was conducted to determine the appropriateness of regressing baseline performance against the improvement with food. A multivariate ANOVA to test for homogeneity of slopes among dietary treatments was conducted. Statistical significance was set at $P < 0.05$. Results are reported as means ± SEMs unless indicated otherwise.

4.3 Results

4.3.1 Characteristics of food ingestion and effects on glucose regulation

All 20 subjects consumed the placebo and glucose drinks within 6 min (placebo, 2.5 ± 0.3 min; glucose, 2.7 ± 0.3 min) and the potatoes and barley within 16 min (potatoes, 9.4 ± 0.7 min; barley, 10.7 ± 0.7 min). The placebo and glucose drinks were entirely consumed by all subjects; 4 subjects did not consume all of the potatoes and 5 subjects did not consume all of the barley. Consumption of potatoes and barley was 95.2 ± 2.3% and 91.3 ± 4.2%, respectively. On a palatability scale of 0 (very pleasant) to 10 (not at all pleasant), the glucose drink was rated by the subjects as most palatable (2.6 ± 0.5), followed by placebo (4.1 ± 0.6; $P = 0.0004$), then potato (6.1 ± 0.6; $P = 0.0245$), which did not differ significantly from barley (6.6 ± 0.6). Subject characteristics and glucose regulation measurements are reported in **Table 4.1**. No significant differences in any of these measures were evident between men and women.

Table 4.1
Characteristics of the subjects[1]

	All subjects ($n = 20$)	Men ($n = 10$)	Women ($n = 10$)
Age (y)	72.3 ± 1.4	74.8 ± 1.9	69.7 ± 1.9
Education (grade)	10.6 ± 0.3	10.1 ± 0.5	11.0 ± 0.4
MMSE (out of 30)	28.0 ± 0.3	27.6 ± 0.5	28.4 ± 0.3
BMI (kg/m^2)	25.1 ± 0.9	25.7 ± 1.3	24.6 ± 1.4
Fasting plasma glucose (mmol/L)	5.4 ± 0.1	5.4 ± 0.2	5.4 ± 0.1
Fasting serum insulin (pmol/L)	52.5 ± 5.6	55.2 ± 7.6	49.8 ± 8.7
β-cell function (%)[2]	78.4 ± 7.1	79.8 ± 7.9	77.0 ± 12.1
Insulin resistance[2]	1.77 ± 0.22	1.90 ± 0.31	1.65 ± 0.30
gAUC[3]	325.1 ± 35.3	333.2 ± 42.3	316.9 ± 56.2

[1]Mean ± SEM. MMSE, Mini-Mental State Examination; gAUC, incremental area under the glucose response curve. There were no significant differences between men and women.

[2]Calculated from fasting plasma glucose and insulin concentrations by using Homeostasis Model Assessment (Matthews et al., 1985).

[3]Determined from plasma glucose concentrations 0, 15, 60, and 105 min after ingestion of a 50-g glucose drink.

The influences of the 4 dietary treatments on plasma glucose at each time point are shown in **Figure 4.1**. As expected, glucose-drink and potato consumption caused the largest rise in plasma glucose concentration, followed by barley and then the placebo drink. Significant differences in incremental area under the glucose response curve were observed among all 4 groups. Mean incremental area under the curve after glucose ingestion (325.1 ± 34.3) > potato (269.6 ± 21.4; $P = 0.033$) > barley (85.3 ± 10.9; $P < 0.0001$) > placebo (-11.1 ± 7.1; $P < 0.0001$).

4.3.2 Relation between baseline cognitive performance and glucose regulation

The relation between baseline cognitive performance (performance during the placebo condition) and measures of glucose regulation in subjects with normal fasting plasma glucose (<6.1 mmol/L) was determined by linear and multiple regression analysis. This analysis included data for 19 of the 20 subjects; one male subject who had impaired fasting glucose (mean of 4 samples: 6.4 mmol/L) was excluded.

Total scores (combining scores at all time points) on each test were used as the response variables for this analysis because only performance with placebo was examined; there was no need to assess a time effect. β-cell function and insulin resistance (assessed from fasting glucose and insulin), gAUC (determined from the blood glucose response to the glucose drink), and body mass index (BMI; in kg/m^2) were used as the predictor variables. The linear regression analyses for each cognitive test are presented in **Table 4.2**, and the linear regressions between total paragraph recall performance (an overall paragraph recall score) and measures of glucose regulation and BMI are shown in **Figure 4.2**. Collectively, these data suggest an association among poor cognitive performance and high gAUC, low insulin resistance (good insulin sensitivity), and low BMI. There was a trend for poorer performance to be associated with poor (low) β-cell function, but no significant associations were observed. No systematic differences were observed between men and women.

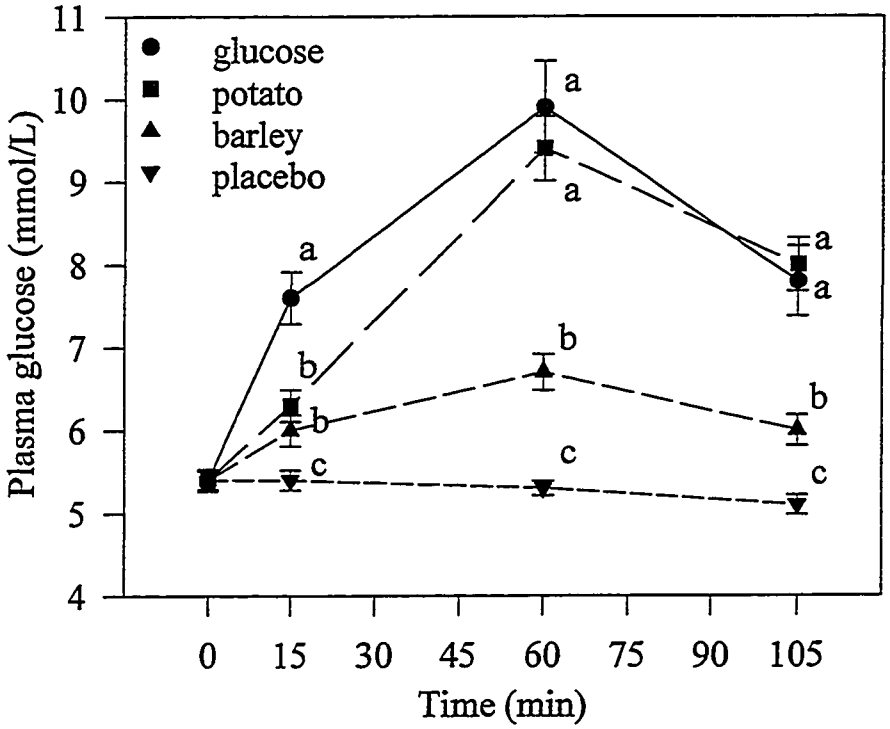

Figure 4.1. Mean ± SEM plasma glucose response to test foods and drinks after initial consumption. Values with different letters at each time point are significantly different: 15 min, $P < 0.0016$; 60 and 105 min, $P < 0.0001$.

Table 4.2

Linear and multiple regressions between cognitive performance and glucose regulation[1]

	gAUC[1]		β-cell function[2]		Insulin resistance[2]		BMI		Multiple regression[2]		
	r	*P*	*r*	*P*	*r*	*P*	*r*	*P*	Significant predictors	R^2	*P*
Paragraph											
Imm[4]	-0.42	0.0712	0.33	NS[5]	0.42	0.0737	0.48	0.0355[3]	gAUC, BMI	0.39	0.0196[3]
Del	-0.41	0.0781	0.30	NS	0.51	0.0244[3]	0.48	0.0377[3]	gAUC, BC, IR, BMI	0.47	0.0506
Total	-0.43	0.0688	0.32	NS	0.48	0.0398[3]	0.49	0.0327[3]	gAUC, BMI	0.40	0.0174[3]
Word list[4]											
1	-0.47	0.0431[3]	0.17	NS	0.25	NS	0.45	0.0534	gAUC, BMI, BC	0.44	0.0292[3]
2	-0.31	NS	0.01	NS	0.13	NS	0.23	NS	none	--	--
3	-0.36	NS	0.01	NS	-0.04	NS	0.20	NS	none	--	--
Total	-0.39	0.0948	0.06	NS	0.10	NS	0.29	NS	none	--	--
Trails[5]											
Total	0.47	0.0405[3]	-0.21	NS	-0.40	0.0918	-0.23	NS	gAUC, IR, BC	0.38	0.0579
Attention											
Total	-0.14	NS	-0.25	NS	-0.16	NS	0.15	NS	none	--	--

[1]gAUC, incremental area under the glucose response curve; imm, immediate recall over both time points (15 and 60 min) combined; del, delayed recall over both time points combined; NS, not significant. β-cell function (BC) and insulin resistance (IR) were calculated from fasting plasma glucose and insulin concentrations by using Homeostasis Model Assessment (Matthews et al., 1985).

[2]Multiple regression analysis (cognitive performance on each test used as response variable and gAUC, BC, IR, and BMI used as predictor variables). The subset of variables with the highest adjusted R^2 are given (actual R^2 shown in table). Predictors had to meet the 0.1500 significance level for entry into the model.

[3]$P \leq 0.05$.

[4]1, 2, and 3 represent scores on the first, second, and third presentations of work lists over all 3 time points (15, 60, and 105 min) combined; total refers to the score over all time points combined.

[5]A lower score (faster) represents better performance.

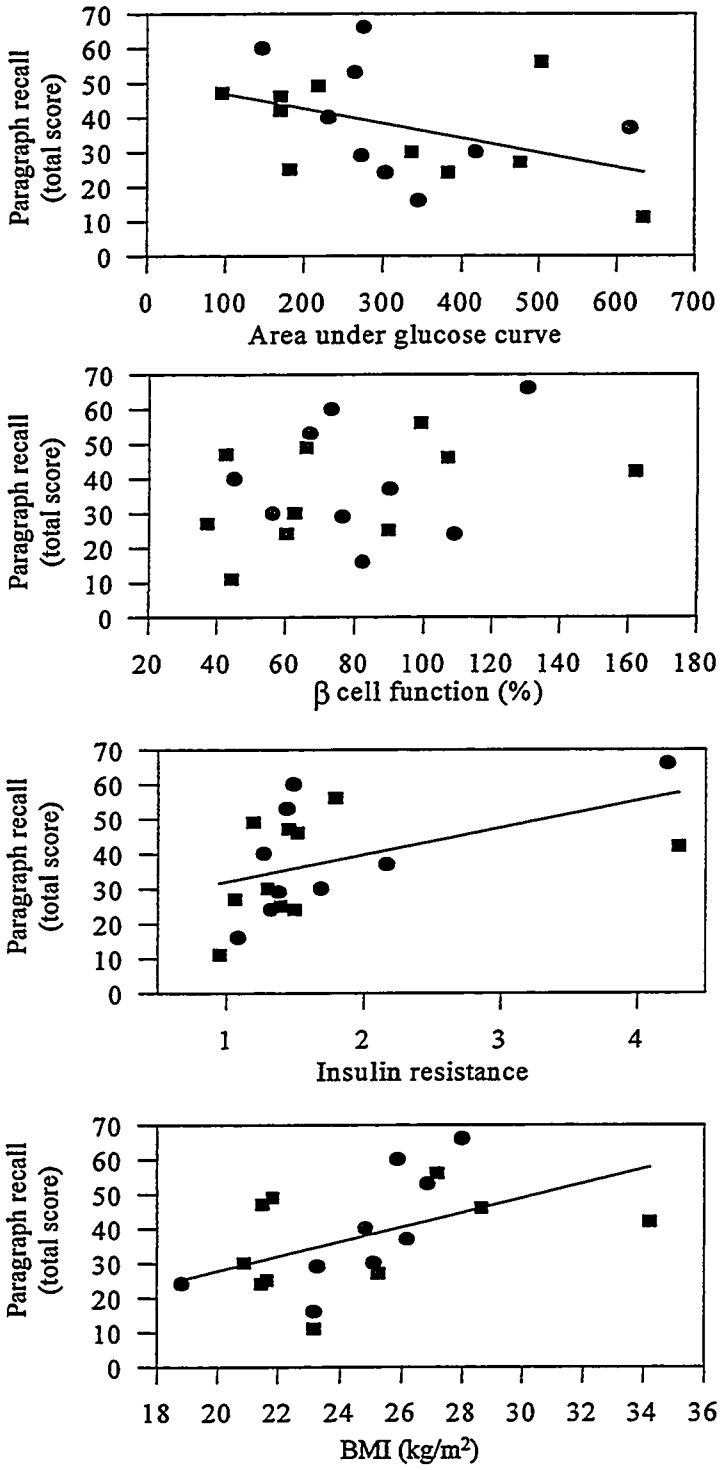

Figure 4.2. Linear regression analyses for regression between paragraph recall performance (total score on all 4 tests: immediate and delayed recall at 15 and 60 min combined) and measures of glucose regulation and body mass index for all 19 subjects. Regression lines shown if $P < 0.10$. Data for men (●) and women (■) are shown for comparison. r and P values for all subjects were as follows: area under glucose curve, $r = 0.43$, $P = 0.0688$; β cell function, $r = 0.33$, $P = 0.1786$; insulin resistance, $r = 0.48$, $P = 0.0398$; and BMI, $r = 0.49$, $P = 0.0327$. Note that the r value for insulin resistance changed only slightly (to 0.44) when the 2 subjects with the highest insulin resistance were removed.

It is important to note the regressions among the predictor variables. BMI was significantly associated with β-cell function ($r = 0.58$, $P = 0.0100$) and insulin resistance ($r = 0.70$, $P = 0.0008$). β-cell function was significantly associated with insulin resistance ($r = 0.79$, $P < 0.0001$). In contrast, gAUC was not associated with any of the other variables. These results indicate that the significant influences of insulin resistance and BMI on cognitive function may be strongly related to each other.

The results of the multiple regression analyses are also shown in Table 4.2. These results indicate that the predictors were able to account for between 38% and 47% of the variation in paragraph recall, word list recall (first presentation only), and Trails performance. An important observation from the multiple regression analyses was that β-cell function, which did not significantly predict performance in the linear regression analyses, was a significant predictor of cognitive performance.

Overall, the linear and multiple regression analyses generally suggested that subjects with relatively high gAUC, poor β-cell function, good insulin sensitivity, and low BMI performed worse on several cognitive tests. The opposite profile of glucose regulation and BMI suggested superior performance.

4.3.3 Effects of glucose and dietary carbohydrates on cognitive performance

When the effects of food consumption on performance were examined, data for all 20 subjects were included. Overall performance after consumption of glucose, potatoes, or barley did not significantly differ from performance after placebo ingestion on any of the cognitive tests (data not shown).

4.3.3.1 Paragraph recall

In contrast, when baseline performance and measures of glucose regulation were factored into the analyses, both poor baseline memory and poor β-cell function were associated with improvements in memory performance for all 3 carbohydrates (glucose, potato, and barley) compared with placebo.

The mean score on immediate paragraph recall for all subjects was 10.3 ± 0.3 and the top score was 20 (out of 25). Thus, no subject reached ceiling performance.

Repeated-measures ANOVA for paragraph recall showed an effect of delay ($P < 0.0001$), as anticipated; performance was better for immediate than for delayed recall. No main effect of food (glucose, potato, or barley), time (15 compared with 60 min), or sex on performance was observed. However, a food X delay interaction ($P = 0.0357$) was apparent, suggesting that the effect of food consumption was dependent on the test (immediate or delay) examined.

Because the results of previous studies suggest that individuals with poor memories and poor glucose regulation may be more sensitive to the cognitive-enhancing effects of glucose than are other individuals, linear and multiple regression analyses were performed using improvement with each food (food score - placebo score) as the response variable and baseline score (combined placebo score on all 4 paragraph recall tests: immediate and delayed recall at 15 min and at 60 min), and measures of glucose regulation (β-cell function, insulin resistance, and gAUC) as predictor variables. The risk of observing regression to the mean by comparing baseline score with improvement with food was determined to be minimal because baseline scores were highly correlated with total paragraph recall scores with each of the other dietary treatments ($r > 0.65$, $P < 0.002$ for all 3 carbohydrates).

Baseline performance and glucose regulation measurements were associated with improvements in performance for all 3 carbohydrates. β-cell function was a better predictor of improvement than was insulin resistance or gAUC. Linear correlations between improvement and baseline score and between improvement and β-cell function are shown in **Table 4.3**. Data are presented separately for the immediate and delayed recall scores at the 15- and 60-min time points and for total scores at each time point and total immediate and total delayed recall scores. There were no significant correlations between improvement and insulin resistance, and only improvement with barley at 15 min delayed recall and 15 min total recall were significantly correlated with gAUC ($P < 0.04$). The results of multiple regression analyses are also shown and suggest that relatively poor β-cell function, poor baseline performance, and good insulin sensitivity were all associated with various measures of improvement. These results show that the associations were generally stronger on tests of delayed recall (long-term memory) than on tests of immediate recall (short-term memory) and were most robust 15 min after ingestion of barley and potato and 60 min after ingestion of glucose.

TABLE 4.3

Linear and multiple regression analyses for paragraph recall

Improvement with dietary treatment[1]	Baseline score[2]		β-cell function[3]		Multiple regression[4]		
	r	*P*	*r*	*P*	Significant predictors	R^2	*P*
Glucose							
15 Imm[5]	-0.21	NS[6]	-0.27	NS	None	--	--
15 Del	-0.17	NS	-0.46	0.0437[6]	BC[3]	0.21	0.0437[6]
15 Tot	-0.20	NS	-0.39	0.0924	BC	0.15	0.0924
60 Imm	-0.10	NS	-0.46	0.0424[6]	BC	0.21	0.0424[6]
60 Del	-0.24	NS	-0.42	0.0686	BC	0.17	0.0686
60 Tot	-0.17	NS	-0.47	0.0345[6]	BC	0.22	0.0345[6]
Imm tot	-0.17	NS	-0.42	0.0630	BC	0.18	0.0630
Del tot	-0.23	NS	-0.51	0.0217[6]	BC	0.26	0.0217[6]
Potato[5]							
15 Imm	-0.60	0.0056[6]	-0.44	0.0526	BS, BC	0.42	0.0094[6]
15 Del	-0.52	0.0191[6]	-0.52	0.0188[6]	BS, BC	0.41	0.0117[6]
15 Tot	-0.58	0.0072[6]	-0.49	0.0283[6]	BS, BC	0.44	0.0073[6]
60 Imm	0.16	NS	-0.15	NS	None	--	--
60 Del	0.00	NS	-0.15	NS	None	--	--
60 Tot	0.10	NS	-0.16	NS	None	--	--
Imm tot	-0.34	NS	-0.42	0.0680	None	--	--
Del tot	-0.44	0.0533	-0.50	0.0264[6]	BS, BC	0.33	0.0327[6]
Barley[5]							
15 Imm	-0.76	0.0001[6]	-0.45	0.0462[6]	BS, BC, I	0.66	0.0005[6]
15 Del	-0.63	0.0030[6]	-0.48	0.0309[6]	BS, BC, I[3]	0.63	0.0009[6]
15 Tot	-0.71	0.0004[6]	-0.48	0.0344[6]	BS, BC, I	0.66	0.0005[6]
60 Imm	-0.12	NS	-0.06	NS	None	--	--

60 Del	0.09	NS	-0.15	NS	None	--	--
60 Tot	-0.01	NS	-0.11	NS	None	--	--
Imm tot	-0.69	0.0007[6]	-0.40	0.0772	BS, BC	0.52	0.0020[6]
Del tot	-0.53	0.0169[6]	-0.54	0.0144[6]	BS, BC	0.43	0.0086[6]

[1] Improvement = score with food or drink score with placebo.

[2] Baseline score (BS) = total score on all 4 paragraph recall tests with placebo.

[3] β-cell function (BC) and insulin resistance (I) were calculated from fasting plasma glucose and insulin concentrations by using Homeostasis Model Assessment (Matthews et al., 1985).

[4] Improvement used as response variable and BS, BC, I, and incremental area under the glucose response curve (gAUC) used as predictor variables. The sub-set of variables with the highest adjusted R^2 are given (actual R^2 shown in table). Predictors had to meet the 0.1500 significance level to be included in the table.

[5] 15 Imm, immediate recall 15 min after consumption; 15 del, delayed recall 15 min after consumption; 15 tot, sum of immediate and delayed recall 15 min after consumption; imm tot, sum of immediate recall of both time points combined; del tot, sum of delayed recall for both time points combined.

[6] $P \leq 0.05$.

Overall, poor β-cell function was associated with improved performance for all 3 carbohydrates compared with placebo, and poorer baseline performance was associated with improvement after potato and barley ingestion. The effects were generally stronger for delayed recall for all carbohydrates, but the strength of the effects at 15 and 60 min varied for each carbohydrate.

The linear regression and 95% mean CI between improvement and β-cell function for all 20 subjects for total delayed recall for all 3 carbohydrates are shown in **Figure 4.3**. Because the regression lines crossed 0 (*y* axis), the CIs were used to determine whether improvements were significant in subjects with poor β-cell function and whether deficits were significant in subjects with good β-cell function. CIs for significant correlations clearly indicated significant improvements in subjects with poor β-cell function (entire CIs generally exceeded 0) and were suggestive of deficits in subjects with good β-cell function. In contrast, when the CIs were examined with respect to the correlations between improvements and baseline performance, significant improvements in subjects with poor baseline performance and significant deficits in subjects with good baseline performance were observed (data not shown). Thus, not only did subjects with poor baseline memories perform better with carbohydrate ingestion, but those with good memories actually performed worse when they consumed carbohydrates.

The response to barley was generally stronger than was the response to glucose or potato. The slopes between improvement and baseline score were stronger for barley than for glucose on 15-min immediate ($P < 0.0001$), delayed ($P = 0.0029$), and total recall ($P = 0.0002$) and on total immediate recall ($P = 0.0128$) and were stronger for barley than for potato on total immediate recall ($P = 0.0342$). The slopes between improvement and β-cell function did not differ significantly among glucose, potato, and barley on any test.

Results of regression analyses performed for men and women separately were similar to the overall data and there were no systematic differences between sexes. In general, stronger associations were evident with barley and glucose in women but with potato in men (Figure 4.3).

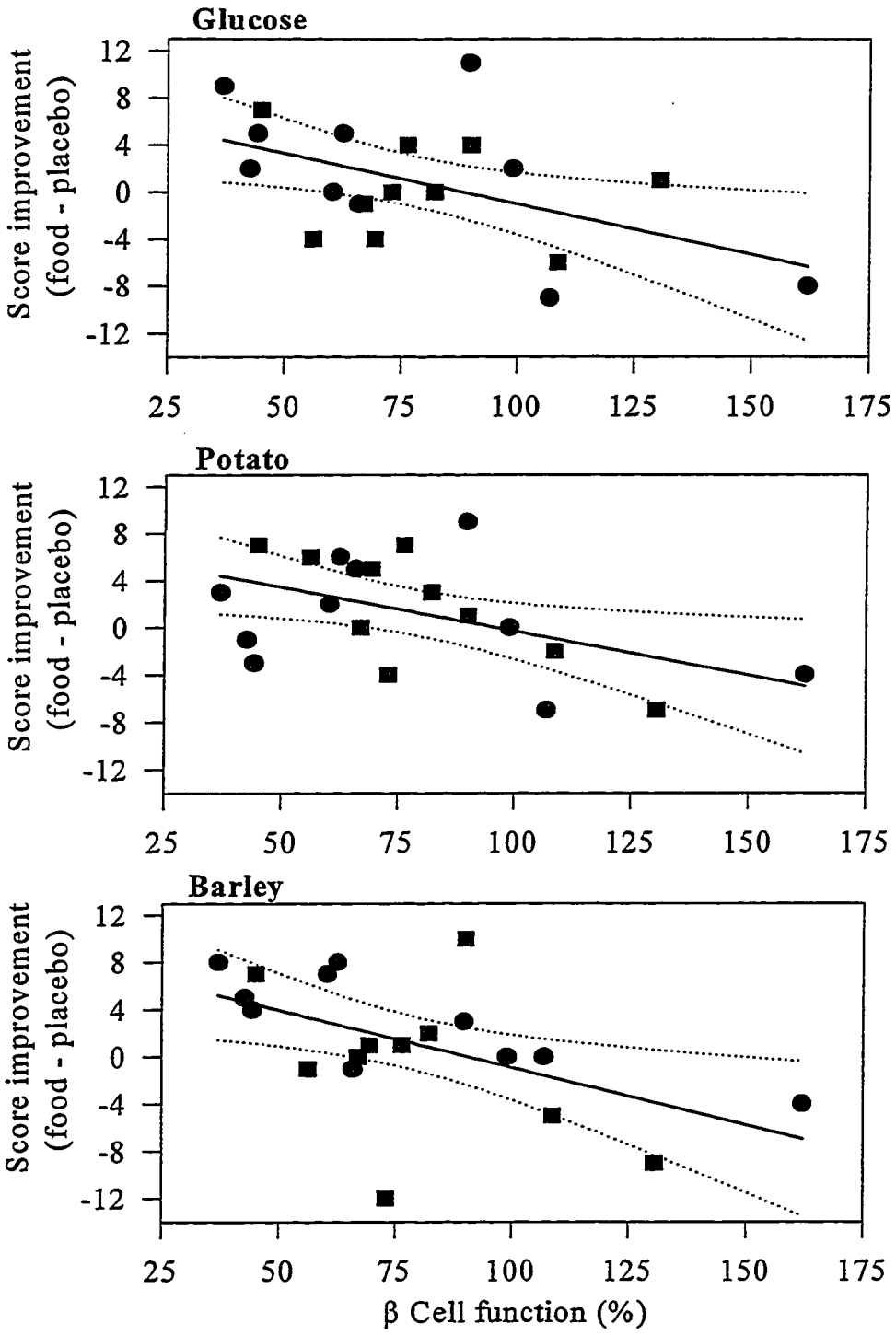

Figure 4.3. Linear regression analyses and 95% mean CIs for regression between improvement with food in score on delayed paragraph recall (15 and 60 min tests combined) and β cell function for all 20 subjects. Higher scores represent better performance. Poorer β cell function was associated with improved performance for all 3 carbohydrates compared with placebo. *r* and *P* values for all subjects were as follows: glucose, 0.51, 0.0217; potato, 0.50, 0.0264; and barley, 0.54, 0.0144. *r* and *P* values for men (■) and women (●) separately were as follows: glucose (men), $r = 0.21$, $P = 0.5659$; glucose (women), $r = 0.63$, $P = 0.0496$; potato (men), $r = 0.76$, $P = 0.0107$; potato (women), $r = 0.35$, $P = 0.3215$; barley (men), $r = 0.43$, $P = 0.2153$; and barley (women) $r = 0.80$, $P = 0.0051$.

4.3.3.2 Word list recall

An association between improvement with dietary treatment and poor glucose regulation was observed for word list recall but was not as robust as for the paragraph recall.

Similar to paragraph recall, no subject reached ceiling performance. Indeed, even by the third recall of the list, the mean and maximum performance were 6.1 ± 0.1 and 11 (out of 12), respectively.

A main effect of time on performance was observed (P = 0.0162), indicating that performance at 15 min was better than that at 60 and 105 min (P = 0.0092), and an effect of sex (P = 0.0305) indicated that women performed better than did men. Not surprisingly, a repeat effect was also evident on word list recall ($P < 0.0001$), indicating that performance was better after more presentations of the list.

The influence of food on the change in score from the first to the second presentation of the word lists, from the second to the third presentation, and from the first to the third presentation were analyzed to capture a measure of learning. In general, there was a trend for all 3 carbohydrates to be beneficial compared with placebo from the first to the second presentation but for placebo to be better from the second to the third presentation and from the first to the third presentation (data not shown). Overall, these data suggest faster learning after carbohydrate ingestion, with catch-up in the placebo group.

Linear regressions between improvement compared with placebo (total score at each time point) and glucose regulation measurements showed that fasting plasma glucose was a better predictor than was baseline score, β-cell function, insulin resistance, or gAUC (data not shown). High fasting plasma glucose values correlated significantly with improvements with glucose at 15 min (r = 0.45, P = 0.0477) and overall (all time points combined: r = 0.46, P = 0.0397) and with barley at 15 min (r = 0.48, P = 0.0307). No consistent strong associations were observed when men and women were analyzed separately (data not shown).

4.3.3.3 Visuomotor test

In general, the results of the visuomotor test were similar to those of the paragraph recall test. Poor β-cell function was associated with greater improvements in visuomotor performance (Trails) with all 3 carbohydrates compared with placebo and there was a trend for subjects with

good insulin sensitivity (low insulin resistance) to improve with food. These associations were much stronger in women than in men.

There were no significant main effects of food on Trails performance. There was a significant effect of time ($P = 0.0007$): performance at 105 min was better than at 15 min ($P = 0.0019$) or 60 min ($P = 0.0204$).

Linear and multiple regression analyses were performed using percentage improvement with each food {[(food score placebo score)/placebo score] X 100} as the response variable and baseline score (combined placebo score on all 3 Trails tests: 15, 60, and 105 min), β-cell function, insulin resistance, and gAUC as predictor variables. It is important to note that a lower score (shorter time) on this test represents better (faster) performance. The risk of observing regression to the mean by comparing baseline score with improvement with food was determined to be minimal because baseline scores were highly correlated with total Trails scores with each of the other dietary treatments ($r > 0.76$, $P < 0.0001$ for all 3 carbohydrates).

Linear correlations between percentage improvement and β-cell function and between percentage improvement and insulin resistance are shown for each of the three Trails tests and for total score in **Table 4.4**. Several strong associations between poor β-cell function and improved performance and between good insulin sensitivity and improved performance were observed for all 3 carbohydrates. A high gAUC was associated with improved performance for barley only (data not shown). There were no significant correlations between improvement and baseline score ($P > 0.05$). Multiple regression analyses indicated that in general, relatively poor β-cell function and baseline performance, high gAUC, and good insulin sensitivity were associated with improvement in performance on various measures of this task (Table 4.4).

The linear regression and 95% mean CIs between percentage improvement and β-cell function for all 20 subjects at 60 min are shown in **Figure 4.4**. CIs indicated that poor β-cell function was generally associated with improved performance after carbohydrate consumption, whereas good β-cell function was associated with poor performance after carbohydrate consumption (the entire CIs were > 0 for good β-cell function). Thus, similarly to the results obtained for paragraph recall, not only did subjects with poor β-cell function improve after carbohydrate ingestion, but those with good β-cell function actually performed worse when they consumed carbohydrates.

Table 4.4

Linear and multiple regression analyses for visuomotor test

Percentage improvement with dietary treatment[1]	β-cell function[2]		Insulin resistance[2]		Multiple regression[3]		
	r	*P*	*r*	*P*	Significant predictors	R^2	*P*
Glucose							
15 min	0.44	0.0520	0.21	NS	BC	0.19	0.0520
60 min	0.60	0.0051[4]	0.59	0.0067[4]	BC, I	0.41	0.0120[4]
105 min	0.19	NS	0.13	NS	gAUC	0.12	0.1317
Total	0.55	0.0125[4]	0.40	0.0813	BC, gAUC	0.36	0.0231[4]
Potato							
15 min	0.09	NS	0.06	NS	None	--	--
60 min	0.52	0.0198[4]	0.40	0.0782	BC,BS, gAUC	0.43	0.0253[4]
105 min	-0.12	NS	0.12	NS	None	--	--
Total	0.25	NS	0.28	NS	None	--	--
Barley							
15 min	0.62	0.0032[4]	0.52	0.0197[4]	BC, BS	0.44	0.0067[4]
60 min	0.45	0.0454[4]	0.41	0.0729	BC	0.20	0.0454[4]
105 min	0.10	NS	-0.03	NS	gAUC	0.26	0.0211[4]
Total	0.52	0.0178[4]	0.39	0.0932	BC, gAUC	0.39	0.0142[4]

[1][(Score with food or drink score with placebo)/score with placebo)] 100. A lower score (faster) represents better performance.

[2]β-cell function (BC) and insulin resistance (I) were calculated from fasting plasma glucose and insulin concentrations by using Homeostasis Model Assessment (Matthews et al., 1985).

[3]Percentage improvement used as response variable and baseline score (BS), BC, I, and incremental area under the glucose response curve (gAUC) used as predictor variables. The subset of variables with the highest adjusted R^2 are given (actual R^2 shown in table). Predictors had to meet the 0.1500 significance level to be included in the table.

[4]$P \leq 0.05$.

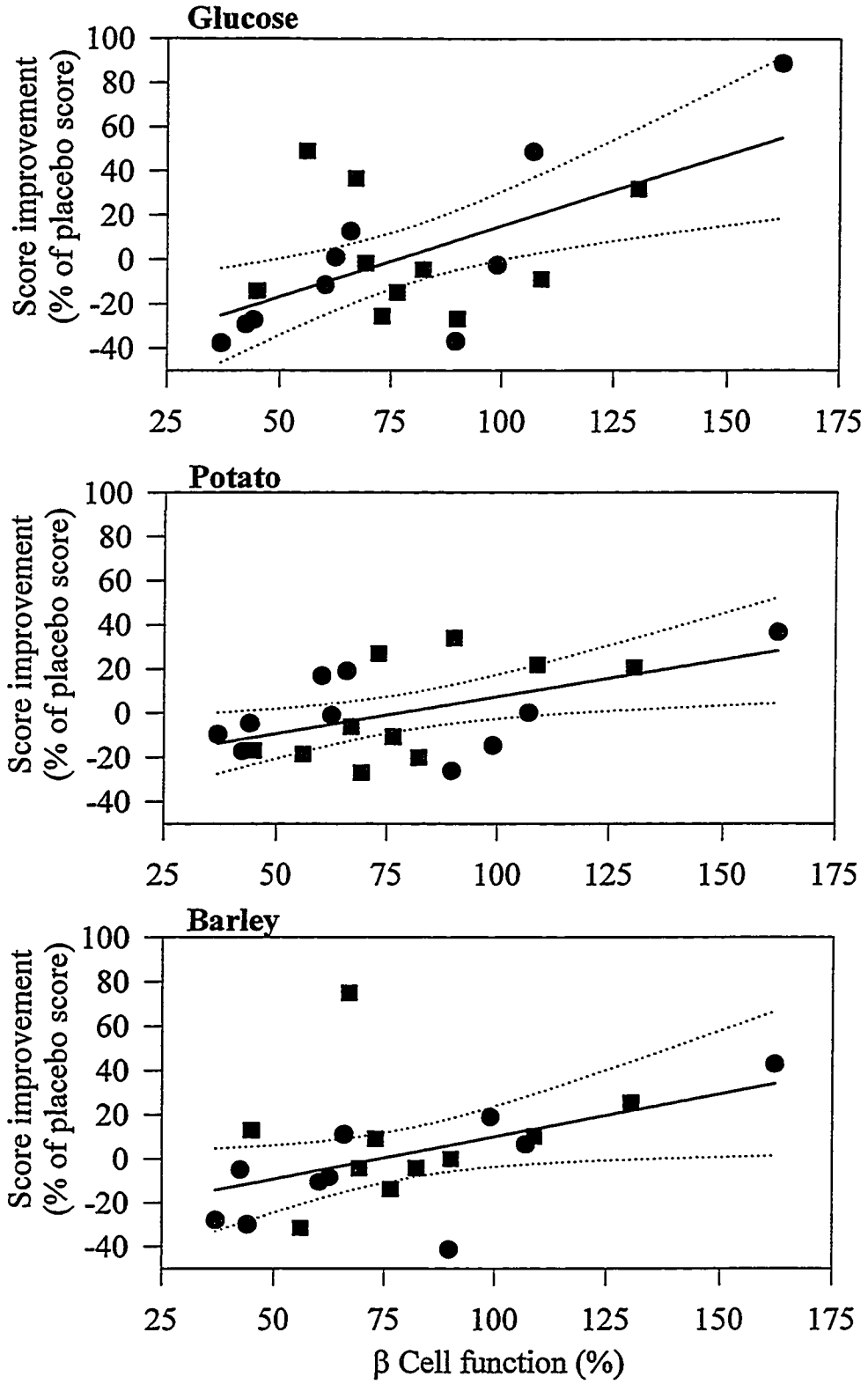

Figure 4.4. Linear regression analyses and 95% mean CIs for regression between improvement with food in score on Trails at 60 min and β cell function for all 20 subjects. A lower percentage represents better performance. Data points for men (■) and women (●) are shown for comparison. Poorer β-cell function was associated with better performance for all 3 carbohydrates compared with placebo. r and P values for all subjects were as follows: glucose, $r = 0.60$, $P = 0.0051$; potato, $r = 0.52$, $P = 0.0198$; and barley, $r = 0.45$, $P = 0.0454$.

The associations between improvement and β-cell function and between improvement and gAUC were generally stronger for barley and glucose than for potato (data not shown). The slopes between improvement and insulin resistance did not differ significantly among glucose, potato, and barley on any test.

Separate regression analyses for men and women showed similar results as the overall data for women only; there were no significant associations for men (data not shown). The r and P values for women were generally stronger than the overall associations because of the lack of a strong association in men.

4.3.3.4 Attention test

No main effects of food on the attention task were found; however, a barley X sex interaction ($P = 0.0261$) was observed, showing that women performed better with barley than with placebo ($P = 0.0135$).

Linear regression analyses between improvement with each food and baseline score, β-cell function, insulin resistance, and gAUC showed no significant associations, except that baseline score was associated with improvement with barley ($r = 0.55$, $P = 0.0128$), suggesting that subjects with poor attention improved with barley only. This same relation was significant for men ($r = 0.80$, $P = 0.0060$) but not for women. Thus, overall, men with poor baseline attention and all women performed better with barley than with placebo.

4.4 Discussion

The present study is the first to show that cognitive performance is associated with glucose regulation in the elderly before the diagnosis of impaired glucose tolerance and that, in addition to glucose, common carbohydrate-containing foods can improve cognition. Importantly, the carbohydrate-enhancing effects of these foods were independent of their effects on plasma glucose.

High gAUC, poor β-cell function, good insulin sensitivity, and low BMI were associated with poor baseline short- and long-term verbal declarative memory and visuomotor performance in cognitively intact elderly subjects with normal fasting plasma glucose. The consumption of 50 g carbohydrate as glucose, potatoes, or barley improved verbal declarative memory in individuals with poor baseline memory or poor β-cell function and improved performance on a

visuomotor task in those with poor β-cell function. Thus, individuals with relatively poor glucose regulation performed worse on cognitive tests than did those with better regulation and were most sensitive to the cognitive-enhancing effects of carbohydrates.

Although no subject obtained the maximum score on any test, it is possible that subjects with good memories had reached their upper capacities of performance at baseline. Thus, these subjects may not have had room for improvement after carbohydrate ingestion. However, it is unlikely that a ceiling effect explains why these individuals did not benefit from carbohydrates, because they actually showed a deficit in performance with carbohydrate consumption.

In the present study, glucose regulation was associated with cognition in healthy elderly subjects, extending previous findings indicating that persons with type 2 diabetes (Strachan et al., 1997) and impaired glucose tolerance (Kalmijn et al., 1995; Vanhanen et al., 1998) perform worse on cognitive tests than do those with better regulation. The novelty of the present study, the results of which are strengthened by a recent report in young adults (Messier et al., 1999), is that the relation was observed in subjects with normal glucose tolerance. Although a cause-and-effect relation cannot be established, the glucose regulatory profile of the subjects who showed poorer performance is consistent with the pathogenesis of type 2 diabetes in nonobese individuals (DeFronzo, 1987), strongly suggesting that brain function may already be impaired in very early stages of glucose dysregulation. Thus, preventing the development of impaired glucose regulation, possibly through dietary means, may help prevent cognitive decline. For instance, reducing saturated fat intake, which is associated with poor glucose regulation (Clandinin et al., 1993) and cognition (Greenwood & Winocur, 1990; Winocur & Greenwood, 1993; Greenwood & Winocur, 1996; Ortega et al., 1997; Winocur & Greenwood, 1999), may be beneficial (Kaplan & Greenwood, 1998).

Several investigators have shown that a glucose drink improves memory in rodents and humans (Korol & Gold, 1998). The results of the present study show that glucose may not be special in this respect because consumption of the same amount of carbohydrate as a high-GI food (potato) or a low-GI food (barley) produced similar cognitive-enhancing effects. In fact, a stronger relation between baseline performance and memory improvement was observed for barley than for glucose or potato, suggesting that low-GI foods may actually have greater benefits.

The effects of carbohydrates on cognitive performance appear to be specific to a subgroup of individuals and to particular cognitive tasks (Korol & Gold, 1998). Several studies, including this one, showed that individuals with poor glucose regulation may be more sensitive to the cognitive-enhancing effects of glucose than are individuals with better regulation (Messier & Gagnon, 1996). Consistent with these data, the present data also suggest that glucose and other carbohydrates have a stronger effect on functions mediated by the medial temporal lobe, such as long-term verbal declarative memory (Gonder-Frederick et al., 1987; Manning et al., 1990; Craft et al., 1992; Manning et al., 1992; Parsons & Gold, 1992; Craft et al., 1994; Manning et al., 1997), than those mediated by other brain regions, such as short-term or working memory (Manning et al., 1990; Craft et al., 1994; Manning et al., 1997), procedural memory (Craft et al., 1994; Manning et al., 1997; Foster et al., 1998), or response inhibition (Benton et al., 1994; Craft et al., 1994). The carbohydrate effects were stronger for the long-term than for the short-term memory tests, which are mediated by the prefrontal lobe (Goldman-Rakic, 1992), and minimal effects were seen on the attention task (mediated by a neural network including the parietal and frontal lobes; (Banich, 1997). Nevertheless, other brain regions may also be influenced, albeit to a lesser extent (Allen et al., 1996).

Performance on Trails, which involves several brain regions, was improved in subjects with poor β-cell function, and there was a trend for word list learning, mediated by the frontal lobe (Shimamura, 1995), to be initially improved with carbohydrates. These Trails data differ from those of another study (Allen et al., 1996), but alternative versions of the task and different measures of glucose regulation were used in the 2 studies. Thus, carbohydrates may reverse memory deficits and improve performance on difficult tasks and in individuals with poor glucose regulation but may have lesser effects on easy tasks and in subjects with good memories and good glucose regulation.

A better measure of glucose regulation (HOMA) was used in this study than in previous studies and extends our understanding of the subgroup of individuals who may be sensitive to the cognitive-enhancing effects of carbohydrates. Relatively poor β-cell function and good insulin sensitivity were correlated with the carbohydrate effects, suggesting that β-cell dysfunction (in the absence of insulin resistance) may be the important physiologic factor that predisposes an individual to be sensitive to the effects of carbohydrate. Although the mechanism is not known, the importance of insulin-secreting cells is consistent with the observation that

circulating insulin may be important in mediating brain function, independently of glucose (Craft et al., 1996).

Actual changes in blood glucose concentrations may not be important in mediating cognitive performance unless a very minor change is necessary. Barley (lowest GI) had the strongest and earliest effect on memory performance, even though plasma glucose rose to only 6.0 mmol/L by 15 min. In addition, blood glucose concentration peaked at 60 min for all 3 carbohydrates, but the strongest effects were not always at this time point. These observations contrast with the hypothesis that the benefits of glucose ingestion are related to blood glucose (Messier & Gagnon, 1996) and that an optimal concentration (8–10 mmol/L) must be obtained (Parsons & Gold, 1992; Manning et al., 1993; Benton et al., 1996). Proponents of this hypothesis argue that glucose may exert its effects by affecting the uptake and utilization of glucose by the brain, which could affect neurotransmitters (Gold & Stone, 1988; Wenk, 1989).

The fact that all 3 carbohydrates enhanced cognition suggests that the provision of energy rather than the effect on blood glucose may be important. On the basis of animal research showing that vagotomy decreases the memory-enhancing effects of peripherally injected drugs (Messier & Gagnon, 1996) and that fructose, which does not cross the blood-brain barrier, enhances memory (Messier & White, 1987; Rodriguez et al., 1994), it has been suggested that the site of action of glucose may be the liver, which could send a neural signal to the central nervous system. Thus, energy intake may be involved in mediating carbohydrate-induced cognitive improvements, possibly via gut peptides or the vagus nerve.

It is not clear why the carbohydrates had their strongest effects at various time points, but it may relate to the influence of proactive interference [i.e., the process through which memory for new material (e.g., 60 min) is disrupted by previously learned material (e.g., at 15 min)]. Healthy elderly adults are highly susceptible to this interference (Schonfield et al., 1983) and an anecdotal observation was that subjects often recalled information from earlier paragraphs and word lists on subsequent tests during the same day. Thus, the dietary treatments may affect proactive interference and therefore differentially influence performance at later time points.

Another issue examined was whether men are more sensitive to the beneficial effects of glucose than are women, which was reported previously (Craft et al., 1994). The present results do not support this finding because no systematic differences between the sexes were observed

for memory performance after carbohydrate intake, and stronger associations were observed for women than for men on the visuomotor task.

An important finding of this study was that subjects with good baseline performance and β-cell function generally performed worse after ingesting carbohydrates. Similar findings after glucose consumption in young adults have been reported (Craft et al., 1994). Thus, carbohydrate ingestion may bring relatively impaired individuals up to an optimal level of functioning but may impair those already at this level.

In summary, high gAUC, poor β-cell function, good insulin sensitivity, and low BMI were associated with poor baseline cognitive performance in healthy elderly subjects with normal glucose tolerance. In addition, carbohydrate-containing foods enhanced cognition, similarly to glucose, in subjects with relatively poor memories or poor β-cell function. Thus, individuals with seemingly minor deficits in glucose regulation appear to perform worse on cognitive tests and are most sensitive to the beneficial effects of carbohydrates.

CHAPTER 5

DIETARY PROTEIN, CARBOHYDRATE, AND FAT ENHANCE MEMORY PERFORMANCE IN THE HEALTHY ELDERLY

These data have been reported in a published document:

Kaplan, R.J., Greenwood, C.E., Winocur, G. & Wolever, T.M.S. (2001). Dietary protein, carbohydrate and fat enhance memory performance in the healthy elderly. *American Journal of Clinical Nutrition.* 74: 687-693.

5. Dietary protein, carbohydrate, and fat enhance memory performance in the healthy elderly

5.1 Introduction

The proportion of North Americans with cognitive impairments is increasing as the population ages. It is important to understand environmental factors, such as nutrition, that may help to prevent or reduce such deficits (Greenwood & Winocur, 1999). Current evidence suggests that poor glucose regulation is associated with poor cognitive performance (Strachan et al., 1997; Messier et al., 1999; Kaplan et al., 2000) and that the consumption of dietary carbohydrates can improve memory in certain situations (Messier & Gagnon, 1996; Korol & Gold, 1998). However, research examining the role of the other macronutrients on cognitive function is lacking (Kanarek, 1997; Bellisle et al., 1998).

Compared with placebo, a 50-g glucose drink improves memory performance 15–20 min after ingestion most consistently in individuals who have relatively poor memories and glucose regulation (Hall et al., 1989; Craft et al., 1992; Craft et al., 1993; Manning et al., 1993; Manning et al., 1997; Kaplan et al., 2000). Blood glucose concentrations between 8 and 10 mmol/L may be optimal for improved memory (Parsons & Gold, 1992; Manning et al., 1993; Benton et al., 1996), and the effects are most robust on tests of declarative memory (conscious recollections of facts or events) (Manning et al., 1990; Craft et al., 1994; Manning et al., 1997; Foster et al., 1998), which is mediated by the medial temporal lobes and related structures (Squire & Zola, 1996). However, we recently found that carbohydrate foods improved memory in the healthy elderly, but the effects were not related to changes in blood glucose (Kaplan et al., 2000). Barley, which only raised blood glucose to 6.7 mmol/L, improved memory similarly to glucose and potatoes, which raised blood glucose to ≈9.5 mmol/L. These results suggest that the ingestion of energy, rather than changes in blood glucose concentration, may be involved in the mechanism mediating enhancements in cognitive performance after carbohydrate intake.

In contrast with the glucose studies, few conclusions were made about the effects of protein and fat on cognition (Kanarek, 1997; Bellisle et al., 1998). Several studies showed that eating breakfast can improve cognitive performance compared with omitting breakfast (Kanarek, 1997; Bellisle et al., 1998), but the effect of each macronutrient was not defined because of various methodologic issues. First, although the effect of meals relatively high in

protein, carbohydrate, and fat on cognition was examined, all meals contained some carbohydrate. Because glucose affects cognitive performance, it is impossible to determine whether the effects of mixed macronutrient meals on performance are related to a carbohydrate-induced increase in blood glucose, to another macronutrient, or to energy intake alone. Second, testing was only conducted 30 min to 4 h after ingestion even though the most robust effects of glucose occur 15–20 min after ingestion. Finally, testing was generally limited to children and young adults, possibly concealing potentially beneficial effects of macronutrients in individuals with poorer baseline memory skills, such as the elderly. Thus, the purpose of the present study was to identify the effect of each macronutrient on cognitive performance. This was accomplished by examining the influence of equal-volume, isoenergetic, pure (> 98% of energy) protein, carbohydrate, and fat drinks on cognitive performance in healthy elderly people.

5.2 Subjects and methods

5.2.1 Subjects

We used a database of previously recruited subjects at the Memory Laboratory of the University of Toronto to contact 11 male and 11 female community-dwelling subjects aged 61–79 y. The subjects participated voluntarily; compensation was provided for travel. All procedures were approved by the ethics committees of the Baycrest Centre for Geriatric Care and the University of Toronto. Only subjects who spoke English as their native language were selected. The level of education ranged from 7 to 12 y, and no subject had evidence of diabetes (fasting plasma glucose ≥7.0 mmol/L; (American Diabetes Association, 1997) or cognitive decline (a score < 25 out of a maximum score of 30 on the Mini-Mental State Examination; (Folstein et al., 1975)).

5.2.2 Procedures

A repeated-measures crossover design was used such that each subject served as his or her own control and participated in all of the 4 sessions. After an overnight (10–12 h) fast during which only water was permitted, the subjects arrived at the testing centre in the morning on the first day to complete a 30-min screening; on the remaining 3 d, subjects arrived 30 min later. Each subject was tested individually with one test drink (placebo, protein, carbohydrate, or fat)

on 4 mornings, each separated by ≈1 wk, and no less than 3 d, to minimize potential carryover effects. The order of the 4 sessions was counterbalanced across test drinks.

During each of the 4 test sessions, blood was collected by finger prick and analyzed for fasting serum insulin at a later date. One additional drop of blood was collected for measurement of fasting plasma glucose. After blood collection, one test drink was given to each subject, who was asked to try to consume the entire amount within ≈5 min. Each subject's plasma glucose was measured 15, 60, and 90 min after the start of consumption of the test drink.

Immediately after the blood collection at 15 min, the subjects underwent 3 verbal memory tests: immediate word list recall and immediate and 20-min delayed paragraph recall. These memory tests were used because glucose was shown to enhance performance on similar tests in healthy elderly subjects (Gonder-Frederick et al., 1987; Hall et al., 1989; Manning et al., 1990; Manning et al., 1992; Parsons & Gold, 1992; Craft et al., 1994; Manning et al., 1997; Messier et al., 1997; Manning et al., 1998; Kaplan et al., 2000). The subjects were first tested on immediate recall of a narrative word list. Immediately after this test, immediate recall of a narrative paragraph was tested. After a 20-min delay, subjects were tested for recall of the same paragraph. The delay period was filled with nonverbal tasks, including the Trail Making Test (or Trails) Parts A and B Adult Form (Reitan & Wolfson, 1985) and an attention test. After blood collection at 60 min, the subjects were tested with alternative versions of the same tests. Thus, each subject was tested on all 3 declarative memory tests, Trails, and the attention test both 15 and 60 min after the start of consumption of the test drink. The assignment of test versions was counter-balanced across test drinks and time of testing.

5.2.3 Test drinks

Subjects were blinded to the content of the 4 test drinks, all of which contained 300 mL, 774 kJ (except placebo), and lemon juice; were of similar sweetness; and were consumed through a straw from opaque cups. The drinks contained the following: 1) placebo: 290 mL water, 10 mL lemon juice, and 23.7 mg sodium saccharin (Hermesetas Original, JL Freeman Inc, Boucherville, Canada); 2) carbohydrate: 260 mL water, 10 mL lemon juice, and 50 g glucose (dextrose monohydrate; Bio-Health, Dawson Traders Ltd, Toronto); 3) protein: 260 mL water, 10 mL lemon juice, 50.5 g whey protein isolate (Ultimate Balance Whey Protein Isolate, BioAdvantex Pharma, Wilmington, DE), and 23.7 mg sodium saccharin; and 4) fat: 248.9 mL

water, 10 mL lemon juice, 41.1 g microlipid (50% safflower oil emulsion; Mead Johnson Nutritionals, Evansville, IN), and 23.7 mg sodium saccharin. The percentages of energy from each macronutrient (manufacturers' analyses) for the 4 test drinks were as follows: 1) placebo: 0 kJ; 2) carbohydrate: 100% as carbohydrate; 3) protein: 98.4% as protein, 1.1% as carbohydrate, and 0.3% as fat; and 4) fat: 100% as fat.

5.2.4 Cognitive tests

5.2.4.1 Memory tests

Word list recall was used to test a form of verbal declarative memory, which is demonstrated by the recall of material immediately after it is presented and is of limited capacity; the information can only be held for a few minutes (Butters et al., 1995). Eight versions of a modified Rey Auditory-Verbal Learning Test (Spreen & Strauss, 1998) were developed (Kaplan et al., 2000), each consisting of one list containing 12 unrelated, but familiar, 2-syllable nouns. Each list was recorded on audiotape; words were spoken at a rate of ≈1/s. Subjects listened to the same list 3 times in succession and were asked to immediately recall as many words as possible. Recalls were tape-recorded to improve scoring accuracy. The number of words recalled was scored for each of the 3 administrations. Differences from the first to the second to the third presentations of the list represent learning (Spreen & Strauss, 1998).

For paragraph recall, memory was assessed immediately after presentation and after a 20-min delay. Overall paragraph recall performance (immediate + delayed) and forgetting (immediate - delayed) were also assessed. Eight paragraphs of comparable difficulty, length, and context, similar to the Logical Memory subtest of the *Wechsler Memory Scale—Revised* (Wechsler, 1987), were used as described previously (Kaplan et al., 2000). Subjects listened to one paragraph on audiotape and were immediately asked to recall as much of the story as they could. After a 20-min delay, the subjects were again asked to recall as much as they could from the paragraph. The subjects' answers were recorded on audiotape. Subjects were distracted with nonverbal stimuli (Trails and attention tests) during the delay period to discourage rehearsing.

5.2.4.2 Trails test

Twelve alternative versions of the standard Trails Parts A and B Adult Form (Reitan & Wolfson, 1985) were used (original plus 11 new versions). This test measures speed for visual

search, attention, mental flexibility, and motor function and is a sound measure of general brain functions (Spreen & Strauss, 1998). For part A, subjects are required to connect 25 encircled numbers, somewhat randomly arranged on a page, in proper order (from 1 to 25) as quickly as they can. This measures visual motor speed. For part B, subjects are required to connect 25 encircled numbers and letters, somewhat randomly arranged on a page, in proper order (1 then A, then 2 then B, and so on) as quickly as they can. Subjects were corrected by the experimenter when mistakes were made; the timer was not stopped during this time. Standard scoring methods were used to assess performance: the time to complete part A, part B, and parts A and B combined (faster times represent better scores) and the difference between the times necessary to complete parts A and B (B - A), which is sensitive to frontal lobe function (smaller differences represent better scores).

5.2.4.3 Attention test

Subjects watched 1 of 4 episodes of a popular situation comedy on videotape during each of the 4 sessions. Subjects watched the first 10 min of each episode during the first delay period (after the tests at 15 min) and the last 10 min during the second delay period (after the tests at 60 min). While watching the television program, the subjects were asked to keep track of, by marking on a page, the number of times specific words were spoken and the number of times doors opened and closed. The percentages correct over the first 10 min, the second 10 min, and the entire 20-min episode were used as the scores on this test.

5.2.5 Blood glucose and insulin analyses

Blood was collected by finger prick with a Penlet II Automatic Blood Sampler lancet device (Lifescan Canada Ltd, Mississauga, Canada). Plasma glucose was measured by using a blood glucose meter (One Touch Basic Meter; Lifescan Canada Ltd). Serum was pooled for each subject, and insulin was analyzed by the Banting and Best Diabetes Core Laboratory, University of Toronto, by using a radioimmunoassay as described previously (Kaplan et al., 2000). Homeostasis model assessment was used to estimate β-cell function and insulin resistance from fasting plasma glucose (average of all 4 sessions) and insulin concentrations (Matthews et al., 1985). The total area under the glucose response curve was determined from the plasma glucose values obtained after the consumption of the glucose drink.

5.2.6 Statistical analyses

Statistical analyses were conducted with SAS 6.12 (SAS Institute, Inc, Cary, NC). Repeated-measures analysis of variance was used to determine the influence of drink, time, delay (paragraph recall), repeat (3 presentations of word lists), and sex and their interactions on performance for each test. Simple contrasts were used to determine the effect of each drink compared with placebo. Linear and multiple regression analyses were conducted to examine the relation between baseline cognitive performance and glucose regulation and between baseline cognitive performance and the response to each drink. An analysis of the risk of regression to the mean (Trochim, 1999) was conducted to determine the appropriateness of regressing baseline performance against the improvement with drink. Statistical significance was set at $P < 0.05$, except when the Bonferroni correction was used for multiple comparisons, in which case statistical significance was set at $P < 0.02$.

5.3 Results

5.3.1 Characteristics of drink ingestion and effects on blood glucose

All subjects consumed each of the 4 drinks within 7 min (mean ± SD: 2.9 ± 1.4 min). No significant differences in the time taken to consume the drinks were observed. One male subject only consumed two-thirds of the protein drink; therefore his cognitive performance data after protein ingestion were excluded from all analyses. On a palatability scale of 0 (very pleasant) to 10 (not at all pleasant), the glucose drink was rated by the subjects as more palatable (mean ± SEM: 3.4 ± 0.4) than the other 3 drinks (placebo: 4.9 ± 0.5; fat: 5.8 ± 0.6; protein: 5.9 ± 0.6; $P < 0.006$).

Subject characteristics and glucose regulation measurements are reported in **Table 5.1.** All subjects had normal fasting plasma glucose values [< 6.1 mmol/L (American Diabetes Association, 1997)]. No significant differences in any of these measures were evident between men and women. As expected, only glucose ingestion caused a significant rise in plasma glucose concentration compared with that after ingestion of placebo (at each time point and as the incremental area under the curve; **Figure 5.1**).

5.3.2 Relation between glucose regulation and baseline cognitive performance

The relation between measures of glucose regulation and baseline (placebo) cognitive performance was examined because previous studies showed that relatively poor glucose regulation is associated with poor cognitive performance in healthy subjects (Messier et al., 1999; Kaplan et al., 2000). Total placebo scores (combining scores at all time points) on each cognitive test were used as the response variables (baseline score), and body mass index, the total area under the glucose response curve, β-cell function, and insulin resistance were used as the predictor variables. There were no significant associations for any of the cognitive tests.

TABLE 5.1
Characteristics of subjects[1]

	All subjects ($n = 22$)	Men ($n = 11$)	Women ($n = 11$)
Age (y)	71.2 ± 1.3[2]	70.0 ± 1.6	72.4 ± 2.2
Education (grade)	10.5 ± 0.3	10.2 ± 0.4	10.7 ± 0.5
MMSE[3] (maximum: 30)	28.2 ± 0.3	27.7 ± 0.4	28.6 ± 0.3
BMI (kg/m^2)	25.6 ± 0.6	25.6 ± 0.7	25.6 ± 0.9
Fasting plasma glucose (mmol/L)	5.2 ± 0.1	5.3 ± 0.1	5.2 ± 0.1
Fasting serum insulin (pmol/L)	51.9 ± 5.5	50.1 ± 8.9	53.6 ± 6.8
β-cell function (%)[2]	82.4 ± 7.5	75.9 ± 11.7	88.8 ± 9.6
Insulin resistance[2]	1.70 ± 0.90	1.66 ± 0.31	1.74 ± 0.24
gAUC (mmol • min/L)[3]	731.6 ± 23.3	730.6 ± 24.7	732.6 ± 40.9

[1]Mean ± SEM. MMSE, Mini-Mental State Examination; gAUC, total area under the glucose response curve. There were no significant differences between men and women.

[2]Calculated from fasting plasma glucose and insulin concentrations with use of the homeostasis model assessment (Matthews et al., 1985).

[3]Values were determined from plasma glucose concentrations 0, 15, 60, and 90 min after ingestion of a 50-g glucose drink.

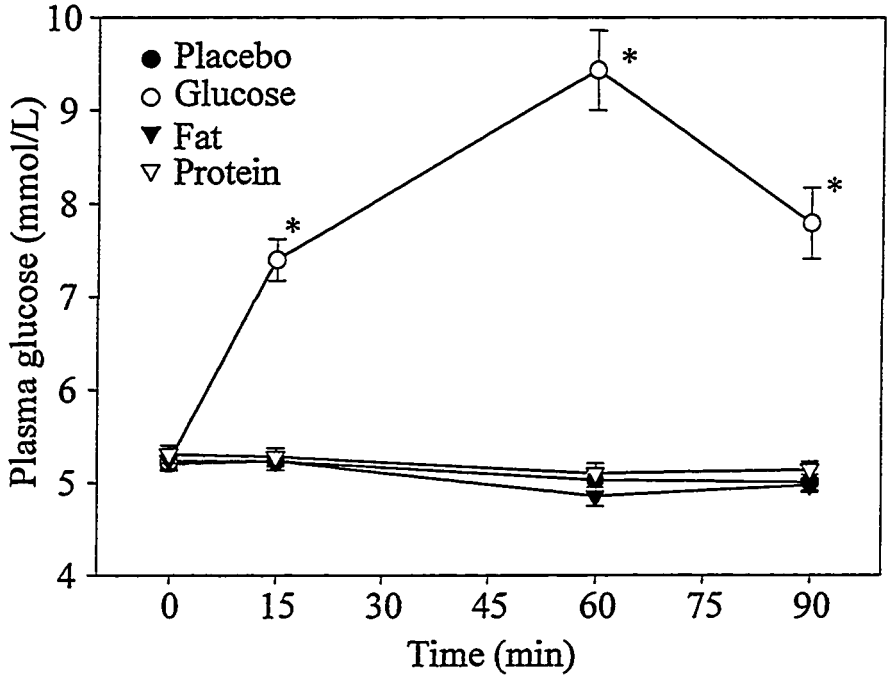

Figure 5.1. Mean (± SEM) plasma glucose concentrations in response to the placebo, glucose, fat, and protein test drinks for men and women combined (n = 21). *Significantly different from placebo, $P < 0.0001$. The incremental area under the curve was greater after glucose ingestion than after ingestion of the other drinks ($P < 0.0001$).

5.3.3 Effects of test drinks on cognitive performance

5.3.3.1 Paragraph recall

Data from one female subject were excluded from the analyses of performance 15 min after ingestion of the fat drink because she had misinterpreted the instructions. The top score was 21 of 25; no subject reached ceiling performance. All subjects were analyzed together because no main effect of sex or interactions with sex was observed. Repeated-measures analysis of variance showed a main effect of drink ($P = 0.01$); an effect of delay ($P = 0.0003$), indicating that performance was better on immediate recall than on delayed recall; a drink X time interaction ($P = 0.03$), indicating that the effect of drink ingestion was dependent on the time of testing (15 or 60 min after ingestion); a trend for a drink X delay interaction, suggesting that the effect of drink ingestion was dependent on the test (immediate or delayed recall); and a drink X time X delay interaction ($P = 0.03$).

Contrast analyses, with significance set at $P < 0.02$ (Bonferroni correction), showed that the ingestion of all 3 macronutrient drinks improved delayed recall (protein: $P < 0.0001$; glucose: $P = 0.001$; fat: $P = 0.0006$) and improved or tended to improve immediate recall (protein: P for trend = 0.04; glucose: $P = 0.02$; fat: $P = 0.008$) compared with the ingestion of placebo 15 min after consumption (**Figure 5.2**). Importantly, the improvements were stronger for each drink on delayed recall than on immediate recall. Analyses of the difference between immediate and delayed recall (forgetting) showed that there was less forgetting 15 min after protein ingestion than after placebo ingestion ($P = 0.002$). Glucose and fat ingestion led to the same rate of forgetting as did the placebo ingestion at 15 min.

In contrast with the data at 15 min, no effect of drink on paragraph recall was found 60 min after ingestion. However, there was a trend for only the glucose drink to improve performance on the composite score (immediate + delayed) when analyzed as the percentage of improvement compared with placebo (Figure 5.2). Rate of forgetting at 60 min did not differ on the basis of the type of drink consumed.

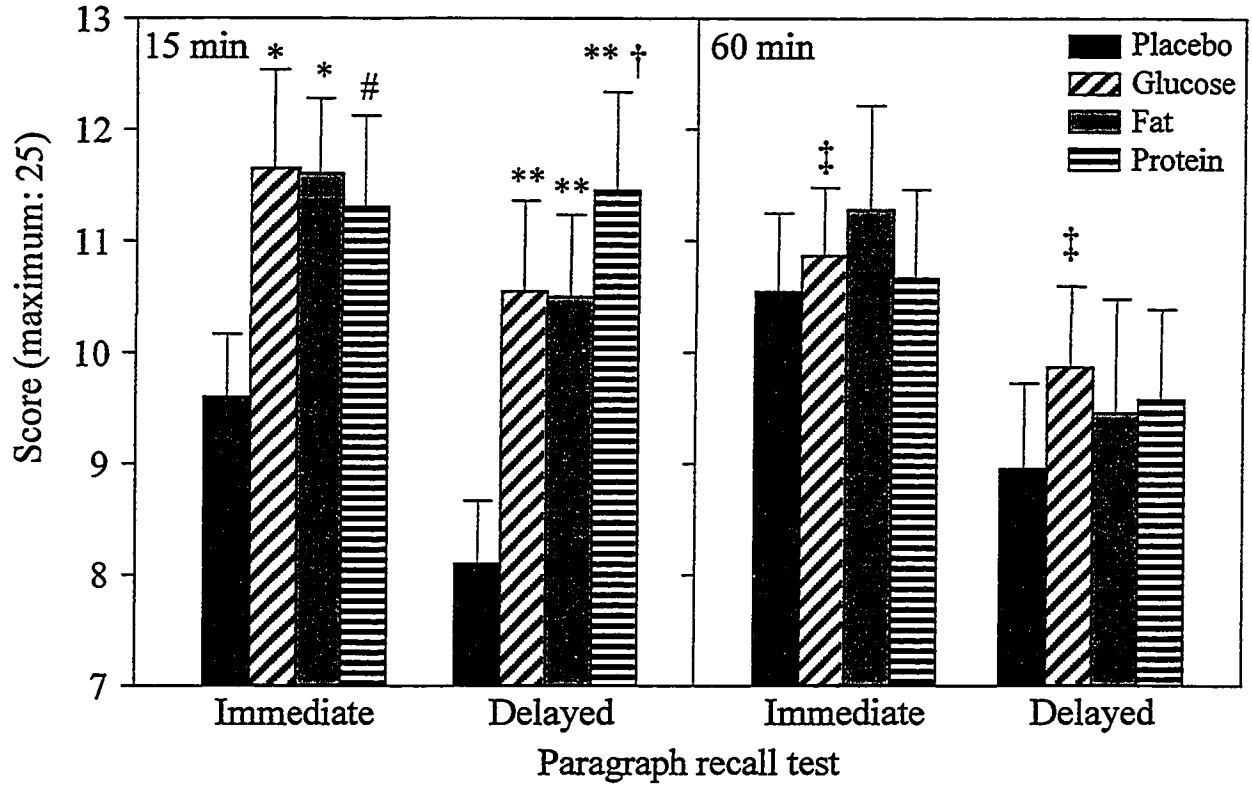

Figure 5.2. Mean (± SEM) scores on the immediate and delayed paragraph recall test 15 ($n = 20$) and 60 ($n = 21$) min after consumption of the placebo, glucose, fat, and protein test drinks for men and women combined. *,#,**,†,‡ Significantly different from placebo: *$P \leq 0.02$, #P for trend = 0.04, **$P \leq 0.001$, †$P = 0.002$ (rate of forgetting, immediate - delayed), ‡P for trend = 0.09 (for composite score, immediate + delayed).

5.3.3.2 Word list recall

Data for one male subject from the first repetition of the word list 15 min after fat ingestion were excluded from the analyses because he had difficulty hearing the list. No main effect of drink was observed. A main effect of time ($P = 0.02$) indicated that performance at 15 min was better than that at 60 min, and a repeat effect ($P < 0.0001$) indicated that, not surprisingly, performance improved after more presentations of the list. The mean ± SD) scores on the first, second, and third repetitions of the list for all data combined were 4.6 ± 1.1, 6.0 ± 1.6, and 7.0 ± 1.8, respectively, at 15 min and 4.3 ± 1.2, 5.7 ± 1.5, and 6.7 ± 1.6, respectively, at 60 min; the possible maximum score was 12. The highest score was 11; no subject reached ceiling performance.

A drink X sex interaction ($P = 0.01$) and a drink X time X repeat X sex interaction ($P = 0.01$) were observed. Contrast analyses, with significance set at $P < 0.02$ (Bonferroni correction), showed that fat ingestion led to impaired performance on total recall (all 3 lists combined) compared with placebo ingestion at 60 min ($P = 0.02$). There was a trend for glucose ingestion to lead to an overall (word lists at 15 and 60 min combined) impairment of performance compared with placebo ingestion in men only ($P = 0.03$). No effect of drink on learning (improvements from list 1 to 2, 2 to 3, and 1 to 3) was observed.

5.3.3.3 Trails test

No main effect of drink was observed. Performance was better at 60 than at 15 min ($P = 0.02$), and as expected, performance on part A was better than on part B ($P < 0.0001$). The mean (± SD) time for all data combined was 48 ± 15 s for part A at 15 min, 47 ± 14 s for part A at 60 min, 98 ± 37 s for part B at 15 min, and 92 ± 32 s for part B at 60 min.

A drink X sex interaction was observed ($P = 0.02$). Further analyses, with significance set at $P < 0.02$ (Bonferroni correction), showed that both fat and glucose ingestion improved performance compared with placebo ingestion in men on part A at 15 min ($P = 0.02$). Improvements in overall performance (parts A + B) were confined to glucose in men at both 15 and 60 min after ingestion compared with placebo (**Figure 5.3**).

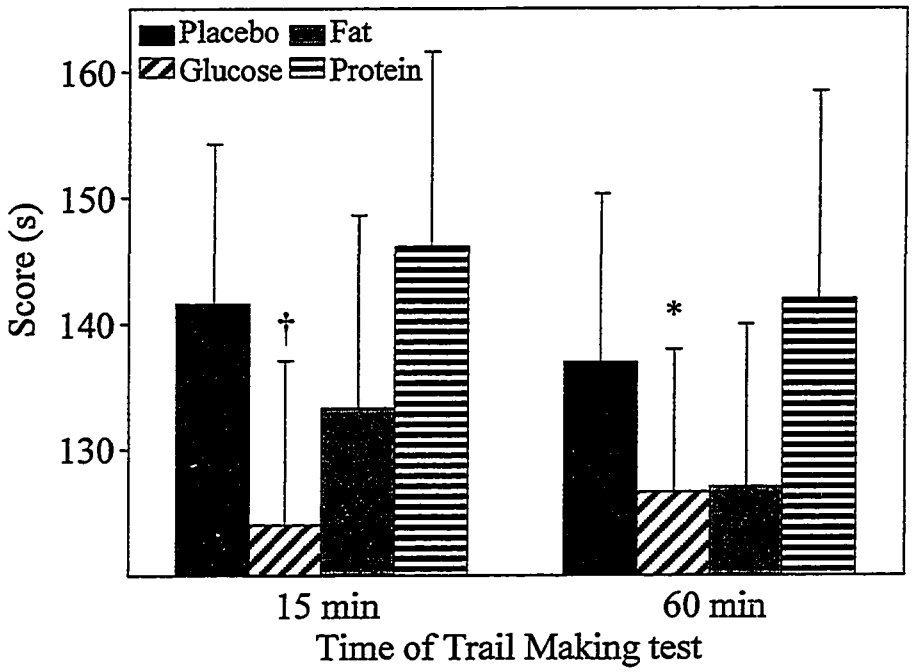

Figure 5.3. Mean ((±SEM) scores on the Trail Making Test (parts A + B) in men ($n = 10$) at 15 and 60 min after ingestion of the placebo, glucose, fat, and protein test drinks. Lower scores represent better performance. †,*Significantly different from placebo: †P for trend = 0.04, *$P = 0.02$.

The relation between baseline scores and improvement with each test drink was analyzed because previous studies showed that carbohydrates selectively improve cognitive performance in individuals with relatively poor baseline cognitive function (Kaplan et al., 2000). Statistical significance was set at $P < 0.02$ (Bonferroni correction). Strong associations between poor baseline performance (overall placebo score on parts A + B at 15 and 60 min combined) and improvement with glucose and fat were observed on several tests. No associations were observed for protein. Significant associations between baseline performance and improvement with glucose were observed for the total score (A + B) at 15 ($r = -0.54$, $P = 0.009$) and 60 ($r = -0.62$, $P = 0.002$) min, for part B at 15 ($r = -0.67$, $P = 0.0007$) and 60 ($r = -0.73$, $P < 0.0001$) min, and for the difference (B - A) at 15 ($r = -0.63$, $P = 0.002$) and 60 ($r = -0.69$, $P = 0.0004$) min. Similar associations were observed for fat: total score (A + B) at 15 ($r = -0.43$, P for trend = 0.04) and 60 ($r = -0.67$, $P = 0.0007$) min, part B at 15 ($r = -0.47$, P for trend = 0.03) and 60 ($r = -0.62$, $P = 0.002$) min, and B - A at 15 ($r = -0.41$, P for trend < 0.05) and 60 ($r = -0.50$, $P = 0.02$) min. Importantly, no relations were observed at 15 or 60 min on part A. The risk of observing regression to the mean by comparing baseline score with improvement with each drink was determined to be minimal because baseline scores were highly correlated with total Trails scores for each of the other test drinks ($r > 0.70$ and $P < 0.0003$ for all 3 drinks).

5.3.3.4 Attention test

Data from one female subject were excluded from the analyses of performance after ingestion of the fat drink because she had misinterpreted the instructions. All subjects were analyzed together because no main effect of sex or interactions with sex was observed. There was no main effect of drink; however, there was a drink X time effect ($P < 0.05$). The performance of subjects at 15 min did not differ on the basis of the type of drink consumed, but there was a trend for performance to be improved with the fat drink at 60 min compared with placebo ($P < 0.05$). The mean ± SD) percentage correct for all data combined was 66 ± 15% at 15 min and 64 ± 12% at 60 min. The highest score was 96%; no subject reached ceiling performance.

5.4 Discussion

To our knowledge, the present study is the first to show that pure dietary protein, carbohydrate, and fat all enhance memory performance. The finding that protein and fat enhanced memory was novel, whereas the benefits of glucose are supported by numerous studies in humans and animals (Messier & Gagnon, 1996; Korol & Gold, 1998). Several studies showed that consuming a mixed macronutrient breakfast can improve cognition compared with not eating breakfast, but some carbohydrate was always included in the meal, with the assumption that blood glucose must increase for an improvement to be observed (Kanarek, 1997; Bellisle et al., 1998). The effects in the present study were clearly independent of increases in blood glucose concentration because it was not affected by protein or fat intake. Thus, the ingestion of energy, regardless of source, appears to improve memory.

Although several authors consistently showed that a glucose drink improves memory in the healthy elderly compared with placebo (Gonder-Frederick et al., 1987; Hall et al., 1989; Manning et al., 1990; Manning et al., 1992; Parsons & Gold, 1992; Craft et al., 1994; Manning et al., 1997; Messier et al., 1997; Manning et al., 1998; Kaplan et al., 2000), the mechanism remains to be elucidated. One common hypothesis is that glucose ingestion may improve memory by increasing plasma glucose concentrations, leading to alterations in glucose uptake and utilization by the brain and ultimately to an increase in glucose-mediated synthesis of acetylcholine in the hippocampus region (Gold & Stone, 1988; Wenk, 1989). Evidence in rodents supports this acetylcholine hypothesis (Messier et al., 1990; Ragozzino et al., 1996; Ragozzino et al., 1998). Others have suggested that the insulin response to an increase in glucose may be responsible for the effects on memory (Craft et al., 1996; Craft et al., 1999). Kaplan et al (Kaplan et al., 2000) recently showed that a low glycemic index carbohydrate (barley), which minimally elevates blood glucose (Wolever et al., 1991), improves memory similarly to high glycemic index carbohydrates (glucose and potatoes), which suggests that energy ingestion could be responsible for the effects. Importantly, our present finding that the ingestion of energy can improve memory independently of elevations in blood glucose does not rule out the acetylcholine or insulin hypotheses but instead suggests that macronutrients may affect cognition by more than one mechanism.

The fact that memory was enhanced soon after the ingestion of energy from any macronutrient may be explained from an evolutionary perspective. A mechanism that would allow an animal to remember the details of a successful hunt for food would clearly be

beneficial for survival (Flood et al., 1987). Any potential mechanism must be consistent with the finding that glucose, protein, and fat all enhanced memory 15 min after ingestion. Within this time period, which precedes fat absorption, activation of the gut-brain axis probably plays an important role (Davenport, 1977). Several gut peptides, including cholecystokinin (Flood et al., 1987) and gastrin-releasing peptide, pancreastatin, and amylin (Morley et al., 1994), influence memory in rodents, probably via stimulation of ascending fibres of the vagus nerve (Flood et al., 1987). Indeed, electrical stimulation of the vagus in human subjects improves declarative memory (Clark et al., 1999), and vagotomy decreases the memory-enhancing effects of glucose (White, 1991) and peripherally injected drugs (Flood et al., 1987). Thus, memory may have been enhanced by all 3 macronutrients via gut-mediated responses, explaining the nonnutrient-specific improvements observed.

Although all of the macronutrients improved paragraph recall 15 min after ingestion, suggesting that energy intake can enhance specific aspects of cognition, other results from this study suggest additional macronutrient-specific effects. That is, in addition to the effects of energy ingestion on memory, each macronutrient enhanced performance on various tasks, possibly via unique mechanisms. For instance, all 3 macronutrients led to an initial, robust improvement on delayed paragraph recall; however, only glucose ingestion trended toward a sustained (60 min) improvement on this task, which is mediated by the medial temporal lobes (Squire & Zola, 1996). Furthermore, for men, only those who ingested glucose had an overall improvement on Trails, supporting the notion that glucose may exert unique effects. The fact that the effect was limited to men is consistent with previous data and may be related to hormonal differences (Craft et al., 1994).

Whereas an overall improvement on Trails was confined to those who ingested glucose, both fat and glucose, but not protein, improved performance on Trails in subjects with poor baseline scores. Importantly, the strongest benefits of glucose and fat were on part B and on the difference between parts B and A, which is sensitive to frontal lobe function (Gaudino et al., 1995), compared with part A alone, which measures visuomotor ability. In addition, at 60 min, fat was the only macronutrient that tended to enhance attention, which is mediated by a neural network including the frontal and parietal lobes (Banich, 1997).

In contrast with glucose and fat, protein was the only macronutrient to influence the rate of forgetting on the paragraph recall test at 15 min; the rate of forgetting is associated with both

the medial temporal and diencephalic regions (Kopelman & Stanhope, 1997). Indeed, after protein ingestion, subjects surprisingly remembered more information during delayed recall than during immediate recall. This finding suggests that some aspect of memory, not shown by the immediate and delayed recall scores, may be enhanced by protein. The immediate, delayed, and forgetting scores all measure aspects of encoding, storage, and retrieval processes to different extents. The inclusion of very specific cognitive tasks in future experiments will be required to decipher the relevance of each aspect of memory.

No benefits of macronutrient ingestion were observed on immediate word list recall, which is mediated by the frontal and medial temporal lobes (Shimamura, 1995). Although the lack of a benefit on this task is consistent with the glucose studies (Manning et al., 1990; Craft et al., 1994; Manning et al., 1997), fat ingestion surprisingly led to an impairment on overall recall at 60 min, and there was a trend for glucose to lead to an impairment in men when the scores at 15 and 60 min were combined. It is unclear why an impairment was observed. In light of the multiple comparisons examined, including the effects on each repeat of each list at 15 and 60 min, total scores at both times, and learning over each repeat of the lists, further research is needed to determine whether these findings are anomalous or reproducible. Although the impairment on immediate word list recall seems contradictory to the effects observed on the other frontal lobe tasks, it must be realized that each task involves several brain regions. Thus, the effects of macronutrient ingestion may be somewhat task-specific depending on the contribution of each brain region.

The activation of the gut-brain axis as well as centrally acting postabsorptive signals, especially at 60 min, may explain the nutrient-specific effects. Specific gut signals may be involved because each macronutrient releases a different profile of peptides; such signals probably occurred throughout the duration of testing because complete gastric emptying of all drinks was estimated to take 60 min (Davenport, 1977). By 60 min, significant absorption of glucose and amino acids, but minimal absorption of fat, would have occurred. The prolonged elevation of blood glucose may have influenced the synthesis of brain neurotransmitters, including acetylcholine (Gold & Stone, 1988; Wenk, 1989), explaining the sustained benefits of glucose. Insulin, which can improve memory in humans (Craft et al., 1996; Craft et al., 1999), and serotonin, which affects cognition (Farr et al., 2000), may also be involved. Indeed, within 20–60 min of ingestion, protein increases hypothalamic extracellular amino acid concentrations

(Choi et al., 1999), and each macronutrient differentially affects hypothalamic insulin (Gerozissis et al., 1997; Gerozissis et al., 1998) and serotonin in rats (Rouch et al., 1999), independently of plasma insulin (Gerozissis et al., 1998). Thus, although the ingestion of energy alone may influence cognition by one mechanism, each macronutrient may improve performance via additional distinct mechanisms involving gut peptides and centrally acting signals.

No relation between glucose regulation and cognition was found in this study. Previous research in healthy and diabetic subjects showed that as glucose regulation worsens, memory performance also worsens (Strachan et al., 1997; Messier et al., 1999). We recently found a relation between glucose regulation and baseline memory in a healthy elderly population similar to the one in the present study (Kaplan et al., 2000). The reason for the failure to observe a similar relation in this study is not clear but may have been due to the greater homogeneity of baseline memory scores in this study (CV in the present study: 28%; CV in the previous study: 40%). Thus, a greater spread in baseline scores may be necessary to observe the association between glucose regulation and baseline scores.

In summary, the ingestion of pure protein, carbohydrate, and fat all improved memory performance 15 min after ingestion in healthy elderly humans. In contrast with the common hypothesis that blood glucose concentrations must be elevated for memory to be improved, these data suggest that the ingestion of energy, in the absence of elevations in blood glucose, can improve memory. In addition, each macronutrient may potentially affect cognition by additional, unique mechanisms.

CHAPTER 6

INFLUENCE OF DIETARY CARBOHYDRATES AND GLYCAEMIC RESPONSE ON SUBJECTIVE APPETITE AND FOOD INTAKE IN HEALTHY ELDERLY PERSONS

These data have been reported in an article accepted for publication:

Kaplan, R.J. & Greenwood, C.E. (2002). Influence of dietary carbohydrates and glycaemic response on subjective appetite and food intake in healthy elderly persons. *International Journal of Food Sciences and Nutrition.* In press.

6. Influence of dietary carbohydrates and glycaemic response on subjective appetite and food intake in healthy elderly persons

6.1 Introduction

Both overeating and obesity, and anorexia and a decrease in body weight affect the health of adults between the ages of 60 and 80 (Morley, 1997). Thus, it is important to understand the regulation of appetite and food intake in this age group. Although many factors are involved in food intake regulation, including social and psychological factors, metabolic and physiologic signals to the brain also play a significant role (Anderson, 1994). The perception, ingestion, digestion, absorption, and metabolism of nutrients can affect subsequent feelings of hunger and satiety and actual food intake (Anderson, 1994). A number of studies suggest that the physiologic regulation of food intake may be impaired in elderly persons (Roberts et al., 1994; Rolls et al., 1995), however, others recently showed that the elderly regulate food intake similarly to younger adults (Zandstra et al., 2000).

Several components of food have been investigated for their effects on subsequent satiety and food intake. Because carbohydrates are the major energy source in our diets, and because the brain requires glucose, it is logical that carbohydrates and the glycaemic response to their ingestion could have an impact on appetite (FAO/WHO, 1998). It has been hypothesized that a low glycaemic index (GI) food (Jenkins et al., 1981), which gradually raises blood glucose, may result in more prolonged satiety than a high GI food, which rapidly raises blood glucose. The GI of a carbohydrate food cannot be predicted by chemical composition alone, but is a function of the type of carbohydrate, the fibre content, the food form and the method of preparation (Ludwig, 2000). It has been hypothesized that the relationship between the GI of a food and satiety may be related to differences in plasma glucose, insulin and cholecystokinin (Holt et al., 1992). Although a number of studies have supported this hypothesis by showing that lower GI foods lead to increased satiety, delayed return to hunger, or decreased ad libitum intake compared with higher GI foods (Ludwig, 2000), others have not supported it (Barkeling et al., 1995; Holt et al., 1996; Stewart et al., 1997). Thus, the effects of foods varying in GI on appetite remain unclear (Roberts, 2000). The effects of foods varying in GI on satiety in the elderly are even less clear (FAO/WHO, 1998). However, it is important to understand these effects in this age group because the incidence of insulin resistance and diabetes is high (Harris

et al., 1987), and consuming low GI foods has been used as part of the treatment for these conditions (Wolever, 2000).

The purpose of the present study was to determine the effects of comparable amounts of available carbohydrate from foods varying in GI on subjective appetite and actual food intake 120 min post-ingestion in healthy elderly persons.

6.2 Subjects and methods

6.2.1 Subjects

Ten male and ten female community-dwelling subjects aged 60-82 y were contacted through a database of previously recruited subjects at the Memory Laboratory of the University of Toronto. Subjects participated voluntarily; compensation was provided for travel. All procedures were approved by the Baycrest Centre for Geriatric Care and University of Toronto ethics committees. Only subjects who spoke English as their native language were selected. Level of education ranged from 8 to 12 y and no subject had evidence of diabetes (fasting plasma glucose $\geq$ 7.0 mmol/L (American Diabetes Association, 1997)) or cognitive decline [below 25 out of 30 on the Mini-Mental State Examination (MMSE; (Folstein et al., 1975))]

6.2.2 Procedure

A within-subjects repeated-measures design was used such that each subject served as his or her own control and participated in all of the four sessions. After an overnight (10-12 h) fast during which only water was permitted, the subjects arrived at the testing centre at 0830 on the first day to complete a 30-min screening and at 0900 on the remaining 3 d. During screening subjects completed the MMSE and the Revised Restraint Scale (Herman & Polivy, 1980), which is a measure of restrained eating or dieting status, and fasting plasma glucose, height and weight were measured.

On each of the four test sessions, blood was collected by finger prick and analyzed for fasting serum insulin and plasma glucose. After blood collection, one test food or drink (preload) was given to the subject, who was asked to try to consume the entire amount within about 5 (drinks) or 10 min (foods). Each subject was tested individually with one test food or drink on four mornings, each separated by approximately 1 wk and no less than 2 d to minimize potential carryover effects. The four test foods and drinks were placebo or one of three foods

containing similar amounts of available carbohydrate (carbohydrate minus dietary fibre). The order of the four sessions was counterbalanced across test foods. Plasma glucose was measured for each subject 15, 60 and 105 min after the start of consumption of the test food or drink.

During each session, subjects were first administered a Sleep Habits and Stress Factors Questionnaire to determine if they were experiencing any unusual stress or illness and to determine their dietary and physical activities from the previous day. To assess subjective appetite, subjects filled out Visual Analogue Scales (VASs) (Rogers et al., 1988; Stewart et al., 1997) before they ingested the test food or drink and throughout a 120-min period after the start of consumption. The Motivation to Eat VAS was filled out 0, 15, 30, 45, 60, 90 and 120 min after the start of ingestion to assess feelings of hunger and satiety. The Physical Discomfort VAS was filled out at times 0, 60 and 120 min to determine level of discomfort. Finally, a Palatability VAS was filled out after consumption of each test food or drink. To assess actual food intake, an ad libitum lunch was provided to subjects after the 120-min period and intake was recorded (at 1100). The three VASs were also administered after lunch. Subjects were also tested on a series of cognitive tests during this interval; the results have been published previously (Kaplan et al., 2000).

6.2.3 Test foods and drinks (preloads)

The four test foods were: 1) placebo: 300 ml lemon beverage (290 ml water and 10 ml lemon juice) sweetened with 23.7 mg sodium saccharin (Hermesetas Original; JL Freeman Inc., Boucherville, Canada), 2) glucose: 300 ml lemon beverage (270 ml water and 10 ml lemon juice) containing 45.5 g glucose (Dextrose monohydrate; Bio-Health, Dawson Traders Ltd., Toronto); 3) potato: 49.5 g of available carbohydrate from instant mashed potatoes (Carnation Mashed potatoes; Carnation Foods Company Ltd., Carberry, Canada), and 4) barley: 46.6 g of available carbohydrate from pearled barley (McNair pearl barley; McNair Products Co. Ltd., Montreal). The available carbohydrate content in potato and barley was determined from a standard nutrient composition database, the Canadian Dietary Information system for food intake analysis (Health Canada, 1996). The placebo and glucose drinks were matched for sweetness in order to blind subjects to their content (Manning et al., 1997). The instant mashed potatoes were prepared by using the package directions, but instead of adding water, milk, butter or margarine, and salt, only water was added (equivalent to recommended amount of water plus

milk); 61 g potato flakes was added to 240 ml water and heated at full power in a microwave oven for 1.5 min. Barley was prepared by adding 60 g barley to 420 ml boiling water; the barley was cooked until all of the water was absorbed (about 30 min). The subjects were given 2.5 g butter (Gay Lea unsalted; Gay Lea Foods, Weston, Canada) and salt and pepper as desired, and 120 ml water (President's Choice Natural Spring Water; Sunfresh Ltd., Toronto) to drink with the barley and potatoes to improve palatability and compliance. Not including the drinking water, the compositions of the test foods are shown in **Table 6.1.**

Table 6.1
Composition of test foods and drinks

	Placebo	Glucose	Potato[1]	Barley[1]
Weight (g)	300	326	312	196
Volume (ml)	300	300	325	200
Energy (kJ)	0	704	978	959
Energy density (kJ/g)	0	2.16	3.14	4.89
Energy density (kJ/ml)	0	2.35	3.01	4.79
Protein (g)	0	0	5.1	5.9
Carbohydrate (g)	0	45.5	49.5	46.6
Fat (g)	0	0	2.2	2.7
Fibre (g)	0	0	3.1	9.4
Glycaemic Index (white bread = 100)[2]	0	142	118	36

[1]Includes 2.5 g butter.

[2]From (Foster-Powell & Miller, 1995).

6.2.4 Visual Analogue Scales

All VASs consisted of several questions (Rogers et al., 1988; Stewart et al., 1997). The Motivation to Eat VAS consisted of the following 4 questions: "How strong is your desire to eat?", "How hungry do you feel?", "How full do you feel" and "How much food do you think you could eat?". Subjects marked an "X" along a 10.0 cm line that was word-anchored at both ends. For example, for the question about desire to eat, one end was anchored with "very weak" and the other with "very strong". The distance on the line was used as the score, with a higher score representing greater satiety for the fullness question, and a lower score representing greater satiety for the other three questions. The Physical Discomfort VAS consisted of five word-anchored questions, which asked if the subjects felt nauseous, if their stomach hurt, if they felt well, if they had gas, or if they had diarrhea. The Palatability VAS consisted of one question: "How pleasant have you found the food?", which was anchored with "very pleasant" and "not pleasant at all".

The incremental areas under the four 120-min Motivation to Eat VAS curves (AUCs) were used to determine the influence of each test food on subjective measures of desire to eat, hunger, fullness and prospective consumption (Holt et al., 1995). AUCs were calculated by using the trapezoidal rule to determine the areas of each 120-min VAS curve above (positive) or below (negative) baseline (fasting VAS value). An average appetite score (Stewart et al., 1997) was also calculated by combining the AUCs for all 4 questions. Average appetite = [desire to eat + hunger + (fullness*-1) + prospective consumption]/4. The change in VAS from fasting to 120 min post-ingestion, or immediately prior to lunch intake, was also used as a measure of subjective appetite for each VAS question.

6.2.5 Lunch

All foods were provided in small portions and weighed before and after consumption. The weight of each food that was consumed was determined by difference and total energy intake and percent of energy from protein, carbohydrate and fat were calculated. Percent compensation at lunch for the energy content of the test food was also calculated. Percent compensation = [lunch intake after placebo (kJ) - lunch intake after test food (kJ)] X 100 / test food (kJ; actual energy consumed was used for subjects who did not consume the entire test food).

All subjects were provided with the following lunch on all 4 sessions unless they

requested a substitution: 1.5 processed cheddar cheese (Sunfresh Ltd.) sandwiches and 1.5 low-fat smoked turkey breast sandwiches (Sunfresh Ltd.) on 100% whole wheat bread (Dempster's; Corporate Foods Ltd., Etobicoke, Canada), each cut into quarters, four mini blueberry muffins (National Grocers Co. Ltd., Toronto), four arrowroot cookies (President's Choice A Better Arrowroot; Sunfresh Ltd.) and as much bottled water (President's Choice Natural Spring Water; Sunfresh Ltd.) as desired. Not including drinking water, the total food provided consisted of approximately 409 g, 1104 kcal (4619 kJ), 44 g protein, 35 g fat and 154 g carbohydrate (manufacturers' analysis).

Eight of 20 subjects requested substitutions, which were kept constant for all four sessions for each subject: two subjects had ham (Maple Leaf, Burlington, Canada) instead of turkey sandwiches; three subjects had ham instead of cheese sandwiches, of which one had no cookies and one had white bread (Dempster's Scone enriched white bread; Corporate Foods Ltd.); one other subject had white bread; one subject had cheese sandwiches only; and one subject had banana instead of blueberry muffins.

6.2.6 Blood glucose and insulin analyses

Blood was collected by finger prick with a Penlet II Automatic Blood Sampler lancet device (Lifescan Canada Ltd., Mississauga, Canada). Each plasma glucose measurement was determined by analyzing one drop of blood with a blood glucose meter (One Touch Basic Meter; Lifescan Canada Ltd.). Serum was pooled from all four sessions for each subject (minimum 250 μL) to determine fasting insulin, which was analyzed by the Banting and Best Diabetes Core Laboratory, University of Toronto by radioimmunoassay as described previously (Kaplan et al., 2000). Homeostasis model assessment (HOMA) was used to estimate relative β-cell function and relative insulin resistance (i.e., insulin sensitivity) from fasting plasma glucose (average of all four sessions) and serum insulin (Matthews et al., 1985).

6.2.7 Statistical analyses

Statistical analyses were conducted with SAS 6.12 (SAS Institute Inc., Cary, NC). Repeated-measures analysis of variance (ANOVA) was used to determine the effects of each test food on plasma glucose; contrasts comparing predicted successive means were used (significance was set at $P < 0.05$). Repeated-measures ANOVAs were conducted between each

pair of test foods to determine the influence of test food on subjective appetite over the entire 120 min post-ingestion period, and at 120 min; statistical significance was set at $P < 0.008$ (Bonferroni correction for multiple comparisons (Pagno & Gauvreau, 1993)). Repeated measures ANOVAs were conducted between each pair of test foods (excluding placebo) to determine the influence of test food on percent compensation at lunch; statistical significance was set at $P < 0.02$ (Bonferroni correction). Repeated measures ANOVAs were conducted to determine the effect of test food on lunch intake. Simple contrasts were used to determine the effect of each food compared to placebo; statistical significance was set at $P < 0.05$. Planned linear regression analyses were conducted among subject characteristics, subjective appetite, and lunch intake; statistical significance was set at $P < 0.05$. Results are reported as means $\pm$ SEM unless indicated otherwise.

6.3 Results

6.3.1 Characteristics of test food ingestion and effects on blood glucose

All subjects consumed the placebo and glucose drinks within 6 min (mean $\pm$ SD: placebo: 2.5 $\pm$ 1.4; glucose: 2.7 $\pm$ 1.4) and the potatoes and barley within 16 min (potatoes: 10.7 $\pm$ 3.2; barley: 9.4 $\pm$ 3.3). The placebo and glucose drinks were entirely consumed by all subjects; 6 subjects did not consume all of the potatoes or barley (4 of these subjects did not consume all of the potatoes and 5 did not consume all of the barley). Mean $\pm$ SD consumption of potatoes and barley was 95.2 $\pm$ 10.3% and 91.3 $\pm$ 18.6%, respectively. The glucose drink was rated as most palatable (2.6 $\pm$ 0.5 cm), followed by placebo (4.1 $\pm$ 0.6 cm; $P = 0.0004$), then potato (6.1 $\pm$ 0.6 cm; $P = 0.0245$), which did not differ from barley (6.6 $\pm$ 0.6 cm).

Analyses were conducted both with all subjects included and with the 6 subjects who did not consume all of the potatoes or barley excluded. Actual test food intake for each subject was factored into the calculations for percent compensation.

Subject characteristics and glucose regulation measurements for all subjects are shown in **Table 6.2.** No differences ($P > 0.05$) were observed between men and women on any of these measurements.

Table 6.2
Characteristics of subjects[1]

	All ($n = 20$)	Men ($n = 10$)	Women ($n = 10$)
Age (y)	72.3 ± 1.4	74.8 ± 1.9	69.7 ± 1.9
Education (grade)	10.6 ± 0.3	10.1 ± 0.5	11.0 ± 0.4
MMSE[3] (out of 30)	28.0 ± 0.3	27.6 ± 0.5	28.4 ± 0.3
BMI (kg/m2)	25.1 ± 0.9	25.7 ± 1.3	24.6 ± 1.4
Restrained eating (0 to 35)	10.4 ± 1.0	9.8 ± 1.0	10.9 ± 1.1
Fasting plasma glucose (mmol/L)	5.4 ± 0.1	5.5 ± 0.2	5.4 ± 0.1
Fasting serum insulin (pmol/L)	52.5 ± 5.6	55.2 ± 7.6	49.8 ± 8.7
β-cell function (%)[2]	78.4 ± 7.1	79.8 ± 7.9	77.0 ± 12.1
Insulin resistance[2]	1.77 ± 0.22	1.90 ± 0.31	1.65 ± 0.30

[1]Mean ± SEM. MMSE, Mini-Mental State Examination.

[2]Calculated from fasting plasma glucose and insulin concentrations by using Homeostasis Model Assessment.

The influences of the 4 test foods on plasma glucose are shown in **Figure 6.1.** As expected, glucose and potato ingestion caused the largest rise in plasma glucose concentration followed by barley and then placebo. Mean incremental area under the curve after glucose ingestion (325.1 ± 34.3) > potato (269.6 ± 21.4; $P = 0.033$) > barley (85.3 ± 10.9; $P < 0.0001$) > placebo (-11.1 ± 7.1; $P < 0.0001$).

6.3.2 Effect of test foods on subjective appetite

In general, the average appetite AUCs and the AUCs for each of the 4 questions from the Motivation to Eat VAS showed that potatoes led to the greatest feelings of satiety over the 120-min post-ingestion period, followed by barley, then glucose, and then placebo. Because each pair of test foods was compared, statistical significance was set at $P < 0.008$.

Based on average appetite, potatoes (-260 ± 62 cm • min) tended to increase satiety (or decrease hunger) more than barley, but this did not reach statistical significance (-115 ± 43; $P = 0.015$) and increased satiety more than glucose (-42 ± 28; $P = 0.0024$) and placebo (18 ± 29; $P < 0.0001$) when all subjects were included (**Figure 6.2**). Barley increased satiety more than placebo ($P = 0.0018$) and glucose tended to increase satiety more than placebo, but this was not significant ($P = 0.027$) (Figure 6.2). When these data were analyzed with the 6 subjects who did not consume all of the potatoes or barley excluded, the results were the same except the differences between potato and glucose ($P = 0.030$) and between barley and placebo ($P = 0.064$) were no longer significant, due to the lower power.

The results for each of the 4 questions analyzed separately were as follows: 1) there was a non-significant trend for potatoes (AUC: -203 ± 78) to lead to less desire to eat than placebo (-4 ± 42; $P = 0.026$), glucose (-47 ± 42; $P = 0.069$) and barley (-45 ± 56; $P = 0.075$); 2) potatoes (-216 ± 76) led to less hunger than placebo (34 ± 39; $P = 0.0023$), and tended to lead to less hunger than glucose (-34 ± 38; $P = 0.044$) and barley (-62 ± 62; $P = 0.064$), but this was not significant; 3) potatoes (397 ± 80) and barley (292 ± 47) both led to greater feelings of fullness than glucose (71 ± 39; $P < 0.0001$) and placebo (-6 ± 51; $P < 0.0001$); there was a non-significant trend for glucose to lead to greater fullness than placebo ($P = 0.091$); and 4) potatoes (-223 ± 47) led to lower ratings of prospective consumption than placebo (37 ± 25; $P < 0.0001$), glucose (-17 ± 27; $P = 0.0018$) and barley (-62 ± 47; $P = 0.0083$); there was a non-significant trend for barley ($P = 0.056$) and glucose ($P = 0.077$) to lead to lower ratings than placebo.

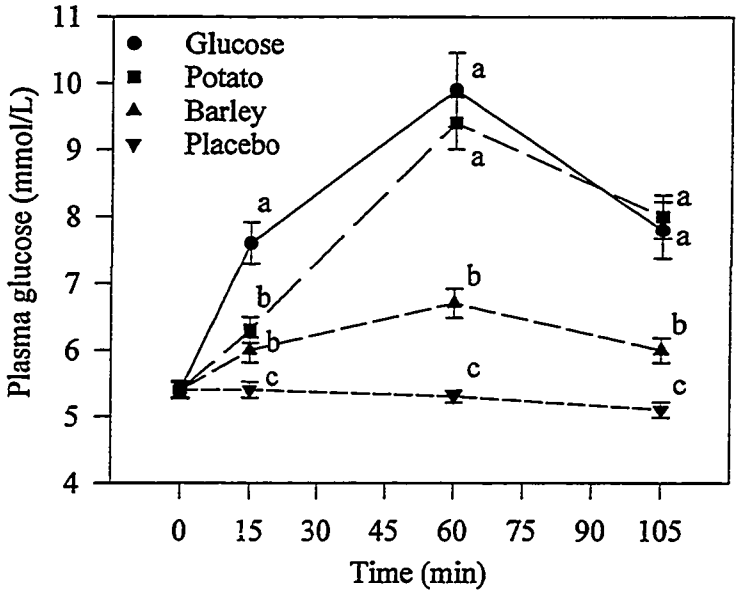

Figure 6.1. Mean ± SEM plasma glucose response to test foods after the start of consumption. Values with different letters at each time point are significantly different: 15 min, $P < 0.0016$; 60 and 105 min, $P < 0.0001$.

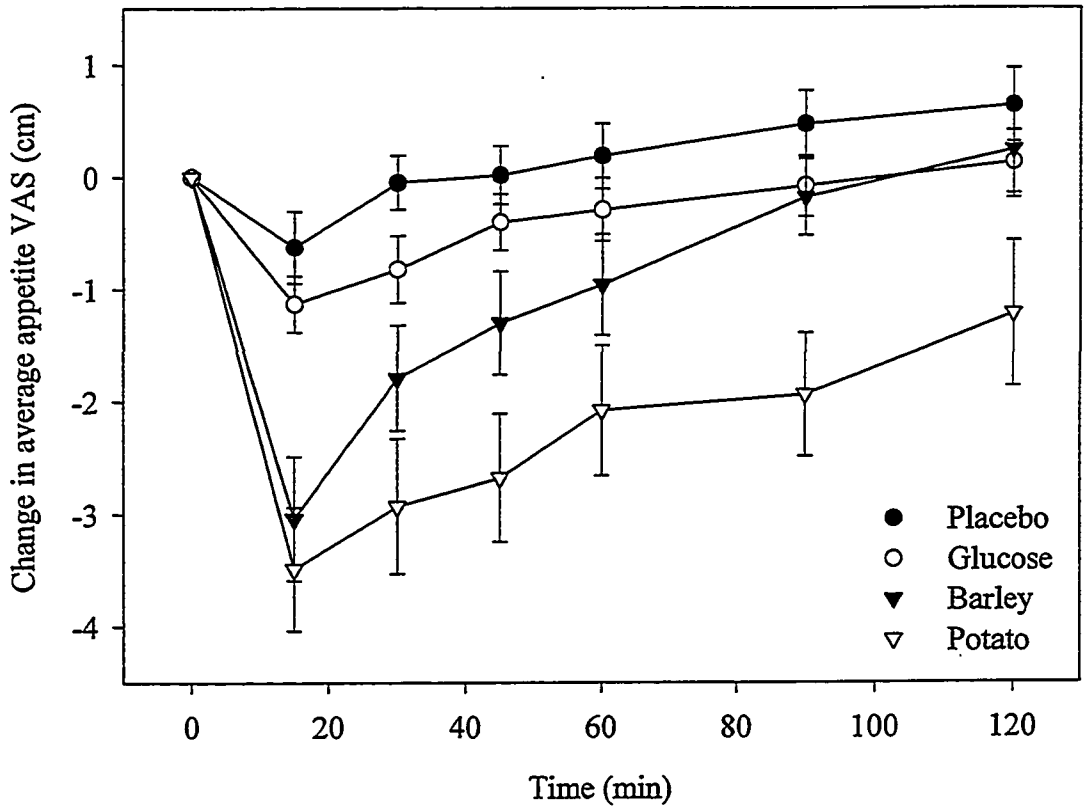

Figure 6.2. Mean ± SEM average appetite values for the Motivation to Eat VAS. Lower values represent greater satiety. The AUC was lower for potato than glucose and placebo, and was lower for barley than placebo ($P < 0.008$). AUCs did not differ between potato and barley, between barley and glucose, or between glucose and placebo.

The influence of the test foods on subjective appetite were also examined 120 min post-ingestion, or immediately prior to lunch intake. In general, potatoes still led to the greatest feelings of satiety, but the effects of barley on satiety diminished by 120 min. Based on average appetite, there was a non-significant trend for potatoes only to increase satiety compared with placebo ($P = 0.014$), barley ($P = 0.021$) and glucose ($P = 0.071$) 120 min post-ingestion (see 120-min timepoint on Figure 6.2). When the 4 questions were analyzed separately, the only significant effects were that potatoes ($P = 0.0002$) and barley ($P = 0.0040$) led to greater fullness than placebo, and potatoes led to greater fullness than glucose ($P = 0.0011$); there was a non-significant trend for barley to lead to greater fullness than glucose ($P = 0.034$). In contrast, the trends observed on the other 3 questions suggested that by 120 min post-ingestion, barley no longer elicited stronger feelings of satiety than glucose or placebo. Specifically, potatoes tended to lead to less desire to eat ($P = 0.086$), less hunger ($P = 0.076$), greater fullness ($P = 0.075$) and lower prospective consumption ($P = 0.068$) than barley and to less prospective consumption than placebo ($P = 0.0090$) and glucose ($P = 0.097$), but these effects were not significant.

None of the test foods had an effect on any of the 5 questions from the Physical Discomfort VAS at 60 or 120 min post-ingestion compared with placebo. The mean $\pm$ SD change from baseline for the average of the 5 questions was 0.13 $\pm$ 0.31 cm at 60 min and 0.09 $\pm$ 0.38 cm at 120 min on the 10.0 cm VAS scale.

6.3.3 Effects of test foods on food intake at lunch

Although potatoes generally had the strongest effect on subjective satiety, both potato (405 $\pm$ 40 kcal (1695 kJ); $P = 0.0031$) and barley (441 + 41 kcal (1845 kJ); $P = 0.031$) ingestion led to less lunch intake than placebo (502 $\pm$ 47 kcal (2100 kJ)) (**Figure 6.3**). Lunch intake after glucose ingestion (482 $\pm$ 43 kcal (2017 kJ)) did not differ from placebo. When these data were analyzed with the 6 subjects who did not consume all of the potatoes or barley excluded, the results were the same except the differences between barley and placebo were no longer significant ($P = 0.068$), due to the lower power.

Percent of energy chosen as each macronutrient at lunch did not differ between any of the 3 test foods and placebo. The mean $\pm$ SD percent of energy selected as each macronutrient was 16.9 $\pm$ 2.8% as protein, 55.1 $\pm$ 3.6% as carbohydrate, and 28.3 $\pm$ 3.3% as fat.

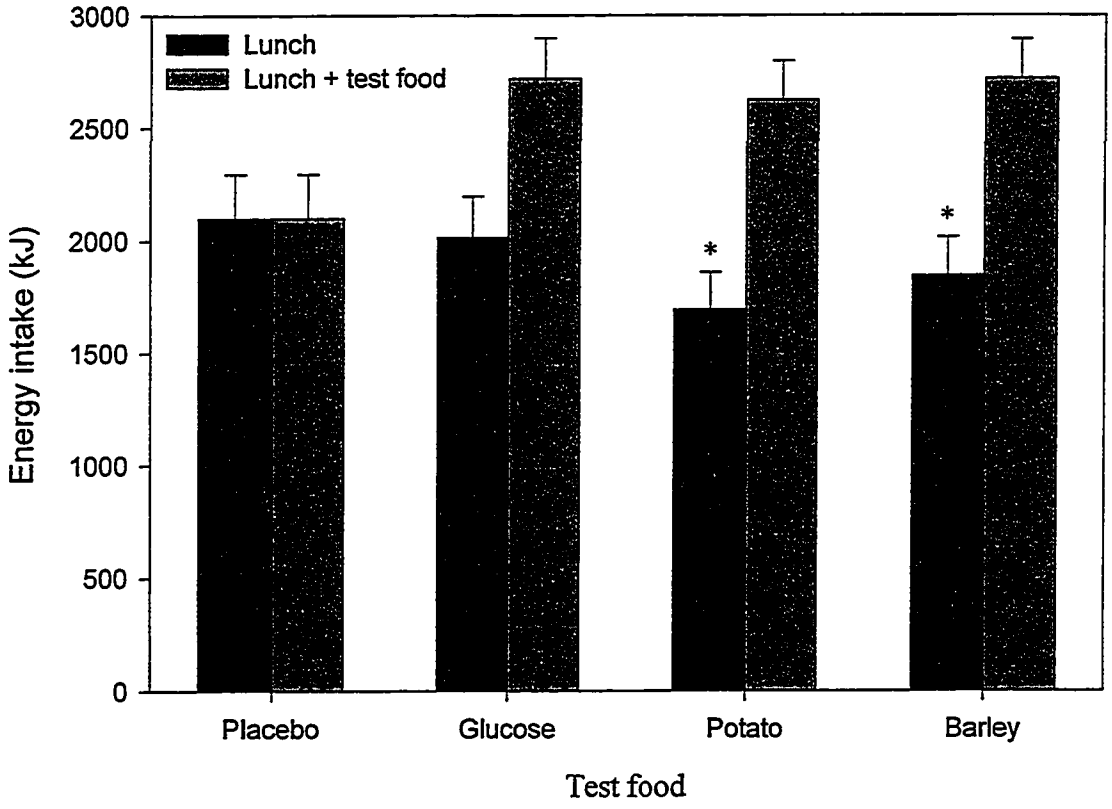

Figure 6.3. Mean ± SEM lunch and total (lunch + test food) intake after each test food. Total intake represents actual energy consumed, incorporating the fact that some subjects did not consume the entire test food. Lunch intake after potato and barley ingestion was significantly different from placebo (*$P <$ 0.031).

Percent compensation at lunch for the energy content of the test food showed that potatoes increased satiety more than glucose, but did not differ from barley. Although both potato and barley ingestion led to the same amount of compensation at lunch (41.9%), only potatoes (41.9 ± 12.3 %) significantly differed from glucose (11.9 ± 9.5 %; $P = 0.016$), due to the greater variability in compensation after barley ingestion (41.9 ± 22.1 %). When these data were analyzed with the 6 subjects who did not consume all of the potatoes or barley excluded, the significant results were the same; the compensations were 46.1 ± 14.9% for potatoes, 22.9 ± 11.5% for barley, and 13.4 ± 12.3% for glucose.

6.3.4 Relations among subject characteristics, subjective appetite and lunch intake

Linear regression analyses showed that restrained eating scores were associated with BMI ($r = 0.57$; $P = 0.0083$) and inversely associated with age ($r = -0.46$; $P = 0.042$) suggesting that more restrained eaters were heavier for their height and younger.

Restrained eating was inversely associated with percent compensation at lunch following barley ingestion ($r = -0.47$; $P = 0.035$) suggesting that less restrained eaters compensated more. However, similar associations between restrained eating and percent compensation were not observed with the other test foods.

Subjective appetite was predictive of actual energy intake at lunch. When data for all 4 testing days for each subject were included in the analyses, prospective consumption at 120 min was associated with energy intake ($P = 0.024$) and a non-significant trend was observed between average appetite AUC and energy intake ($P = 0.073$). When each food was analysed separately, there were non-significant trends for prospective consumption to be associated with lunch intake after potato ($r = 0.38$, $P = 0.097$), barley ($r = 0.43$, $P = 0.055$) and placebo ($r = 0.38$, $P = 0.098$) ingestion at 120 min, and fullness ratings were associated with lunch intake after barley ingestion ($r = 0.46$, $P = 0.041$).

Interestingly, percent of energy chosen as protein at lunch was associated with BMI following ingestion of all 4 test foods ($r > 0.49$; $P < 0.028$), and with insulin resistance following ingestion of potatoes, barley and glucose ($r > 0.48$; $P < 0.035$), but was not related to restrained eating. Energy intake at lunch, and percent of energy from carbohydrate and fat were not associated with BMI, insulin resistance or restrained eating. Importantly, BMI was strongly associated with insulin resistance ($r = 0.72$; $P = 0.0004$).

6.4 Discussion

The results of the present study suggest that the elderly are sensitive to the effects of carbohydrate foods on subjective satiety and food intake, but do not support the hypothesis that lower GI foods lead to greater satiety and less food intake than higher GI foods. The findings show that among three foods containing similar amounts of available carbohydrate and a placebo, mashed potatoes (high GI) led to the greatest subjective feelings of satiety, followed by barley (low GI), glucose (high GI) and placebo over a 120-min period after ingestion. The difference between potatoes and the other three test foods was much greater by the end of the 120-min period. Although potatoes led to the strongest effects on subjective satiety, both potatoes and barley led to less energy intake at lunch after 120 min compared with placebo. The effect of glucose ingestion on subsequent energy intake did not differ from placebo.

A decline in the ability to regulate food intake occurs as people age, partly explaining the anorexia that occurs in ageing (Morley, 1997). It has been suggested further that food intake may be primarily influenced by external, rather than physiologic factors, in the elderly (De Castro, 1993). However, although no direct comparisons were made between young and old subjects in the present study, these results and others (Zandstra et al., 2000) suggest that physiologic signals regulating appetite exert a considerable influence on food intake behaviour in the elderly. Zandstra et al. (Zandstra et al., 2000) showed that the ability to regulate food intake 90 min post-ingestion did not differ between older (61-86 y) and younger (18-26 y) subjects. These authors found that energy compensation at lunch for four different preloads ranged from 15 to 44% in young adults and from 17 to 23% in the elderly, but these differences were not significant. In the present study, energy compensation ranged from 12 to 42% for the three carbohydrate preloads, which is more similar to the range in compensation of the younger subjects in the noted study, and suggests that after 120 min, elderly subjects were sensitive to the different preloads. Additional evidence in the present study, showing that subjective appetite ratings were predictive of energy intake at lunch, supports the notion that physiologic signals are involved in the food intake behaviour of elderly subjects.

These results differ from those of another study, which found that young men compensated at lunch more precisely than elderly men after receiving various preloads (Rolls et al., 1995). Differences in the size of the preload (500 g) and the time interval between test food

consumption and lunch presentation (30 min) may explain the discrepancies. Thus, it is possible that physiologic effects on appetite are stronger in younger than in older subjects soon after preload consumption, but that the differences are less pronounced after a longer period has passed. Although the reason for these differences can not be determined from these studies, they may be related to the delayed gastric emptying that is observed in ageing (Clarkston et al., 1997).

It is important to note that even though anorexia is seen in advanced ageing, the healthy elderly subjects in the present study and in those previously mentioned (Rolls et al., 1995; Zandstra et al., 2000) did not completely compensate for the energy content of any of the preloads at lunch. In other words, they consistently overate at lunch after the carbohydrate preloads so that total intake (test food + lunch) was higher than after the placebo, suggesting that compensation was imprecise.

Restrained eating scores were associated with BMI and inversely associated with age in this study, suggesting that, similarly to young adults (Tuschl et al., 1990), individuals over 60 years old who are heavier for their height are also more restrained eaters. That is, restrained eating, which is often associated with dieting, concerns about body image, and overweight appears to continue into this age group. Nevertheless, even within this age group, younger individuals were more restrained. Because restrained eaters have been shown to be more sensitive to external cues than unrestrained eaters (Fedoroff et al., 1997), subjective appetite and food intake data were analyzed with highly restrained eaters (restrained eating score $\geq$ 14) excluded, but the same results were observed as when all subjects were included. There was, however, some evidence that more restrained eaters were less sensitive to physiologic signals than less restrained eaters. Indeed, there was an inverse association between restrained eating and percent compensation at lunch following barley ingestion when all subjects were included, suggesting that less restrained eaters compensated more for test food intake.

An interesting finding from the present study was that the percent of energy chosen as protein at lunch was significantly associated with BMI and insulin resistance. There are at least two possible explanations for these data. The most intriguing, from a physiologic standpoint, is that subjects with poorer insulin resistance selectively chose higher protein diets because it may be beneficial to them. This possibility is supported by studies in rodents, which have shown that when given the opportunity to self-select, rats with poor glucose regulation choose higher

intakes of protein and lower intakes of carbohydrate than normal rats (Siegel et al., 1980; Tepper & Kanarek, 1985; Kaplan et al., 1996). An alternative explanation is that the subjects with higher BMIs, which associated with restrained eating, consumed a greater percentage of energy as protein because they selectively avoided the low protein dessert-like foods. That is, the more restrained eaters may have consumed more of the cheese and meat sandwiches and less of the cookies and muffins at lunch because they perceive cookies and muffins to be more unhealthy or "fattening" than the sandwiches. Although this hypothesis seems plausible, if this were the case, restrained eating would be expected to be associated with percent of energy selected as protein, which was not found. Taken together, although the exact reason why subjects with higher BMIs and insulin resistance selected a higher percentage of energy as protein is not clear from this study, it may be important to further investigate the impact of insulin resistance on appetite regulation in the elderly because this disorder increases with ageing (Harris et al., 1987).

Although the present study is limited by the small number of foods tested, these findings do not support the hypothesis that lower GI foods lead to greater feelings of satiety and less food intake than higher GI foods in the elderly. Despite the fact that barley had a very gradual impact on blood glucose in comparison with potatoes, potato ingestion led to greater feelings of satiety, particularly after 120 min, and both foods decreased subsequent intake compared with placebo. Thus, although a recent review noted that 15 studies have found support for the GI hypothesis (Ludwig, 2000), the present study is at least the sixth that has not supported it (Krishnamachar & Mickelson, 1987; Barkeling et al., 1995; Holt et al., 1996; Stewart et al., 1997; Anderson et al., 2001). Consistent with the present findings, one study showed potatoes to have the strongest effect on satiety among 38 isoenergetic foods of varying GI (Holt et al., 1995). Although it remains possible that low GI foods can increase satiety in certain situations, several other components of food are likely as important as GI.

In addition to low GI, it has been suggested that high volume (Black et al., 1993; Rolls et al., 2000) and weight (De Graaf & Hulshof, 1996), low energy density (Drewnowski, 1998; Rolls, 2000), high protein (Holt et al., 1995), low fat (Holt et al., 1999), high fibre (Burton-Freeman, 2000), and low palatability (Holt et al., 1995; Drewnowski, 1998) are associated with increased satiety and decreased food intake. Potential mechanisms for each of these factors are discussed in these articles and elsewhere (Stubbs, 1999). Because the purpose of the present study was to provide similar amounts of carbohydrate in foods with a wide range of GI values,

these other factors were not controlled. Nevertheless, the present data are not consistent with any one single factor controlling satiety, but instead suggest that several or all of these factors could be involved. That is, although in general, the order in effects on subjective satiety was potatoes > barley > glucose > placebo, and both potatoes and barley, but not glucose, decreased food intake compared with placebo, no specific factor varied in this order. For example, potatoes and barley were similar in composition with respect to total energy and protein content. But, potatoes had higher weight and volume, and lower energy density than barley and more than three times the GI and less than one-third the fibre content of barley, yet both reduced intake to the same degree. Similarly, glucose and potatoes had comparable weights and volumes, yet, only potato reduced lunch intake. However, the lower total energy and liquid form (Hulshof et al., 1993) of the glucose drink may partly explain its lesser effect on satiety. Another factor that could explain the lesser effect of glucose on satiety is palatability, which was highest for glucose. However, a recent study showed that although palatability has an impact on satiation, which is the amount eaten in a meal, it does not affect satiety, which is the effect of a meal on subsequent intake (De Graaf et al., 1999). Thus, the present data suggest that GI alone does not explain the effects of these foods on satiety in healthy elderly persons. Furthermore, no individual factor appears to explain the effects, suggesting that a combination of factors are likely involved in mediating the effects of these foods on subjective satiety and food intake.

In summary, the present study suggests that among three foods containing similar amounts of available carbohydrate, mashed potatoes led to the greatest feelings of satiety, followed by barley, and then glucose over a 120-min post-ingestion period in healthy elderly persons. Furthermore, both potato and barley decreased food intake 120 min post-ingestion compared with placebo. In conclusion, these findings suggest that elderly individuals are sensitive to different carbohydrate preloads and that the effects of these foods on satiety and food intake were not predicted by GI. Nevertheless, low GI foods may be beneficial for the elderly with respect to their effects on glycaemic control (Wolever, 2000).

CHAPTER 7

INFLUENCE OF PURE PROTEIN, CARBOHYDRATE, AND FAT DRINKS OF EQUAL ENERGY AND VOLUME ON SUBJECTIVE APPETITE AND FOOD INTAKE IN HEALTHY ELDERLY PERSONS

These data have been reported in an article submitted for publication:

Kaplan, R.J. & Greenwood, C.E. (2002). Influence of pure protein, carbohydrate, and fat drinks of equal energy and volume on subjective appetite and food intake in healthy elderly persons. *Appetite*. Submitted.

7. Influence of pure protein, carbohydrate, and fat drinks of equal energy and volume on subjective appetite and food intake in healthy elderly persons

7.1 Introduction

The aging population and importance of diet in the development of chronic diseases has increased the need to understand appetite regulation in the elderly. Current evidence suggests that the ability to regulate food intake declines with aging (Roberts et al., 1994; Rolls et al., 1995), such that social and psychological factors may become more important than physiologic signals (De Castro, 1993). These studies demonstrate that under experimental conditions, seniors are less able to respond appropriately to excess energy consumption by reducing subsequent intake, or to a shortage of energy by increasing intake, placing them at risk for either weight gain or weight loss depending on the environmental circumstances.

Despite these observations, there have been few studies examining appetite responses to macronutrient ingestion in older adult populations. The major energy-providing macronutrients, protein, carbohydrate, and fat, have important physiologic impacts on subsequent hunger, satiety and energy intake in young adult subjects (reviewed in (Anderson, 1994; Stubbs, 1999)). Results of numerous studies consistently show that when isoenergetic diets are consumed high protein foods induce higher subjective ratings of satiety or lead to decreased subsequent food intake relative to high carbohydrate and fat foods (Booth, 1970; Barkeling et al., 1990; Stubbs et al., 1996; Poppitt et al., 1998; Latner & Schwartz, 1999; Stubbs et al., 1999; Marmonier et al., 2000), with few exceptions (Geliebter, 1979; De Graaf et al., 1992). By contrast, the effects of carbohydrate and fat on satiety are less clear. Some studies have shown carbohydrate to induce higher satiety than fat (Green et al., 1994; Rolls et al., 1994; Stubbs et al., 1996; Stubbs et al., 1999; Woodend & Anderson, 2001), whereas others have found no difference (De Graaf et al., 1992; Crovetti et al., 1998; Poppitt et al., 1998; Rolls & Bell, 1999; Marmonier et al., 2000; Rolls, 2000). It has been suggested that when energy content and energy density are controlled, the difference between fat and carbohydrate may no longer be evident (Rolls, 2000).

To date, no study has examined the effects of all three macronutrients on satiety and food intake in elderly humans. Thus, the purpose of the present study was to determine the effects of ingesting pure (>98% energy) protein, carbohydrate, and fat drinks of equal energy, energy density, and volume, on subjective appetite and food intake 90 min post-ingestion in healthy

elderly persons.

7.2 Subjects and methods

7.2.1 Subjects

Eleven male and 11 female community-dwelling subjects aged 61-79 y were contacted through a database of previously recruited subjects at the Memory Laboratory of the University of Toronto. Subjects participated voluntarily; compensation was provided for travel. All procedures were approved by the Baycrest Centre for Geriatric Care and University of Toronto ethics committees. Only subjects who spoke English as their native language were selected. Level of education ranged from 7 to 12 y and no subject had evidence of diabetes (fasting plasma glucose $\geq$ 7.0 mmol/L (American Diabetes Association, 1997)) or dementia [below 25 out of 30 on the Mini-Mental State Examination (MMSE; (Folstein et al., 1975))]. While subjects were scored on the Revised Restraint Scale (Herman & Polivy, 1980), restrained eating was not used as an exclusion criterion. This exclusion criterion is often used in studies examining appetite in young adults because restrained eaters may respond less to physiologic signals than non-restrained eaters (Fedoroff et al., 1997). However, there is no data on the prevalence of restrained eating in the senior population or an inherent understanding of how restrained eating may influence appetite responses in this population. Therefore, planned analyses were conducted with all subjects included as well as with highly restrained eaters [scores $\geq$ 14 on the Revised Restraint Scale (Herman & Polivy, 1980)] excluded.

7.2.2 Procedure

A within-subjects repeated-measures design was used such that each subject served as his or her own control and participated in all of the 4 sessions, each separated by approximately 1 wk and no less than 3 d. After an overnight (10-12 h) fast during which only water was permitted, the subjects arrived at the testing centre in the morning on the first day to complete a 30-min screening, and started 30 min later on the remaining 3 d (between 0730 and 0930). During screening subjects completed the MMSE and the Revised Restraint Scale (Herman & Polivy, 1980), and fasting plasma glucose, height and weight were measured. On each session, 1 test drink (preload) was given to the subject, who was asked to try to consume the entire amount within about 5 min. The 4 test drinks were placebo or 1 of 3 isoenergetic drinks

containing pure protein, carbohydrate, or fat. The order of the 4 sessions was counterbalanced across test drinks.

During each session, subjects were first administered a Sleep Habits and Stress Factors Questionnaire to determine if they were experiencing any unusual stress or illness, and to determine their dietary and physical activities from the previous day. To assess subjective appetite, subjects filled out Visual Analogue Scales (VASs) (Rogers et al., 1988; Stewart et al., 1997) before they ingested the test drink and throughout a 90-min period after the start of consumption. The Motivation to Eat VAS was filled out 0, 15, 30, 45, 60, and 90 min after the start of ingestion to assess feelings of hunger and satiety. The Physical Discomfort VAS was filled out at times 0, 60 and 90 min to determine level of discomfort. Finally, a Palatability VAS was filled out after consumption of the test drink. To assess actual food intake, an ad libitum lunch was provided to subjects after the 90-min period and intake was recorded. Subjects were also tested on a series of cognitive tests that were completed while sitting during this interval; the results were published elsewhere (Kaplan et al., 2001).

7.2.3 Test drinks (preloads)

Subjects were blinded to the content of the 4 test drinks, which all contained 300 mL, 774 kJ (except placebo), lemon juice, were of similar sweetness, and were consumed through a straw from opaque cups. The 4 drinks were 1) placebo: 290 mL water, 10 mL lemon juice and 23.7 mg sodium saccharin (Hermesetas Original; JL Freeman Inc.), 2) carbohydrate: 260 mL water, 10 mL lemon juice and 50 g glucose (Dextrose monohydrate; Bio-Health, Dawson Traders Ltd.), 3) protein: 260 mL water, 10 mL lemon juice, 50.5 g whey protein isolate (Ultimate Balance Whey Protein Isolate; provided by BioAdvantex Pharma, Wilmington, DE) and 23.7 mg sodium saccharin, and 4) fat: 248.9 mL water, 10 mL lemon juice, 41.1 g microlipid (50% safflower oil emulsion; Mead Johnson Nutritionals, Evansville, IN) and 23.7 mg sodium saccharin. The compositions of the drinks are shown in **Table 7.1.**

Table 7.1

Composition of test drinks

	Placebo	Protein	Carbohydrate	Fat
Volume (mL)	300	300	300	300
Energy (kJ)	0	774	774	774
Energy density (kJ/mL)	0	2.58	2.58	2.58
Protein (%energy)	0.0	98.4	0.0	0.0
Carbohydrate (%energy)	0.0	1.1	100.0	0.0
Fat (%energy)	0.0	0.3	0.0	100.0
Saccharin (mg)	23.7	23.7	0	23.7

7.2.4 Visual Analogue Scales

All VASs consisted of several questions (Rogers et al., 1988; Stewart et al., 1997). The Motivation to Eat VAS consisted of the following 4 questions: "How strong is your desire to eat?", "How hungry do you feel?", "How full do you feel" and "How much food do you think you could eat?". Subjects marked an "X" along a 10.0 cm line that was word-anchored at both ends. For example, for the question about desire to eat, one end was anchored with "very weak" and the other with "very strong". The distance on the line was used as the score, with a higher score representing greater satiety for the fullness question, and a lower score representing greater satiety for the other 3 questions. The Physical Discomfort VAS consisted of 5 word-anchored questions, which asked if the subjects felt nauseous, if their stomach hurt, if they felt well, if they had gas, or if they had diarrhea. The Palatability VAS consisted of 1 question: "How pleasant have you found the food?", which was anchored with "very pleasant" and "not pleasant at all".

The incremental areas under the 4 90-min Motivation to Eat VAS curves (AUCs) were used to determine the influence of each test drink on subjective measures of desire to eat, hunger, fullness and prospective consumption (Holt et al., 1995). An average appetite score (Stewart et al., 1997) was also calculated by combining the AUCs for all 4 questions. Average appetite = [desire to eat + hunger + (fullness*-1) + prospective consumption]/4. The change in VAS from fasting to 90 min post-ingestion, or immediately prior to lunch intake, was also used as a measure of subjective appetite for each VAS question.

7.2.5 Lunch

All foods were provided in bite-sized portions and weighed before and after consumption. The weight of each food that was consumed was determined by difference and total energy intake was calculated. Percent compensation at lunch for the energy content of the test drink was also calculated. Percent compensation = [lunch intake after placebo (kJ) - lunch intake after test drink (kJ)] X 100 / test drink (kJ).

At each of the 4 sessions, lunch consisted of an entire package of microwaveable Deluxe Pizza Bundles (Pillsbury Canada Ltd., Markham, Canada) containing meat, cheese and vegetables, which included 21-24 bundles, and as much drinking water as desired. This food was chosen because it is bite-sized, consistent in macronutrient and energy content relative to

providing a range of foods, was easy to prepare, and pizza is a common lunch food in Canada. Not including drinking water, the total food provided consisted of approximately 348 g, 3520 kJ, 29 g protein, 29 g fat, and 117 g carbohydrate (manufacturers' analysis). Three subjects, who requested cheese-only pizzas, were provided with an entire package of microwaveable Cheese Pizza Pops (Pillsbury Canada Ltd.) at each session, which included 4 Pops, each cut into quarters, and as much drinking water as desired. Each package contained approximately 448 g, 4800 kJ, 44 g protein, 48 g fat, and 132 g carbohydrate.

7.2.6 Statistical analyses

Statistical analyses were conducted with SAS 6.12 (SAS Institute Inc., Cary, NC). Repeated measures analyses of variance (ANOVA) were used to determine the effect of test drink on subjective appetite over the entire 90-min post-ingestion period, and at 90 min. Simple contrasts were used to determine the effect of each test drink compared with placebo; statistical significance was set at $p < 0.05$. Repeated-measures ANOVAs were conducted between each pair of test drinks (excluding placebo) to determine the influence of test drink on percent compensation at lunch; statistical significance was set at $p < 0.02$ (Bonferroni correction for multiple comparisons (Pagno & Gauvreau, 1993)). Repeated measures ANOVAs were conducted to determine the effect of test drink on lunch intake. Simple contrasts were used to determine the effect of each food or drink compared to placebo; statistical significance was set at $p < 0.05$. Planned linear regression analyses were conducted among subject characteristics, subjective appetite, and lunch intake; statistical significance was set at $p < 0.05$. Because men and women may have different physiological and psychological appetite signals (Zylan, 1996), all analyses were conducted with sex included and not included as a between-subject variable; no significant effects of sex were observed. Results are reported as means $\pm$ SEM unless indicated otherwise.

7.3 Results

7.3.1 Characteristics of subjects and test drink ingestion

All subjects consumed each of the 4 drinks within 7 min (mean $\pm$ SD: 2.9 $\pm$ 1.4 min). One subject only consumed two-thirds of the protein drink. Analyses were conducted both with this subject included and excluded. Actual test food intake for this subject was factored into the

calculations of percent compensation. The carbohydrate drink was rated as more palatable (3.4 $\pm$ 0.4 cm) than the other 3 drinks (placebo: 4.9 $\pm$ 0.5 cm; fat: 5.8 $\pm$ 0.6 cm; protein: 5.9 $\pm$ 0.6 cm; $p < 0.006$).

Subject characteristics for all subjects are shown in **Table 7.2.** No significant differences ($p > 0.05$) were observed between men and women on any of these measurements.

Table 7.2

Characteristics of subjects[1]

	All subjects ($n = 22$)	Men ($n = 11$)	Women ($n = 11$)
Age (y)	71.2 ± 1.3	70.0 ± 1.6	72.4 ± 2.2
Education (grade)	10.5 ± 0.3	10.2 ± 0.4	10.7 ± 0.5
MMSE (out of 30)	28.2 ± 0.3	27.7 ± 0.4	28.6 ± 0.3
BMI (kg/m^2)	25.6 ± 0.6	25.6 ± 0.7	25.6 ± 0.9
Restrained eating (0 to 35)[2]	9.0 ± 0.8	9.1 ± 1.5	8.9 ± 0.9
Fasting plasma glucose (mmol/L)	5.2 ± 0.1	5.3 ± 0.1	5.2 ± 0.1

[1]Mean ± SEM. MMSE, Mini-Mental State Examination.

[2]Lower value represents less restrained.

7.3.2 Effects of test drinks on subjective appetite

The data showed that both protein and fat, but not carbohydrate, induced greater subjective feelings of satiety than placebo over the 90-min post-ingestion period. The strength of the effects was more pronounced when highly restrained eaters were excluded from the analyses.

When the 4 questions were analyzed separately, both fat (-26 $\pm$ 24; p = 0.022) and protein (-3 $\pm$ 21; p = 0.043) led to lower ratings of prospective consumption than placebo (37 $\pm$ 16) over the 90-min post-ingestion period. By contrast, no significant effects were observed on the other 3 VAS questions or on the average of all 4 questions (average appetite) between any of the three macronutrient drinks and placebo. The same results were obtained when the subject who did not consume all of the protein drink was excluded.

Because restrained eaters may respond less to their physiological signals than non-restrained eaters (Fedoroff et al., 1997), subjective appetite analyses were conducted with 3 highly restrained eaters (Revised Restraint Scale scores $\geq$ 14) excluded. These subjects had restraint scores of 14, 14, and 16.

When the highly restrained eaters were excluded (n = 19), protein was found to lead to less desire to eat (p = 0.017) and less hunger (p = 0.051) than placebo. Fat led to greater satiety based on average appetite (p = 0.055; **Figure 7.1**) and specifically led to less prospective consumption (p = 0.044) than placebo. The effects of carbohydrate on subjective appetite still did not differ from placebo.

The influence of the test drinks on subjective appetite 90 min post-ingestion, or immediately prior to lunch intake, showed that only protein still induced significantly higher satiety than placebo. That is, protein led to less hunger than placebo (p = 0.026).

None of the test drinks had an effect on any of the 5 questions from the Physical Discomfort VAS at 60 or 90 min post-ingestion compared with placebo. The mean $\pm$ SD change from baseline for the average of the 5 questions was 0.49 $\pm$ 0.63 cm at 60 min and 0.14 $\pm$ 0.39 at 90 min on the 10.0 cm VAS scale.

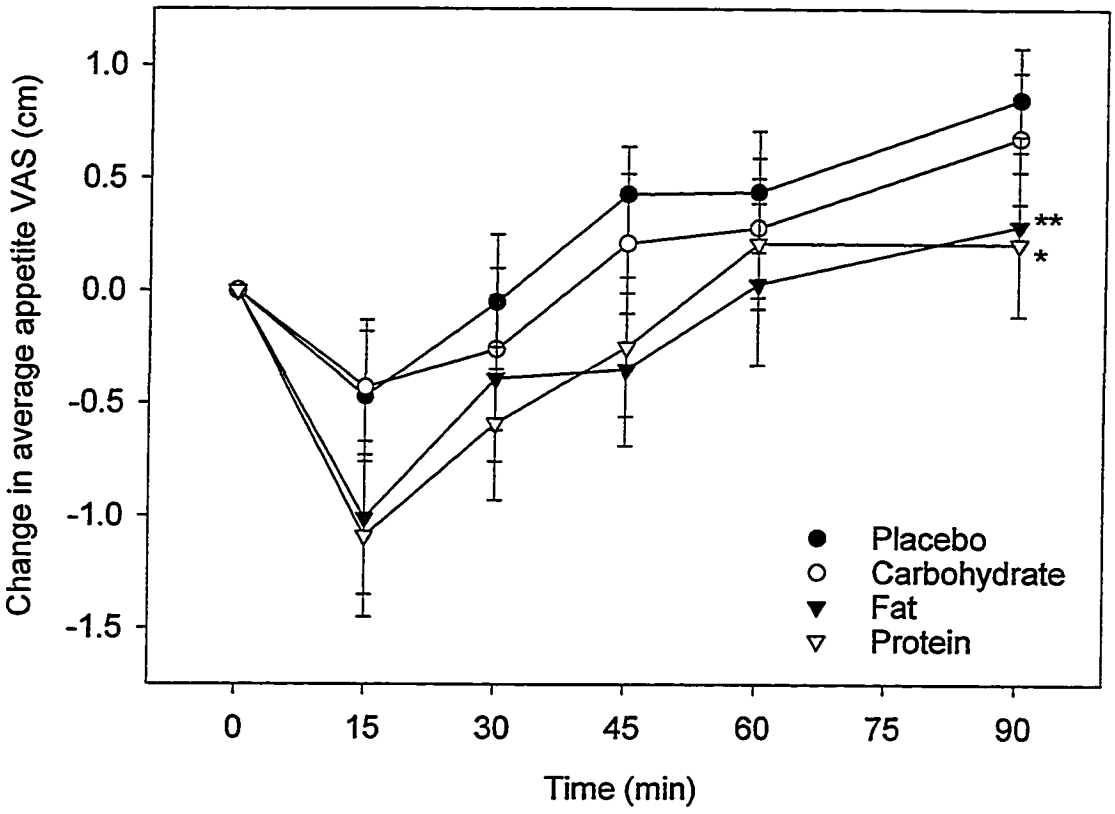

Figure 7.1. Mean ± SEM average appetite values for the Motivation to Eat VAS when highly restrained eaters were excluded. Average appetite = [desire to eat + hunger + (fullness*-1) + prospective consumption]/4. Lower values represent greater satiety. AUCs after fat and protein ingestion were lower (**$p = 0.055$) and trended to be lower (*$p = 0.091$) relative to placebo.

7.3.3 Effects of test drinks on food intake at lunch

Three subjects who did not wish to eat the lunch and 1 subject who ate the entire lunch on all 4 sessions were excluded from the analyses ($n = 18$). Although both protein and fat led to greater subjective feelings of satiety than placebo, there was no significant difference between intake at lunch after ingestion of protein (1460 $\pm$ 222 kJ), fat (1519 $\pm$ 230 kJ), or carbohydrate (1544 $\pm$ 213 kJ) compared with placebo (1703 $\pm$ 251 kJ). However, when the highly restrained eaters were excluded ($n = 15$) the difference in lunch intake between protein (1268 $\pm$ 163 kJ) and placebo (1531 $\pm$ 180 kJ) was significant ($p = 0.037$) (**Figure 7.2**). This effect was evident when sex was included as a between subject variable. The same results were obtained when the subject who did not consume all of the protein drink was excluded.

Percent compensation at lunch for the energy content of the test drink did not differ between pairs of drinks (30.9 $\pm$ 16.8 % for protein; 24.1 $\pm$ 18.7 % for fat; and 20.7 $\pm$ 16.2 % for carbohydrate). No differences were evident when the highly restrained eaters were excluded or when the subject who did not consume all of the protein drink was excluded.

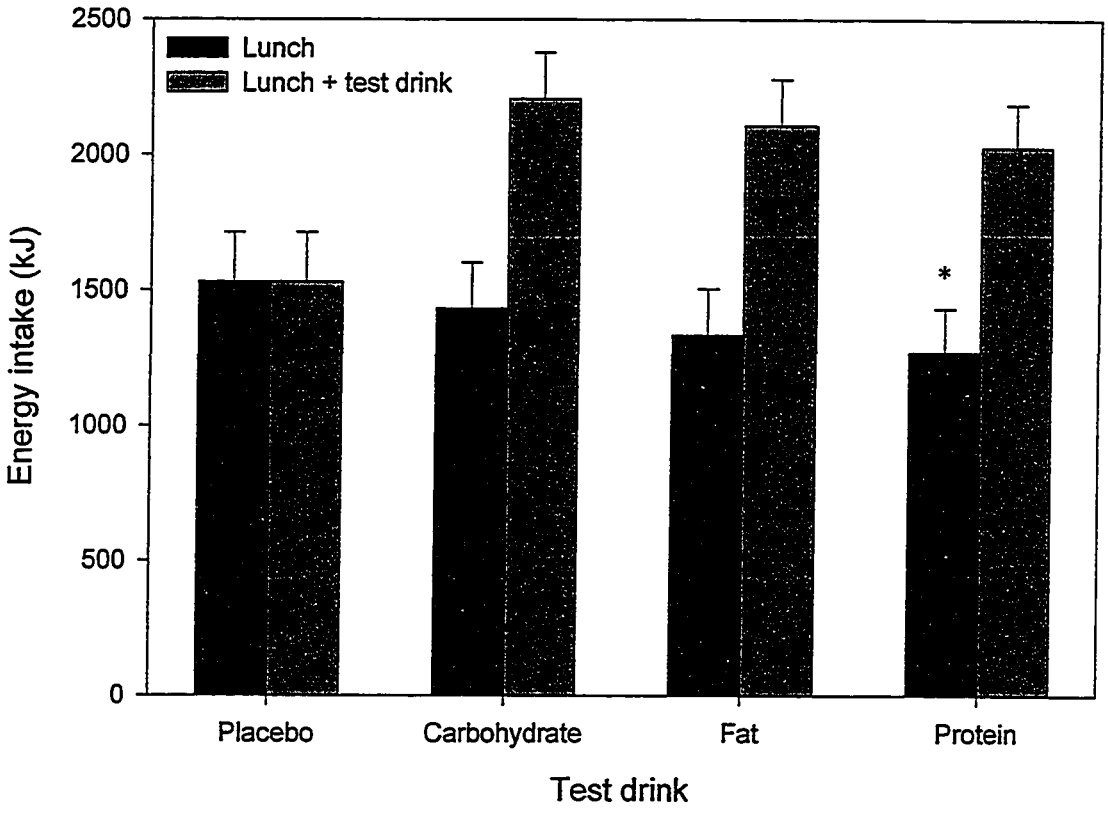

Figure 7.2. Lunch and total (lunch + test drink) intake after each test drink when highly restrained eaters were excluded. Lunch intake after protein ingestion significantly differed from placebo (*$p = 0.037$).

7.3.4 Relations among subject characteristics, subjective appetite and lunch intake

Restrained eating scores were associated with BMI ($r = 0.46$; $p = 0.031$) suggesting that more restrained eaters were heavier for their height. Restrained eating was not associated with percent compensation at lunch for any of the test drinks.

Subjective appetite was predictive of actual energy intakes at lunch. Lunch intake was associated with prospective consumption after carbohydrate ($r = 0.48$, $p = 0.045$) and with desire to eat after fat ingestion ($r = 0.67$, $p = 0.0025$), at the 90-min measurements. When highly restrained eaters were excluded, significant associations with intake were also evident for hunger and average appetite after placebo ingestion and for prospective consumption and average appetite after fat ingestion (data not shown). Average appetite AUC ($r = 0.48$; $p = 0.043$) and desire to eat AUC ($r = 0.64$, $p = 0.004$) were also associated with lunch intake following fat ingestion. In addition, percent compensation at lunch was significantly associated with several subjective appetite AUCs and at 90 min ($p < 0.05$; data not shown). These data were similar when highly restrained eaters were excluded.

7.4 Discussion

The present study was the first to examine the effects of pure sources of all three macronutrients on subjective appetite and food intake in the elderly. The ingestion of protein (whey) and fat (safflower oil) drinks, but not a carbohydrate (glucose) drink of equal energy and volume, induced greater subjective ratings of satiety during the next 90 min compared with placebo. However, only protein decreased ad libitum energy intake compared with placebo after 90 min in non-restrained eaters. These findings suggest that protein has the greatest effect on satiety on a per joule basis in the elderly and demonstrate that under experimental conditions the elderly respond to macronutrient specific signals in terms of both subjective satiety and subsequent intake.

The effects of the macronutrients on subjective satiety and food intake are comparable to those previously reported in younger adults, however exceptions were observed. With respect to food intake, these data are consistent with numerous studies in younger adults showing that high protein foods decrease subsequent intake more than high carbohydrate or fat foods (Booth, 1970; Rolls et al., 1988; Teff et al., 1989; Barkeling et al., 1990; Vandewater & Vickers, 1996; Porrini et al., 1997; Poppitt et al., 1998; Latner & Schwartz, 1999; Stubbs et al., 1999).

Similarly, the inability of either the carbohydrate or fat drinks to influence food intake in the current study supports the notion that these two macronutrients do not differ in satiety responses (De Graaf et al., 1992; Crovetti et al., 1998; Poppitt et al., 1998; Rolls & Bell, 1999; Marmonier et al., 2000; Rolls, 2000) when energy content and energy density are controlled (Rolls, 2000). One exception was observed in a recent study comparing pure isoenergetic (1254 kJ), equal energy density liquid preloads, which showed that sucrose suppressed food intake more than a safflower oil preload 60 min after ingestion (Woodend & Anderson, 2001). The discrepancy with the present study may be related to the different age groups, different carbohydrate sources, the smaller size of the preload in the present experiment, or the longer time between preload and food intake in the present experiment. Several physiologic mechanisms may account for the effects of each macronutrient on satiety (reviewed in (Stubbs, 1999)). For instance, protein satiety is associated with the stimulation of glucagon (Rocha et al., 1972) and cholecystokinin (Liddle et al., 1986), the rate of protein oxidation (Stubbs et al., 1996), and the thermic effect of protein (Crovetti et al., 1998).

While the food intake data were consistent with the subjective appetite data for protein and carbohydrate, the fat drink induced higher subjective satiety than placebo in the absence of a decrease in food intake. This finding is in contrast with most studies in younger adults, which have reported that protein induces higher subjective satiety than fat (Rolls et al., 1988; Holt et al., 1995; Stubbs et al., 1996; Crovetti et al., 1998; Poppitt et al., 1998; Stubbs et al., 1999; Marmonier et al., 2000). It is not clear why the fat drink induced higher subjective satiety without a decrease in intake, however, a lack of statistical power may be partly responsible because fat ingestion led to a non-significant decrease in intake at lunch of 196 kJ compared with placebo (Figure 7.2). The reason for observing a stronger effect of fat on satiety than in previous studies may be related to the fact that physiologic changes occur in aging, including increases in cholecystokinin, insulin, and amylin concentrations, a decreased ability of opioids to influence intake, a decline in hypothalamic neuropeptide-Y concentrations, and an impaired ability to detect hypoglycaemia (reviewed in (Morley, 1997; Morley & Thomas, 1999)). These data may also be partly explained by the fact that others have not controlled for energy density, or have used mixed macronutrients. Indeed, fat has been shown to induce higher satiety than carbohydrate when given directly into the small intestine of healthy young adults (Cook et al., 1997; Andrews et al., 1998). More studies with a larger sample size are required to determine if

the effect of fat on subjective satiety is reproducible and whether it extends to an actual decrease in food intake.

Although appetite regulation is impaired in the elderly relative to younger adults (De Castro, 1993; Roberts et al., 1994; Rolls et al., 1995; Keene et al., 1998), the present study showed that the elderly are sensitive to appetite sensations. Because no comparison was made to a younger group, no direct conclusions can be made about the relative ability of the elderly to respond to the effects of macronutrients on satiety and food intake. Nevertheless, because the subjects were blinded to all four drinks the observation that they were influenced by macronutrient ingestion suggests that they responded to physiologic signals. Furthermore, their subjective ratings were predictive of actual intake. That is, individuals with greater feelings of hunger ate more at lunch showing that they behaved in response to their subjective feelings. The observation that appetite responses are functional in aging may be important for regulating body weight because adaptive thermogenesis associated with excess energy intake appears to be blunted (Roberts et al., 1996). Thus, unlike younger adults, seniors are likely more dependent on maintaining body weight through adjustments in intake because thermogenesis cannot override the adverse effects of over-consumption.

The ability to observe a physiologic response to food ingestion in the elderly may be related to the fact that gastric emptying is delayed in aging (Clarkston et al., 1997). Indeed, the 90-120 min period between preload and lunch in the present experiments and in others that showed minimal appetite deficits in the elderly (Zandstra et al., 2000) was longer than in studies that have found greater impairments in the elderly (e.g., 30-60 min (Rolls et al., 1995; Keene et al., 1998)). Thus, extending the time between eating occasions may benefit the elderly by allowing them to adjust their intakes based on physiologic signals.

While often not assessed in the elderly, our present and previous findings (Kaplan & Greenwood, 2002) showed that restrained eating is evident in older adults and that it is associated with BMI. This suggests that individuals who are heavier for their height are more concerned with their diet, which is similar to what is observed in young adults (Tuschl et al., 1990). Moreover, the effects of the test drinks on satiety and food intake were stronger when highly restrained eaters were excluded from the analyses, suggesting that similarly to younger subjects (Fedoroff et al., 1997), elderly restrained eaters respond less to their physiologic signals than non-restrained eaters.

The design of this study differed from others because all three macronutrients were tested within the same study, energy density was controlled, subjects were blinded to the content of the test drinks, and pure macronutrients were used. The one previous study that investigated the effects of ingesting pure isoenergetic macronutrients found no differences among the preloads on subjective appetite and food intake in healthy young men, aged 21-36 y (Geliebter, 1979). The discrepancy may be related to the age of the subjects or to methodological differences, including the fact that the previous study used larger energy and volume preloads (1184 kJ and 450 mL vs. 774 kJ and 300 mL), different macronutrient sources (corn oil, cornstarch, and egg albumin vs. safflower oil, glucose, and whey protein), a shorter time between preload and lunch (70 vs. 90 min), and different lunch foods (liquid meal vs. pizza) than the present study.

The effects of pure macronutrients have rarely been examined because of the potential for these preloads, particularly fat, to taste unpleasant and to cause nausea, which could affect satiety (Rolls & Bell, 1999). In the present study, liquid preloads were used because this was the easiest way to develop palatable preloads containing pure macronutrients of equal energy, volume, and similar viscosity. Similar preloads have also been used previously to study appetite in young adults (Woodend & Anderson, 2001). Palatability was improved by adding a sweetener and lemon juice, which also masked the taste of the drinks, the drinks were consumed from opaque cups to blind subjects from their content, and the fat and protein sources were specifically chosen because these commercial products mixed easily with water. Although viscosity was not measured, all ingredients completely dissolved in water and there were no obvious differences in fluidity. The drinks were all rated as reasonably palatable, and the Physical Discomfort VAS showed that none of the drinks caused nausea or other discomfort.

Despite our efforts to control potentially confounding variables, the composition of the drinks may have limited the results. The liquid nature of the preloads may have made it more difficult for the subjects to adjust their food intake compared with solid preloads, because liquid preloads have a lesser effect on satiety than solids (Hulshof et al., 1993). Another potential limitation was that subjects rated the carbohydrate drink as more pleasant than the others. It has been suggested that greater palatability induces less satiety (Drewnowski, 1998). However, a recent study showed that palatability influences satiation, which is the effect on the duration of a meal, but not satiety, which is the effect on subsequent intake (De Graaf et al., 1999).

Furthermore, it is likely that if palatability affected satiety, it would occur soon after ingestion. In this study, by 90 min post-ingestion, protein still led to less hunger than placebo, and there was a trend for fat to induce higher subjective satiety. Finally, the protein and fat drinks had almost identical palatability ratings, yet they differentially affected food intake. Thus, although the present findings do not appear to be due to differences in palatability, it would be beneficial to repeat this study while matching palatability among all drinks.

Another limitation is that only one source of each macronutrient was tested. This study showed the effects of whey protein, safflower oil and glucose on satiety and food intake, however these effects cannot necessarily be extended to other sources of the macronutrients. The absence of an effect of glucose on satiety in the elderly is consistent with our previous work (Kaplan & Greenwood, 2002), however, different carbohydrates (Ludwig, 2000; Kaplan & Greenwood, 2002), fats (Van Wymelbeke et al., 1998; Lawton et al., 2000), and possibly proteins (Lang et al., 1998) exert different effects on satiety.

In summary, the present study showed that the ingestion of pure protein and fat drinks of equal energy and volume induced greater subjective satiety than a non-energy placebo drink in the healthy elderly while a carbohydrate drink did not affect satiety compared with placebo. Moreover, only the protein drink decreased food intake 90 min post-ingestion compared with placebo. Although this study did not address whether the magnitude of these effects would be relevant to the maintenance of a healthy body weight in the elderly, these results support the notion that protein induces satiety and that these responses are evident in older adults.

CHAPTER 8

GENERAL DISCUSSION

8. GENERAL DISCUSSION

8.1 Introduction

A summary of the hypotheses tested is presented in the next section, followed by three sections discussing the present research and potential future research regarding glucose regulation and baseline cognitive performance, macronutrient ingestion and cognitive performance, and macronutrient ingestion and appetite regulation respectively. In addition, a brief section discussing the interaction between cognitive function and appetite regulation is presented.

8.2 Summary of hypotheses tested

The hypothesis that energy ingestion from carbohydrates (glucose, potato, and barley), protein (whey), and fat (safflower oil) enhances cognitive performance compared with placebo in healthy elderly persons was supported by the experiments conducted in this thesis. In experiment 1, the ingestion of glucose and other carbohydrates did not lead to an overall improvement in cognitive performance, but did improve performance in subjects with relatively poor glucose regulation and baseline cognitive performance. In experiment 2, glucose, protein and fat improved overall performance compared with placebo. The cognitive-enhancing effects observed in both experiments suggest that energy ingestion improves performance independently of elevations in plasma glucose concentration.

The second hypothesis tested, which was that carbohydrates (glucose, potato, and barley) influence satiety differently from each other, and protein (whey) induces higher satiety than carbohydrate (glucose) or fat (safflower oil) in healthy elderly persons was also supported. Similar amounts of dietary carbohydrate provided in different forms affected subjective appetite and food intake differently from each other, however, the effects were independent of GI. In addition, protein ingestion, but not carbohydrate or fat ingestion, decreased subsequent energy intake compared with placebo.

8.3 Glucose regulation and baseline cognitive performance

An important and novel finding from the first experiment, which was not reproduced in the second experiment, was that relatively poor glucose regulation was associated with poor

baseline cognitive performance in cognitively intact elderly subjects with normal fasting plasma glucose concentrations. Specifically, subjects with relatively high incremental gAUC, poor β-cell function and good insulin sensitivity, which was related to low BMI, performed worse on tests of short- and long-term verbal declarative memory and on the Trails test after ingestion of the placebo than subjects with the opposite glucose regulatory and BMI profile. The glucose regulatory profile of the subjects who had poorer cognitive performance is consistent with the early stages of the pathogenesis of type 2 diabetes (i.e., β–cell dysfunction generally develops before insulin resistance in non-obese individuals (reviewed in (DeFronzo, 1987) and 18 of 19 subjects in the present study had a BMI below 29). Thus, although a cause-and-effect relationship cannot be established, certain brain functions may be impaired in very early stages of glucose dysregulation, prior to attaining clinical standards of impaired glucose regulation. Recent reports of similar findings in healthy young adults, published after the present experiment was completed, support these data (Messier et al., 1999; Donohoe & Benton, 2000).

The results of experiment 1 extend those of previous studies that have shown that greater deficits in glucose regulation are also associated with deficits in cognition. Indeed, individuals with type 2 diabetes (reviewed in (Strachan et al., 1997)) and non-diabetic subjects with impaired glucose tolerance (Kalmijn et al., 1995; Vanhanen et al., 1998) or an increased post-ingestion blood glucose concentration (Hall et al., 1989; Manning et al., 1990; Craft et al., 1992; Parker & Benton, 1995; Messier et al., 1997) perform worse on cognitive tests than those with better glycaemic control.

Additional evidence suggests that more advanced deficits in glucose regulation lead to more advanced cognitive decline, and that cognitive deficits can be reversed by improved glucose regulation. Indeed, type 2 diabetes greatly increases the risk of vascular dementia (Landin et al., 1993; Mortel et al., 1993; Tatemichi et al., 1993; Nielson et al., 1996) and AD, independent of other factors including atherosclerosis, blood pressure, and antihypertensive drug treatment (Yoshitake et al., 1995; Ott et al., 1996; Kuusisto et al., 1997; Leibson et al., 1997; Ott et al., 1999). Moreover, cognitive performance improves in individuals with type 2 diabetes by improving glucose regulation with oral hypoglycaemic agents (Gradman et al., 1993; Meneilly et al., 1993). Several observations may explain these relationships. These include reports that chronic hyperglycaemia can result in a loss of cortical neurons leading to neuroglycopenia (Strachan et al., 1997), hyperinsulinaemia can inhibit synaptic activity (Strachan et al., 1997),

and glucose dysregulation is associated with deficits in acetylcholine synthesis (reviewed in (Messier & Gagnon, 1996; Finch & Cohen, 1997; Messier & Gagnon, 2000)). Diabetes has also been linked to the development of AD by the association between chronic hyperglycaemia and the excessive formation of advanced glycation end-products, which may increase oxidative stress and result in cellular damage (Finch & Cohen, 1997; Ott et al., 1999).

The implications of these data are that preventing the development of poor glycaemic control, even before clinical signs of impairment are evident, may not only be beneficial for reducing the risk of diabetes but may also reduce the risk of memory decline and ultimately dementia and AD. Perhaps, recommendations for decreasing the risk of developing diabetes, which include maintaining a healthy body weight, exercising, increasing fibre intake, and decreasing saturated fat intake, should also be recommended to healthy elderly individuals to minimize the risk of cognitive decline. Some evidence showing a link among nutrition, glycaemic control, and cognition supports this suggestion. For instance, total dietary fat and saturated fat intake are associated with both poor glucose regulation (Clandinin et al., 1993) and impairments in cognitive performance in rodents (Greenwood & Winocur, 1990; Winocur & Greenwood, 1993; Greenwood & Winocur, 1996; Winocur & Greenwood, 1999; Greenwood & Winocur, 2001); reviewed in (Kaplan & Greenwood, 1998). Recent reports in human subjects have also shown that the same dietary factors that influence glucose regulation influence cognition. Increased fruit and vegetable intake decreases the risk of diabetes (Ford & Mokdad, 2001) and the long-term consumption of a diet high in fat and cholesterol and low in carbohydrate, fibre and some vitamins and minerals is associated with poor cognitive function in elderly subjects (Ortega et al., 1997; Kalmijn, 2001). Thus, similar nutrition and lifestyle behaviours appear to be beneficial for reducing the risk of both diabetes and cognitive decline. Nevertheless, long-term intervention studies are required to determine whether such environmental factors can actually reduce cognitive decline.

Despite the observation that glucose regulation was associated with baseline cognitive performance in the first experiment, these results must be reproduced in the healthy elderly because this association was not found in the second experiment. The reason for this is not clear because both studies used similar designs and subjects, but may be related to differences in baseline memory scores. The average age of the subjects in experiment 1 and 2 were 72.3 and 71.2 y respectively, and measures of glucose regulation were remarkably similar in the two

subject groups, making it unlikely that these factors explained the differences. Indeed, the mean fasting plasma glucose, fasting insulin, β-cell function (HOMA), and insulin resistance (HOMA) values were 5.4 mmol/L, 52.5 pmol/L, 78.4%, and 1.77 respectively in experiment 1 and 5.2 mmol/L, 51.9 pmol/L, 82.4%, and 1.70 in experiment 2. One possible explanation for the discrepancy between the two experiments is that baseline memory scores differed between the two studies. That is, a greater homogeneity of baseline memory scores was observed in the second study (CV for paragraph recall was 28%) than in the first (CV was 40%). Thus, a greater spread in baseline scores may be necessary to observe the glucose regulation-baseline score association in healthy individuals who have normal fasting plasma glucose concentrations.

Different methods of subject selection could be used in future studies to determine whether the inability to reproduce the results of experiment 1 in experiment 2 was related to the small variation in memory performance. One method would be to simply include more subjects, which would likely result in a larger range of baseline scores and glucose regulation among subjects. Another method, which would limit the number of subjects required, would be to specifically pre-select subjects who had a wide range of memory abilities and glucose regulation based on screening measurements.

Better methods of measuring glucose regulation are also required to validate the results. The present studies used a more standard method than used previously by employing the HOMA to estimate insulin resistance and β-cell function from fasting plasma glucose and insulin measurements. The incremental and total gAUCs were also calculated as other measures of glucose regulation estimating changes in blood glucose over time (only incremental gAUC was reported in Chapter 4 because it associated with memory, whereas only total gAUC was reported in Chapter 5 because neither variable associated with memory and total gAUC is a more standard measure). However, more sophisticated methods of analyzing glucose regulation, such as the glucose clamp method (Bergman et al., 1985), are needed to determine the precise level of β-cell function, insulin resistance, or glucose tolerance that is associated with cognitive impairment. Such studies would provide a better understanding of the mechanism associated with these observations and will be helpful for potential clinical applications.

In summary, evidence from previous studies suggests that impaired glucose tolerance and diabetes are clearly associated with cognitive impairment and that glucose dysregulation may be a causative factor. An important follow up question is to determine how early a

cognitive deficit can be observed throughout the progression from normal glucose regulation to diabetes. Identifying this stage could be helpful in developing strategies to prevent or treat memory decline by improving glucose regulation as early as possible with dietary, lifestyle, or medical interventions. Results from the first experiment presented here, though not reproduced in the second experiment, provide preliminary evidence that indeed glucose regulation is associated with cognitive function in the elderly even before the onset of clinically diagnosed stages of diabetes. Recent studies in young adults support this association (Messier et al., 1999; Donohoe & Benton, 2000), however these subjects may have had unusually poor glucose regulation for this age group (Messier et al., 1999). Thus, more studies are needed to determine how early in the development of glucose dysregulation this association can be observed, and to determine whether the effects are causally related. The ability to detect an association in healthy subjects will likely be difficult because the magnitude and range of the deficits in glucose regulation and cognitive function will be expected to be very minor and narrow. Nevertheless, if such studies demonstrate that early detection of glucose dysregulation can lead to intervention strategies that will prevent or slow the progression of cognitive decline and dementia, enormous strides in our ability to enhance the quality of life of the elderly will have been made.

8.4 Macronutrient ingestion and cognitive performance

Several observations were made regarding the effects of macronutrient ingestion on cognitive performance adding to the growing body of literature showing that nutrition has a significant impact on brain function. First, in addition to pure glucose, carbohydrate foods (potato and barley) improved declarative memory 15 min after ingestion and improved performance on Trails 60 min after ingestion compared with placebo in subjects with relatively poor baseline performance and glucose regulation (experiment 1). Second, experiment 2 showed that energy ingestion from protein (whey), carbohydrate (glucose), or fat (safflower oil) improved declarative memory 15 min post-ingestion. The effects of food ingestion on memory in both studies were independent of elevations in blood glucose concentration suggesting that any energy source improved memory. Third, in addition to the effects of the macronutrients on memory, each macronutrient enhanced performance on other cognitive tasks, suggesting that they may act via unique mechanisms (e.g., only glucose led to an overall improvement on Trails in men, only fat improved attention at 60 min, and only protein led to less forgetting at 15 min).

Finally, in both studies, energy ingestion improved some cognitive tasks, but not others, suggesting that the effects were specific to certain cognitive tasks and brain regions rather than indicative of a general enhancement of all brain functions.

Results from the present and previously reported studies suggest that glucose improves cognitive performance, and that the improvement may be dependent on glucose regulation and baseline performance. Both experiments showed that glucose improved performance in the healthy elderly, however, the effects were limited to subjects with relatively poor glucose regulation and baseline memories in experiment 1, but were evident in the overall subject group in experiment 2 (see **Table 8.1** for a comparison between the two experiments). The results of experiment 1 are consistent with previous studies that suggest that the beneficial effects of glucose are strongest in those with relatively poor memories and glucose regulation, including the healthy elderly (Gonder-Frederick et al., 1987; Hall et al., 1989; Manning et al., 1990; Craft et al., 1992; Manning et al., 1992; Parsons & Gold, 1992; Craft et al., 1993; Allen et al., 1996; Manning et al., 1997) and patients with AD (Craft et al., 1992; Manning et al., 1993), and showed that the effects are also observed with other carbohydrates. By contrast, the effects of glucose on memory in experiment 2 were evident in the overall subject group independent of glucose regulation and baseline performance. However, the effects of glucose on Trails was dependent on baseline performance in experiment 2 and one explanation for the discrepancy between the experiments is that glucose may have improved memory in the overall subject group in experiment 2 because the subjects had lower baseline memory scores than those in experiment 1. Indeed, mean baseline scores on immediate and delayed paragraph recall at 15 min post-ingestion, which was the most sensitive test, were 10.0 and 8.8 in the first study, and 9.4 and 7.9 in the second study respectively. Thus, if this explanation is valid, both studies are consistent with the hypothesis that a subgroup of subjects who have relatively poor glucose regulation and/or baseline memories are the only subjects who are sensitive to the cognitive-enhancing effects of glucose. These results must be reproduced using a larger sample size with a wider range of memory scores to clearly determine whether the effects are actually dependent on glucose regulation or baseline memory as suggested by experiment 1, or if they can be observed in the general elderly population as suggested by experiment 2.

TABLE 8.1

Comparison of the effects of each test food/drink on cognitive performance between experiments 1 and 2[1]

Cognitive test	Experiment 1						Experiment 2					
	Placebo	Glucose	Potato	Barley	Improvement vs. placebo[2] Overall[2]	Regression[3]	Placebo	Carbohydrate (glucose)	Protein (whey)	Fat (safflower)	Improvement vs. placebo Overall	Regression
Immediate paragraph recall (score out of 25)												
15 min	10.0±1.0	10.6±1.0	10.4±0.8	10.2±0.6	NS	Po, B	9.4±0.6	11.0±0.9	10.7±0.9	11.1±0.7	G, Pr, F	NS
60 min	10.3±0.8	10.7±0.9	9.7±1.0	10.6±0.9	NS	G	10.5±0.7	10.9±0.6	10.5±0.8	11.3±0.9	NS	NS
Total (15 + 60)	20.2±1.7	21.3±1.9	20.1±1.6	20.8±1.2	NS	B	19.9±1.1	21.9±1.3	21.2±1.4	22.4±1.5	G, F	NS
Delayed paragraph recall (score out of 25)												
15 min	8.8±1.0	9.0±1.1	9.7±0.9	9.5±0.8	NS	G, Po, B	7.9±0.6	9.9±0.9	10.9±0.9	9.7±0.9	G, Pr, F	NS
60 min	8.3±0.8	9.0±0.9	8.8±0.9	8.8±1.1	NS	NS	9.0±0.8	9.9±0.7	9.2±0.9	9.5±1.0	NS	NS
Total (15 + 60)	17.1±1.7	18.0±1.9	18.4±1.6	18.3±1.5	NS	G, Po, B	16.8±1.2	19.8±1.4	20.1±1.6	19.2±1.7	G, Pr, F	NS
Total (immediate+delayed) paragraph recall (out of 50)												
15 min	18.8±2.0	19.6±2.1	20.1±1.7	19.7±1.4	NS	Po, B	17.2±1.1	21.0±1.7	21.6±1.8	20.8±1.5	G, Pr, F	NS
60 min	18.6±1.5	19.7±1.8	18.5±1.9	19.4±1.8	NS	G	19.5±1.4	20.7±1.3	19.7±1.6	20.7±1.9	NS	NS
Immediate word list recall (score out of 12)												
15 min												
List 1	4.0±0.3	4.1±0.4	4.2±0.3	4.2±0.3	NS	NS	4.5±0.2	4.5±0.3	4.9±0.3	4.4±0.2	NS	NS
List 2	5.5±0.4	5.4±0.3	5.8±0.3	5.6±0.4	NS	NS	6.0±0.3	5.9±0.3	6.2±0.4	6.0±0.4	NS	NS
List 3	7.0±0.4	6.5±0.4	6.4±0.4	6.1±0.4	NS	NS	6.7±0.4	7.2±0.4	7.1±0.4	6.8±0.4	NS	NS
Total (1 + 2 + 3)	16.5±0.9	15.9±0.9	16.4±0.8	15.9±1.0	NS	NS	17.1±0.8	17.6±0.8	18.3±0.9	17.2±0.8	NS	NS
60 min												
List 1	4.1±0.3	4.0±0.3	4.0±0.3	4.4±0.2	NS	NS	4.6±0.2	4.3±0.2	4.3±0.3	4.1±0.3	NS	NS
List 2	5.1±0.3	5.4±0.4	4.9±0.4	5.4±0.3	NS	NS	6.0±0.3	5.9±0.2	5.8±0.4	5.2±0.3	F worse	NS
List 3	6.0±0.3	5.8±0.4	6.5±0.4	6.0±0.4	NS	NS	7.2±0.3	6.4±0.3	6.8±0.4	6.3±03	F worse	NS
Total (1 + 2 + 3)	15.1±0.8	15.2±0.9	15.3±1.0	15.8±0.8	NS	NS	17.8±0.8	16.5±0.7	17.0±0.9	15.6±0.8	F worse	NS
105 min												
List 1	3.7±0.2	4.0±0.3	3.9±0.2	4.0±0.3	NS	NS	—	—	—	—	—	—
List 2	5.4±0.3	5.3±0.3	5.1±0.2	5.4±0.3	NS	NS	—	—	—	—	—	—
List 3	6.2±0.3	5.6±0.4	5.5±0.4	6.3±0.4	NS	NS	—	—	—	—	—	—
Total (1 + 2 + 3)	15.2±0.6	14.8±1.0	14.5±0.7	15.6±0.8	NS	NS	—	—	—	—	—	—
Total 15+60/15+60+105	46.8±1.9	45.9±2.5	46.1±2.1	47.2±2.2	NS	NS	35.0±1.4	34.1±1.3	35.2±1.6	32.8±1.5	G-men worse	NS
Trail making test [time to complete (s)]												
15 min												
Part A	—	—	—	—	—	—	47±3	47± 3	50±3	48±3	G-, F-men	NS
Part B	107±9	103±8	104±10	100±6	NS	G, B	100±10	93±7	101±9	103±8	NS	G, F
B - A	—	—	—	—	—	—	52±9	46±6	45±5	55±7	NS	G, F
A + B	—	—	—	—	—	—	147±11	140±9	143±9	152±10	G-men	G, F
60 min												
Part A	—	—	—	—	—	—	47±3	46±3	48±3	45±3	NS	NS
Part B	102±9	100±9	98±7	101±9	NS	G, Po, B	96±9	89±6	96±10	94±7	NS	G, F
B - A	—	—	—	—	—	—	48±8	43±5	40±4	50±6	NS	G, F
A + B	—	—	—	—	—	—	143±11	136±8	135±9	139±8	G-men	G, F
105 min												
Part A	—	—	—	—	—	—	—	—	—	—	—	—
Part B	99±7	96±8	93±8	90±6	NS	B	—	—	—	—	—	—
Total 15+60+105	308±22	299±22	294±24	291±20	NS	G, B	—	—	—	—	—	—
Attention test (% correct)												
15 min	—	—	—	—	—	—	67.7±3.5	69.5±2.4	65.2±3.7	61.8±3.1	NS	NS
60 min	—	—	—	—	—	—	62.7±2.2	62.7±2.8	62.6±2.5	68.2±2.9	F	NS
15 + 60 min	69.0±2.6	67.3±2.8	65.9±3.2	70.0±2.6	B-women	B-men	—	—	—	—	—	—

[1]Mean±SEM.

[2]Overall effect of food compared with placebo. NS, not significant; letters indicate significantly different from placebo (G, glucose; Po, potato; B, barley; Pr, protein; F, fat). Improvement observed unless noted as "worse". "Men" or "women" indicates that the effect was limited to that gender.

[3]Letters indicate that regression analyses showed that glucose regulation and/or baseline performance was associated with improved performance with food.

The preceding discussion raises the question of whether the cognitive-enhancing effects of food ingestion observed in these experiments were dependent on baseline scores, glucose regulatory status, or both. Considering that poor baseline memory was associated with glucose regulation in experiment 1 and that glucose regulation was not associated with the cognitive-enhancing effects of glucose in experiment 2, it is possible that poor baseline memory was the important factor in determining sensitivity to the effects of glucose (and the other macronutrients) independently of glucose regulation. Unfortunately, because baseline performance was associated with glucose regulation, the independent effects of these factors cannot be determined, hence, the results of the present studies cannot resolve this issue. Nevertheless, the present data and those of previous studies, discussed earlier, suggest that glucose regulation is an important factor in determining the sensitivity of subjects to the cognitive-enhancing effects of glucose. Data from experiment 1 (Tables 4.3 and 4.4) showed that β-cell function, insulin resistance, and gAUC, but not baseline performance, was associated with the improvements observed after glucose ingestion on both the paragraph recall and Trails tests. These data along with those of many previous studies indicate that both factors are likely important in determining the sensitivity of subjects to the cognitive-enhancing effects of glucose and other macronutrients. Future studies testing subjects with similar baseline memories but different glucose regulatory profiles, and conversely, testing subjects with similar glucose regulatory profiles but different baseline memories, are needed to resolve this issue.

The idea that individuals with relatively poor glucose regulation are the most sensitive to the effects of glucose on memory suggests that glucose brings relatively impaired individuals up to an optimal level of functioning but does not improve functioning if it is already optimal. This suggestion seems somewhat counterintuitive because of the potential negative effects of hyperglycaemia on cognition in people with diabetes (Ryan et al., 1992; Sachon et al., 1992). Indeed, research conducted by our laboratory in subjects with type 2 diabetes (Appendix 1), showed that although carbohydrate ingestion initially improved memory 15 min after ingestion, memory was impaired 30 min post-ingestion when blood glucose concentrations peaked. These results and others (Craft et al., 1992; Craft et al., 1994; Allen et al., 1996; Messier et al., 1999) suggest that there exists an optimal range of glucose regulatory status that is sensitive to the memory-enhancing effects of glucose ingestion. In other words, as observed previously (Craft et al., 1994), individuals with very good or very poor glucose regulation, such as healthy young

adults and diabetics respectively, may not show cognitive improvements after ingesting glucose or other carbohydrates, whereas those with a minor impairment in glucose regulation may be the only subgroup that shows improvements. It is not yet possible to define this minor impairment, however, it appears that healthy elderly individuals who have not been diagnosed with impaired glucose regulation (e.g., impaired fasting glucose, impaired glucose tolerance, or diabetes), but who have poorer regulation than healthy young subjects, fit within this optimal range of people who are most sensitive to the cognitive-enhancing effects of carbohydrates.

The mechanism explaining the observation that carbohydrate-induced improvements only occur in subjects with relatively poor glucose regulation is not known. However, the effects may be related to an increased degradation and decreased synthesis of acetylcholine in individuals with poor glucose regulation, along with the ability of glucose to enhance acetylcholine synthesis (reviewed in (Messier et al., 1999)). Thus, carbohydrate ingestion may increase acetylcholine synthesis in individuals with poor glucose regulation and depressed cholinergic activity leading to improved memory, but have no effect or even a negative effect in those with normal glucose regulation and acetylcholine concentrations. Differences in the ability of glucose to elevate central insulin concentrations in subjects with different glucose regulatory abilities has also been proposed as a mechanism to explain the selective effects of glucose on cognition (Craft et al., 1994).

A comparison of the effects of glucose ingestion in the two studies shows that glucose consistently and robustly improves performance on some aspects of cognition but the effects on other aspects are variable (Table 8.1). With respect to paragraph recall, an improvement was observed in both studies on 15 min delayed paragraph recall, however, an overall (15 + 60 min) improvement was evident only on delayed recall in experiment 1, but on immediate and delayed recall in experiment 2. In addition, an overall effect was observed at 60 min in experiment 1, but at 15 min in experiment 2. These results suggest that there is a clear enhancement on delayed paragraph recall at 15 min, but that the effects on immediate recall and at 60 min are less robust. These results are consistent with studies showing that the effects are stronger on tests mediated by the medial temporal lobes, such as delayed recall (Manning et al., 1990; Craft et al., 1994; Manning et al., 1997; Foster et al., 1998) and suggest that the ability to observe effects on immediate recall and at 60 min may be related to the specific subject groups tested. It is more difficult to compare the effects on Trails because different scoring methods were used in

the two studies, however, in general, there was an overall benefit of glucose at 15 and 60 min in experiment 1, but the effects in experiment 2 were limited to men. Perhaps the effects are evident in both genders, but are more pronounced in men, which is consistent with one previous study (Craft et al., 1994). These results support previous data showing that glucose consistently enhances performance on long-term verbal declarative memory tasks, but further research is required to define the other aspects of cognition that are affected.

The results from both studies indicated that energy ingestion from any macronutrient improved memory independently of elevations in blood glucose. These data differ from previous reports that suggested that blood glucose must increase substantially for glucose (Parsons & Gold, 1992; Manning et al., 1993) or mixed macronutrient meals (Kanarek, 1997; Bellisle et al., 1998) to improve cognitive performance, and suggest that mechanisms responsive to any energy ingestion are involved in mediating these acute effects of food ingestion. It has been suggested that the memory-enhancing effects of a glucose drink in the elderly (Gonder-Frederick et al., 1987; Hall et al., 1989; Manning et al., 1990; Manning et al., 1992; Parsons & Gold, 1992; Craft et al., 1994; Manning et al., 1997; Messier et al., 1997; Manning et al., 1998) may be explained by an increase in plasma glucose concentration that could increase acetylcholine synthesis in the hippocampus region (Gold & Stone, 1988; Wenk, 1989; Messier et al., 1999), or increase centrally acting insulin (Craft et al., 1996; Craft et al., 1999). However, the activation of gut peptides and stimulation of the vagus nerve could explain the effects of glucose and the other macronutrients on memory (Flood et al., 1987; Clark et al., 1999). This hypothesis does not rule out the acetylcholine or insulin hypotheses but suggests that more than one mechanism may be involved.

Activation of the gut-brain axis as well as the effects of centrally acting post-absorptive signals could explain the macronutrient-specific effects on cognition that were observed. Each macronutrient would be expected to release a different profile of peptides throughout the duration of testing and the previously mentioned acetylcholine and insulin hypotheses could explain the specific post-absorptive effects of glucose. Other mechanisms explaining these effects can be hypothesized based on the observations that each macronutrient differentially affects hypothalamic insulin (Gerozissis et al., 1997; Gerozissis et al., 1998) and serotonin (Rouch et al., 1999). Thus, although the ingestion of energy may influence cognition by one

mechanism, each macronutrient could improve performance via additional distinct mechanisms involving gut peptides and centrally acting signals.

These results are limited by the specific sources of each macronutrient tested. Although it has been suggested here that all macronutrients can improve memory, albeit with some distinct differences, these results are limited to the specific sources of each macronutrient tested (i.e., glucose, potato, barley, whey, and safflower oil). Some recent studies in younger subjects suggest that other sources of the macronutrients may have distinct effects on memory (Catherine, 2000; Woodend, 2000; Tecimer, 2001). In these studies from the same laboratory, sucrose improved immediate recall of a word list 15 min after ingestion compared with other carbohydrates (amylose, amylopectin, polycose, glucose-fructose) and a control in one set of experiments (Catherine, 2000), but not in others (Woodend, 2000; Tecimer, 2001). In experiments comparing macronutrients, safflower oil improved performance on delayed word list recall compared with sucrose, but neither treatment differed from a water control (Woodend, 2000), and soy protein weakly improved memory compared with whey and egg protein on one component of a word list test, but did not differ from sucrose or control (Tecimer, 2001). Thus, individual sources of macronutrients may have different effects on memory, at least in younger subjects. More research is needed to determine if the results presented in this thesis can be extended to other sources of the macronutrients in elderly subjects.

Consistent with previous reports of the effects of glucose ingestion on cognition (Manning et al., 1990; Craft et al., 1994; Manning et al., 1997; Foster et al., 1998), the most robust cognitive enhancements were observed on a long-term declarative memory task (paragraph recall), which is mediated by the medial temporal lobe and related structures (Squire & Zola, 1996). By contrast, tasks mediated by other brain regions, such as attention and immediate word list recall, were not consistently improved by macronutrient ingestion. These findings suggest that the macronutrient effects are related to specific cognitive tasks mediated by specific brain regions, rather than to a general enhancement of brain function. However, it is also possible that the lack of an observed benefit on word list recall and attention are related to the fact that these tests are of less complexity and difficulty than the paragraph recall test. Indeed, some have suggested that glucose ingestion improves performance on tasks such as attention if they are of sufficient difficulty (reviewed in (Korol & Gold, 1998)). The evidence to date indicates that the medial temporal lobe, including the hippocampus and related structures, is

most sensitive to the effects of glucose on cognitive function, but that glucose may enhance performance on other tasks to a lesser extent.

Although the lack of a benefit of glucose ingestion on word list recall is consistent with previous studies (Manning et al., 1990; Craft et al., 1994; Manning et al., 1997), glucose and fat ingestion actually led to an impairment on some of the word list scores. However, because of the large number of scores used to analyze this test, the few impairments observed may have been anomalies. The more consistent finding was that no improvements were observed on most scores from this test, supporting the notion that the effects of each macronutrient may be somewhat task specific depending on the brain regions involved. Better tests of sufficient difficulty that specifically test individual cognitive skills and types of memory (e.g., encoding, storage, or retrieval) are required to define the range of brain functions that are affected by glucose and other macronutrients.

The studies presented here were unique in that they were designed to test the effects of various nutrients on cognitive function at more than one post-ingestive time point. This paradigm was used because of the potential for each nutrient to have different effects at different time points. Previous studies investigating the effects of glucose on cognitive performance have generally administered a series of tests 15-20 min post-ingestion, whereas in these studies separate batteries of tests were administered at 15 and 60 min post-ingestion in both experiments and at 105 min in the carbohydrate study. To accommodate this, it was necessary to develop several new versions of each cognitive task. In general, the results of the two experiments suggested that the most robust effects, which occurred with any energy source, were on the paragraph recall test 15 min post-ingestion. In addition, each macronutrient had different effects 60 min post-ingestion. As mentioned, the individual effects of each macronutrient are likely related to pre- and post-absorptive factors involved in the digestion and absorption of each macronutrient. No significant beneficial effects of carbohydrates were observed after 105 min.

Despite the stronger effects of food ingestion on memory 15 min after ingestion, the design of the studies may have caused substantial proactive interference at 60 and 105 min post-ingestion. That is, memory performance at the longer post-ingestive time points may have been diminished because of interference from material that was learned at the earlier time points. Thus, to more accurately determine the specific effects of each nutrient on cognitive function after 60 min, it would be necessary to administer tests at 60 min only.

The first study was also designed to examine the effects of food ingestion on cognition within a shorter period after ingestion than had been tested previously. That is, previous studies that have examined the effects of meals on cognitive performance have only tested the effects 30 min to 4 h post-ingestion with mixed results regarding the effects on cognitive performance (Smith et al., 1988; Michaud et al., 1991; Benton & Sargent, 1992; Lloyd et al., 1994; Rogers & Lloyd, 1994; Smith et al., 1994; Green et al., 1995; Lloyd et al., 1996). The fact that beneficial effects were observed at the earlier post-ingestion time points in the present studies and not at 105 min may partly explain the mixed results of previous studies. Nevertheless, to determine the specificity or generality of the effects of macronutrients on memory, future studies need to examine more post-ingestion time points and at various times of the day because circadian rhythm may also affect cognition (May et al., 1993).

The possibility that sex differences exist regarding the effects of nutrients on cognitive function was tested in these studies, as both studies included half male and half female subjects. In contrast to a previous study that reported that men, but not women, were sensitive to the cognitive enhancing effects of glucose ingestion, possibly related to differences in glucose regulation between the sexes (Craft et al., 1994), the present data did not show any consistent differences between genders. There was some evidence of different effects between the sexes, such as the finding that glucose benefited Trails performance in men, but not women, in the second study, but no general conclusions about different effects can be made from these data. Overall, these findings indicate that cognitive performance can be influenced by macronutrients in both elderly men and women.

The significance of this work is that it emphasizes the fact that nutrition has a significant impact on brain function. This research extends our understanding of the effects of energy ingestion and of each macronutrient on cognition with respect to the tasks and brain regions involved, the importance of post-ingestion time periods, and the relationship with glucose regulation. More specifically, these findings suggest that it may be important to determine if it is beneficial for elderly individuals to consume regular meals without going long periods between meals in order to maintain normal functioning. This may be important particularly because of the anorexia of aging, or decrease in energy intake, that can occur in this population (Morley, 1997). Hopefully, understanding the role of an environmental influence such as

nutrition on cognitive function will lead to a better understanding of potential dietary changes that can prevent cognitive decline and dementia or reverse cognitive deficits.

Although it is important to understand the acute effects of nutrition on cognitive function as presented in these studies, it is ultimately more important to determine the longer-term effects of nutrition. Understanding the long-term effects will hopefully lead to clinical applications for the prevention and treatment of cognitive deficits and dementia. The evidence indicating that poor glucose regulation impairs cognition, and that improvements in glucose regulation with medication can reverse the cognitive impairments, leads to the suggestion that preventing the development of poor glucose regulation or improving it with dietary and lifestyle changes can also help prevent cognitive decline. At this point, the findings that high saturated fat and low fibre diets are associated with cognitive impairments (Ortega et al., 1997; Kalmijn, 2001) suggest that dietary intervention is promising, but this must be tested. Thus, long-term intervention studies are needed to determine whether lifestyle factors that improve glucose regulation can also prevent declines in cognitive function or improve cognition. Potential interventions include increasing exercise, weight loss, and fibre intake, and decreasing saturated fat intake. The amount of change required to lead to such effects as well as the age that such intervention would be effective are important considerations for future research.

8.5 Macronutrient ingestion and appetite regulation

In addition to examining the effects of macronutrients on cognitive function, the present studies tested the effects of macronutrients on subjective satiety and subsequent food intake. Although several interesting observations were made regarding appetite regulation in the elderly, the studies were specifically designed to test cognitive function such that the investigation of appetite regulation was a secondary objective. Thus, some components in the design of these studies may not have been ideal to examine the tested hypothesis and objectives. Limitations of the present results due to design issues have been noted in the following discussion.

The results of the present experiments suggest that the elderly were able to respond to macronutrient-specific physiologic appetite signals in terms of both subjective satiety and subsequent energy intake. The first experiment showed that among foods containing similar amounts of carbohydrate, potatoes (high GI) induced the greatest subjective feelings of satiety,

followed by barley (low GI), and then glucose (high GI) relative to placebo over a 120-min post-ingestion period. However, both potatoes and barley, but not glucose, decreased energy intake after 120 min compared with placebo. Experiment 2 showed that the ingestion of protein (whey) and fat (safflower oil) drinks, but not a carbohydrate (glucose) drink of equal energy and volume, induced greater subjective ratings of satiety during the next 90 min compared with placebo. However, only protein decreased ad libitum energy intake compared with placebo after 90 min in non-restrained eaters. Although limited by the macronutrient sources used, these findings suggest that protein induces higher satiety than carbohydrate or fat on a per joule basis in the elderly and that different carbohydrate sources have different effects on satiety, independent of GI.

Although appetite regulation has been shown to be regulated less precisely in healthy elderly subjects than in younger subjects (De Castro, 1993; Roberts et al., 1994; Rolls et al., 1995; Keene et al., 1998), the present results suggest that the elderly are able to respond to physiologic appetite signals. Because no younger comparison group was included in these studies, no conclusions can be made about the relative ability of the elderly to respond to the effects of macronutrients on satiety and food intake. Nevertheless, the observations in both studies that subjective appetite and energy intakes 90 or 120 min after preload ingestion differed depending on the composition of the preloads, and that these effects were observed in experiment 2 even though subjects were blinded to the content of the test drinks, suggests that the subjects responded to physiologic signals. In addition, subjective appetite ratings were predictive of energy intake at lunch supporting the notion that physiologic signals were involved. However, the subjects did not completely compensate at lunch for the energy content of the preloads in both studies. That is, they consistently overate at lunch so that total intake (preload + lunch) was higher than after the placebo. Thus, although previous studies have shown that physiologic signals regulating appetite are suppressed in the elderly, these studies suggest that they still exert a considerable influence on food intake behaviour.

The ability to observe a significant physiologic response to food ingestion in the elderly may be stronger when there is a longer time period between preload and test food ingestion. One reason for this may be because gastric emptying is delayed in aging (Clarkston et al., 1997). Indeed, the 90-120 min period between preload and lunch in the present experiments and in those that showed minimal appetite deficits in the elderly (Zandstra et al., 2000) was longer than

in studies that have found greater impairments in the elderly (e.g., 30-60 min (Rolls et al., 1995; Keene et al., 1998)). Thus, extending the time between eating occasions may permit the elderly to respond better to their physiologic signals. This longer time period may potentially benefit the elderly by allowing them to adjust their intakes appropriately.

The ability to respond to physiologic signals may be more important for body weight regulation in the elderly than in younger adults because of differences in adaptive thermogenesis between age groups. Adaptive thermogenesis can help to override the adverse effects of excess energy consumption in young adults even if they do not accurately alter their intake in response to physiologic signals. By contrast, adaptive thermogenesis associated with excess energy intake is blunted in the elderly (Roberts et al., 1996). Thus, minor alterations in energy intake may affect body weight to a greater extent in the elderly thereby increasing the importance of the ability to regulate food intake in response to physiologic appetite signals.

The present studies suggest that concerns about food intake and body weight are present in the elderly and may impact appetite regulation. Using a restrained eating questionnaire that has primarily been used in young adults (Herman & Polivy, 1980), but that has been used in the elderly (Polivy, personal communication), the present studies showed that some elderly subjects are restrained eaters, and that restrained eating was associated with BMI. That is, individuals who were heavier for their height were more concerned with their diet and body image, similarly to what is observed in young adults (Tuschl et al., 1990). Moreover, the effects of the test foods and drinks on subjective satiety and food intake were stronger when highly restrained eaters were excluded from the analyses, suggesting that as in younger subjects (Fedoroff et al., 1997), more restrained eaters may respond less to their physiologic signals than less restrained eaters. Thus, it may be important to consider these factors when designing research studies and in developing dietary guidelines for maintaining or losing weight in seniors.

The results of experiment 2 showing the effects of macronutrients on subjective satiety and subsequent food intake were consistent with most studies in younger subjects. Using equal energy and volume drinks, protein (whey) ingestion, but not carbohydrate (glucose) or fat (safflower oil) ingestion, suppressed subsequent energy intake compared with placebo. These findings showed that protein induces higher satiety than the other macronutrients on a per joule basis in seniors, supporting the results of previous studies in young adults that have shown that high protein foods increase satiety more than high carbohydrate or fat foods (Booth, 1970; Rolls

et al., 1988; Teff et al., 1989; Barkeling et al., 1990; Vandewater & Vickers, 1996; Porrini et al., 1997; Poppitt et al., 1998; Latner & Schwartz, 1999; Stubbs et al., 1999). In particular, these data are consistent with a recent study in young adults that showed that pure whey and soy protein, but not egg protein or sucrose, suppressed food intake compared with a control 60 min after ingestion (Tecimer, 2001). Thus, not only macronutrient content, but also the specific source is important; whey protein in particular may have a strong effect on satiety. These data also suggest that when energy density is controlled, carbohydrate and fat do not differ in their effects on food intake, which is consistent with some studies in young adults (Rolls, 2000) but not with others that have shown that pure carbohydrate (sucrose) suppresses intake more than pure fat (safflower oil) (Woodend & Anderson, 2001). Again, the discrepancy between the present and latter study may be related to the different carbohydrate sources used, but may also be related to differences in the age of the subjects, sizes of the preloads, or the time between preload and food intake between these studies. Clearly, more research is needed to address the generality of the effects of each macronutrient on satiety in light of the noted differences among specific sources of each macronutrient.

One surprising finding was that although the fat drink did not suppress food intake, it induced higher subjective satiety than placebo. By contrast, the subjective satiety mirrored the actual intake data for protein and carbohydrate. Most studies in younger adults have reported that protein induces higher subjective satiety than fat (Rolls et al., 1988; Holt et al., 1995; Stubbs et al., 1996; Crovetti et al., 1998; Poppitt et al., 1998; Stubbs et al., 1999; Marmonier et al., 2000). A lack of statistical power may partly explain these findings because fat ingestion led to a non-significant decrease in intake at lunch compared with placebo. The reason for observing a stronger effect of fat on satiety than in other studies may be related to the several physiologic changes that occur in aging (reviewed in (Morley, 1997; Morley & Thomas, 1999)) or to differences in methodology with previous studies. More studies using a larger sample size are required to determine whether fat induces higher satiety than placebo in the elderly.

The effect of glucose ingestion on appetite was examined in both experiments and showed that glucose did not induce higher subjective satiety or decrease subsequent food intake compared with placebo. These data differ from those in young adults that have shown that similar doses of glucose (50-75 g) suppress food intake one hour after ingestion (Rogers et al., 1988; Rogers & Blundell, 1989; Anderson et al., 2001). The discrepancy between the present

and previous studies may be related to the age of the subjects or to methodological differences. Glucose may not suppress intake in the elderly because of actual differences in appetite regulation between the age groups or it may simply be more difficult to observe an appetite suppressing effect in the elderly because appetite regulation is blunted. The fact that very minor, though not significant, effects of glucose on subjective appetite and intake were evident in the present studies (Figures 6.2, 6.3, 7.1 and 7.2) suggests that the latter suggestion is possible; a larger sample size is required to determine whether or not this is the case. Alternatively, the 90 and 120 min time period between preload and lunch in the present experiments may have been too long to observe an appetite suppressing effect of glucose, which may only occur within the first 60 min (Anderson, 1995). Finally, subjects in both studies found the glucose drinks to be more palatable than the placebo, which may have minimized the effects of glucose on satiety (Holt et al., 1995; Drewnowski, 1998). Thus, future studies attempting to more clearly define the effects of glucose on appetite in the elderly should attempt to control palatability and should test the effects within one hour after ingestion.

As mentioned, although most studies reported in the literature have compared the general effects of protein, carbohydrate, and fat, the effects of individual sources of each of these macronutrients may differ substantially. Indeed, the first experiment showed that similar amounts of carbohydrate provided as different sources had different effects on subjective appetite and food intake regulation, but that GI did not explain the effects. Although these findings are limited by the food sources used, they are inconsistent with the conclusion of a recent review article that suggests that low GI foods increase satiety more than high GI foods (Ludwig, 2000). However, Ludwig (Ludwig, 2000) did not review the complete literature on the topic, and in fact, the present study is at least the sixth that has not supported this hypothesis (Krishnamachar & Mickelson, 1987; Barkeling et al., 1995; Holt et al., 1996; Stewart et al., 1997; Anderson et al., 2001). Although it remains possible that low GI foods can induce higher satiety in certain situations, the present study and others suggest that several components of food are likely as important as GI, including volume (Black et al., 1993; Rolls et al., 2000), weight (De Graaf & Hulshof, 1996), energy density (Drewnowski, 1998; Rolls, 2000), fibre content (Burton-Freeman, 2000), palatability (Holt et al., 1995; Drewnowski, 1998), and liquid or solid form (Hulshof et al., 1993). These findings are important because the incidence of insulin resistance and diabetes is high in the elderly (Harris et al., 1987), and consuming low GI foods

has been used as part of the treatment for these conditions (Wolever, 2000). Future studies should determine whether there are specific situations in which GI has a greater effect on satiety. For instance, increasing the time period between the ingestion of a low GI preload and food intake may lead to greater satiety relative to a high GI preload if the effects on satiety are related to the hypoglycaemia associated with high GI foods.

Results from the carbohydrate study showed a relationship between higher insulin resistance and higher protein intake. However, no other associations between measures of glucose regulation and appetite were observed in either study. It is unclear whether this finding was related to the tendency for subjects with higher BMIs to avoid dessert foods at lunch or whether an actual physiological effect influenced them to choose the higher protein diet. Although the cause cannot be deciphered from this experiment, this observation again raises the point that it may be important to study the relationship between glucose regulation and appetite regulation in this age group, which is at greater risk for insulin resistance. Studies are needed to determine why those with poorer insulin sensitivity would select more protein. Such findings may help or hinder people with diabetes from adhering to their prescribed diets.

As mentioned, the interpretation of these studies is limited by some components in the designs due to the fact that the primary objective was to examine cognitive performance rather than appetite. The many differences between the designs of the two studies also make it impossible to compare them. In the first study, the effects of similar amounts of carbohydrate from different sources varying in GI were examined. However, although the GI of the foods differed substantially among the foods, many other factors that can impact satiety also differed making the interpretation of the specific effects of GI difficult. These factors include the fact that one source (glucose) was a liquid and the others were solids (potato and barley), that subjects were not blinded to the foods, that palatability differed, and that the foods differed in volume, weight, energy, protein, fat and fibre content (see Table 6.1). To more accurately investigate the specific effect of the GI of foods on appetite in the elderly, future studies need to control all of these factors. One method, used in a study in young subjects, would be to compare equal energy and volume beverages containing different pure carbohydrate sources (Anderson et al., 2001). The second study was designed to control for most of these variables by using pure macronutrients, by blinding subjects to the content of the drinks, by using easily dissolvable macronutrients, and by controlling for energy, volume, weight, and weight. Unfortunately,

despite efforts to match palatability, which has been shown to affect satiety, it differed among the drinks.

Other variables in both studies that may not have been ideally suited to test appetite in the elderly were the doses used, the time period between preload and lunch intake, and the foods used for lunch. The size of each preload was based on the amount of carbohydrate shown in previous studies to improve memory (50 g or 774 kJ), whereas the amount needed to produce an effect on satiety may be higher. Indeed, a minimum amount of sucrose is needed to produce an effect on satiety in young adults, and the effects are dose dependent (Woodend & Anderson, 2001). A study using several doses of the same preload would be required to determine the ideal dose for this age group. Lunch was provided after the cognitive testing had been completed, but perhaps a different time may have been more or less sensitive to the effects on appetite considering the delayed rates of gastric emptying in the elderly. Again, various post-ingestion time points would have to be tested to determine the most suitable time. Finally, the ability to detect a difference in the effects of the preloads on food intake may be related to the amount of food consumed at lunch. Young subjects have been shown to consume over 4000 kJ 60 min after ingesting a non-energy drink after an overnight fast (Anderson et al., 2001; Woodend & Anderson, 2001), whereas the subjects in the present studies consumed just over 2000 and 1700 kJ 120 or 90 min after ingestion of the placebo in experiment 1 and 2 respectively. Increasing the intake at lunch by possibly providing better tasting or more energy-dense foods may be helpful, but this will be difficult considering the much smaller sized meals that people in this age group are used to eating. The amounts consumed in the present studies may therefore be a more realistic measure of the effects of preloads on subsequent intake in this age group.

As with the cognitive research, although these acute preload studies are helpful for increasing our understanding of the effects of nutrition on appetite, long-term studies are required to determine if the effects are clinically relevant. For instance, it is important to understand whether a high protein diet can be beneficial in designing weight loss programs over the long term, or whether people only respond to other factors such as energy density, volume, palatability, and environmental cues outside the laboratory.

8.6 Interaction between cognitive function and appetite regulation

Although the studies conducted were designed to independently investigate the effects of nutrition on cognitive function and on appetite regulation, it is interesting to look at the relationship between the two. Some previous animal research conducted by our laboratory and by others has attempted to link these seemingly divergent behaviours. For instance, we and others found that a high intake of saturated fatty acids compared with other fatty acids increases the subsequent selection of protein and decreases the selection of carbohydrate in rats (McGee & Greenwood, 1990; Mullen & Martin, 1990). Our laboratory has also shown that saturated fatty acids impair cognitive performance independently of other fatty acids (Greenwood & Winocur, 1990; Greenwood & Winocur, 1996). We have proposed that these behaviours may be linked by a common mechanism (Kaplan & Greenwood, 1998). That is, saturated fatty acids have been shown to impair glucose regulation (Clandinin et al., 1993), which may impact on the CNS and lead to both increased protein intake and poorer cognitive performance. Others have found a link between the effects of peripheral hormones on feeding and cognitive behaviours (Morley et al., 1994). These authors showed that food ingestion and CCK administration improves memory in rodents and that the effect is abolished when a CCK antagonist is administered. They have concluded that the meal-induced memory enhancement is associated with the release of CCK, and furthermore, that the effect is dependent on the stimulation of the vagus nerve. These authors have suggested that their findings can be explained from an evolutionary perspective; it is reasonable to believe that a mechanism that would allow an animal to remember the details of a successful hunt for food would be beneficial for survival (Flood et al., 1987).

Because of these potential links between feeding and cognitive behaviours, analyses were conducted comparing the appetite and cognition results of the present experiments. Linear regression analyses were conducted comparing average percent compensation at lunch for the preloads compared with immediate and delayed paragraph recall performance at 15 and 60 min post-ingestion in both experiments. However, no significant relationships were observed (experiment 1: $r < +/-0.17$, $p > 0.48$; experiment 2: $r < 0.37$, $p > 0.13$). These results suggest that among the healthy elderly individuals tested, a poor ability to regulate appetite was not related to poor memory skills. Although these data support a study in young adults that also showed no association between subjective appetite or food intake and memory scores

(Catherine, 2000), the present studies were not specifically designed to test this relationship. Thus, it may be premature to suggest that there is no link between these behaviours. Subjects with greater deficits in appetite regulation and/or cognition may need to be tested for such a relationship to be observed.

CHAPTER 9

SUMMARY AND CONCLUSIONS

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9.1 Summary

1. Measures of glucose regulation were associated with baseline cognitive performance in elderly subjects with normal fasting plasma glucose concentrations in one experiment suggesting that very minor alterations in glucose regulation can impair performance. However, these findings were not reproduced in a second experiment.

2. In one experiment, glucose and other carbohydrates with very different GIs (potatoes and barley) did not improve cognitive performance in the overall subject group, but did improve performance in subjects with relatively poor glucose regulation and baseline cognitive performance independently of elevations in plasma glucose concentration. In a second experiment, glucose improved performance compared with placebo in the overall subject group.

3. In addition to carbohydrate (glucose), protein (whey) and fat (safflower oil) improved memory performance compared with placebo further suggesting that energy ingestion can improve performance independently of elevations in plasma glucose concentration. Notably, each macronutrient had unique effects on cognitive performance.

4. Similar amounts of available carbohydrate from foods varying in GI differentially affected subjective appetite and food intake, but the effects were not predicted by GI. Potatoes (medium GI) induced the highest subjective satiety, followed by barley (low GI), then glucose (high GI), which trended towards inducing higher satiety than placebo. Both potatoes and barley decreased food intake 120 min after ingestion compared with placebo.

5. Equal energy and volume protein (whey) and fat (safflower oil) preloads, but not a carbohydrate (glucose) preload, increased subjective satiety in elderly subjects compared with placebo but only protein decreased food intake 90 min after ingestion.

9.2 Conclusions

1. Glucose, other carbohydrates (potato and barley), protein (whey) and fat (safflower oil) improved memory performance compared with placebo suggesting that energy ingestion can improve performance independently of elevations in plasma glucose concentration.

2. Similar amounts of dietary carbohydrate provided in different forms (glucose, potato, and barley) affected subjective appetite and food intake differently from each other, however, the effects were independent of GI. In addition, protein (whey) ingestion, but not carbohydrate (glucose) or fat (safflower oil) ingestion, decreased subsequent energy intake compared with placebo.

CHAPTER 10
REFERENCES

10. REFERENCES

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11. APPENDICES

Appendix 1

Published abstracts from two additional studies that were not reported in this thesis, but that were conducted during the development of the thesis:

Greenwood, C.E., Hebblethwaite, S., Kaplan, R.J., Papanikolaou, Y. & Jenkins, D.J.A. (2000). Carbohydrate induced memory impairment in individuals with type 2 diabetes. ***The FASEB Journal.*** **14: A260.**

Adults with type 2 diabetes experience a memory impairment that can be reversed by pharmacologic treatment and improvement in overall glycemic control. The degree to which carbohydrate (CHO) consumption can contribute to or exacerbate memory dysfunction has not been examined. Thus, the objective of this study was to determine the impact of providing 50 g of available CHO (as ½ bagel + white grape juice) at breakfast to adults with type 2 diabetes. Subjects (n = 20; age 63 ± 9; BMI 26.1 ± 4.5) were tested, under fed and fasted conditions, on declarative memory using both word list and paragraph recall tests [immediate and delayed (10 minute) recall], Trails Part B as a measure of general brain function, and mood (subjectively monitoring global vigor and affect). CHO ingestion impacted on measures of delayed, but not immediate, recall in a time dependent fashion (time X food) (word list, $p = 0.046$ and paragraph, $p = 0.044$) such that delayed recall was improved at 15 minutes post ingestion but was impaired at 30 minutes. These changes in declarative memory were not accompanied by changes in either trails scores ($p = 0.17$) or mood (affect, $p = 0.68$ and vigor, $p = 0.45$). These results demonstrate that consumption of high glycemic index foods can impair declarative memory, at least during the absorptive state, in adults with type 2 diabetes. (Supported by NSERC).

Greenwood, C.E., Papanikolaou, Y., Kaplan, R.J., Fletcher, D., Bruce, B. & Spiller, G.A. (2000). Breakfast consumption enhances long-term declarative memory in seniors. ***Society for Neuroscience Abstracts.*** **26: 2013.**

Glucose consumption enhances cognitive function in seniors, with the most robust and consistent effects being observed on measures of declarative memory. Despite these data relating to glucose treatments, little information is available on the impact of consuming high carbohydrate (CHO) containing foods. This study examined the effects of eating 50 g of available CHO [as cereal (24 g of whole wheat flakes) + 1% milk (1/2 cup) + white grape juice (123 mL)] at breakfast in 41 healthy free-living seniors. All subjects had normal fasting glucose levels and were tested under fed and fasted (250 mL water) conditions. Testing included declarative memory, using both word list and paragraph recall [immediate and delayed (20 minute) recall], and Trails Parts A and B as a measure of general brain function. In agreement with previous studies, fasting blood glucose level was a negative predictor of paragraph recall performance ($R^2 = 0.18$), measured under placebo conditions. Breakfast ingestion enhanced measures of delayed, but not immediate recall (word list, $p = 0.007$ and paragraph, $p = 0.003$) monitored at 35 and 55 minutes post ingestion. These changes in declarative memory were not accompanied by changes in Trails scores ($p = 0.522$). These results parallel those previously reported for glucose administration and demonstrate that CHO consumption enhances cognitive performance in seniors with tests relying on hippocampal function being the most sensitive. (Supported by General Mills USA).

Appendix 2

The attached Letter to the Editor (Das) and Reply (Kaplan et al) were previously published (Am J Clin Nutr 2001; 74: 409-411). The Letter was written in response to the published data (Kaplan et al 2000, Am J Clin Nutr; 72: 825-836) that were presented in Chapter 4 of this thesis.

Cognitive performance and glucose

Dear Sir:

A recent report by Kaplan et al (1) suggested that glucose enhances cognitive performance. This work is supported by extensive evidence that modest increases in circulating glucose concentrations enhance the formation of new memories in rodents and humans (reviewed in reference 2). Glucose enhances memory for several different tasks in rodents. In humans, glucose enhances memory in healthy young and elderly persons and in persons with Alzheimer disease or Down syndrome (2). The effect of glucose on cognitive functions across species and tasks suggests that glucose might act on the areas of the brain important for memory formation, which may be in addition to glucose's being the major source of energy for the central nervous system. This suggestion is supported by the observation that microinjections of glucose into the septohippocampal system of rats enhance mnemonic functioning (3). In this context, it is interesting to note that glucose is critical for the production of acetyl-CoA, a precursor of acetylcholine (4), and that decreases in glucose concentrations result in decreases in brain acetylcholine (5). Thus, one strong possibility is that glucose enhances memory processes by increasing acetylcholine synthesis and release (2). This is substantiated by the observation that glucose can modify the effects of cholinergic drugs on various behavioral and neural measures (2). Furthermore, extracellular brain glucose concentrations vary with neuronal activity, indicating that glucose may be critical in modulating memory functioning (6). This is supported by the report that hippocampal acetylcholine release is increased in rats during a spatial task (2).

Insulin receptors are present in brain cells and may play a role in brain cognitive functions (7), including learning and memory. Insulin is also a potent stimulator of endothelial nitric oxide formation (8) and an inhibitor of tumor necrosis factor α (TNF-α) synthesis (9). One of the functions of insulin in the brain could be to stimulate nitric oxide formation and at the same time to down-regulate TNF-α synthesis so that neurons are protected from the neurotoxic actions of TNF-α (10) and memory formation is aided. Thus, one important function of insulin, insulin receptors, and glucose in the brain may be to protect neurons from the death signals of TNF-α. This is in addition to the role of glucose in improving memory. The finding that hyperinsulinemia improves memory in patients with Alzheimer disease (11) supports this view. Furthermore, nitric oxide is also believed to play a role in memory formation. On the basis of this evidence, I suggest that there is a close interaction between glucose, insulin, insulin receptors in the brain, endothelial nitric oxide, TNF-α, and neuronal survival and memory formation.

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Reply to UN Das

Dear Sir:

We agree with Das that glucose ingestion was shown to improve memory performance within ≈1 h of ingestion compared with a non-energy-containing placebo in a range of human and rodent populations (1). The strongest effects in humans are observed in elderly subjects and in those with existing memory deficits or relatively poor glucose regulation (2) and may be limited to declarative memory tasks (3), which are mediated by the medial temporal lobe and related structures (4). For instance, in the study that Das referred to, we found that the beneficial effects of glucose ingestion were most evident in healthy elderly subjects with normal fasting plasma glucose concentrations who had the poorest β cell function (5). Furthermore, we extended the glucose data by showing that other carbohydrate foods could also improve memory.

Several mechanisms have been suggested to explain the effects of glucose ingestion on memory performance. Das discussed some of these and presented another potential mechanism. One common hypothesis suggests that glucose ingestion may improve memory by increasing plasma glucose concentrations, leading to alterations in glucose uptake and utilization by the brain and ultimately to an increase in the glucose-mediated synthesis of acetylcholine in the hippocampus region (6). As mentioned by Das, research with rodents supports this hypothesis (7). Others have suggested that the insulin response to an increase in glucose may be responsible for the effects on memory (8). Das suggested further that insulin may improve memory by stimulating endothelial nitric oxide formation and inhibiting the synthesis of tumor necrosis factor α. Although each of these mechanisms may be involved in mediating the effects of glucose on memory, our work suggests that other mechanisms are also involved.

The important finding of our study, which Das failed to refer to, is that barley ingestion improved memory similarly to the ingestion of glucose and mashed potatoes even though it had a minimal effect on blood glucose. Although it has been commonly argued that blood glucose must increase to 8–10 mmol/L for memory to improve (9), we found that barley, which increased blood glucose to only 6.7 mmol/L, improved memory similarly to glucose and potatoes, which increased blood glucose to ≈9.5 mmol/L. In addition, although not measured, we anticipate that barley would also minimally affect insulin concentrations (10). Consequently, the aforementioned mechanisms are unlikely to account for the memory-enhancing effects of barley. Instead, our data suggest that the provision of energy, independently of elevations in blood glucose, can improve memory.

The effects of energy ingestion on gut-mediated responses could explain our findings. Several gut peptides, including cholecystokinin (11), gastrin-releasing peptide, pancreastatin, and amylin (12), influence memory in rodents, likely via stimulation of ascending fibers of the vagus nerve (11). Furthermore, electrical stimulation of the vagus in human subjects improves declarative memory (13), and vagotomy decreases the memory-enhancing effects of glucose (14) and peripherally injected drugs (11). Thus, the ingestion of any energy source may improve memory by these mechanisms, independently of elevations in blood glucose. Importantly, this mechanism does not rule out the acetylcholine or insulin hypotheses, but instead suggests that carbohydrates affect cognition by more than one mechanism. Our current work, examining the effects of protein, carbohydrate, and fat on memory performance, will increase our understanding of the effects of energy ingestion on cognition compared with the individual effects of each macronutrient.

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Dietary ratio of animal to vegetable protein and rate of bone loss and risk of fracture in postmenopausal women

Dear Sir:

We reported recently in the Journal that in free-living elderly white women, bone loss and hip fracture rates were greatest in those consuming diets with the highest ratio of animal to vegetable protein content (1). Those diets represented diets with the highest ratios of animal to vegetable foods consumed. Because animal foods tend to be net acid producing and vegetable foods net base producing (2), our results support the hypothesis that diet-dependent net endogenous acid production (NEAP) is a risk factor for bone loss and hip fracture in elderly white women.

In an accompanying editorial, Heaney (3) offers comments that might lead some readers to discount our findings and interpretation. He contends that opponents of the use of animal products "have had a disproportionate effect both on public consciousness and on the agenda of nutritional science itself," and that it "would be surprising if the study had not been influenced to some extent by currents in the larger society."

To allay the concerns of Journal readers, we offer a public opinion we published before having had any reason to suspect our article would be interpreted by anyone as having been unduly influenced by animal activist press:

> By referring to the American diet as "protein-rich," and linking dietary protein's acid yield to bone damage, [the article entitled] "Could Diet Attack Bones? It's a Beef About Meat" [(4)] might lead some readers to believe that Americans are eating too much protein. In fact, the protein content of American diets is below the evolutionary norm for humans, and therefore may be overall nutritionally suboptimal. For bone, the problem may not be too much acid from protein, but too little acid-neutralizing base from those types of plant foods that are rich in base, such as roots, tubers, fruits, and vegetable fruits and leaves. The plant foods that Americans eat most are cereal grains, such as wheat and rice, which are unusual plant foods in that they yield acid, not base. To boot, grains crowd out base-rich plant foods from the diet, helped in that by all those empty-calorie foods Americans eat, such as refined sugars and separated fats. In the acid attack on bone, the beef therefore is not so much with meat, as with grain and empty-calorie foods.

Nevertheless, Heaney may prove to have been prescient in predicting that "it is virtually certain that [the article by Sellmeyer et al] will be used by some to 'prove' that animal protein is positively harmful." But if some do so, it will be because they failed to recognize that our findings speak only to the ratio of animal to vegetable foods consumed, as indexed by the ratio of animal to vegetable protein in the diet, and not specifically to the absolute amounts of animal food or protein consumed. Our hypothesis is that the rate of NEAP is a risk factor for bone loss and hip fracture. In our study, the ratio of animal to vegetable protein consumed was used as a surrogate for that risk factor because animal foods are richer in acid precursors than in base precursors and because many vegetable foods are richer in base precursors than in acid precursors (2). It would not be inconsistent with our findings or hypothesis if large increases in total animal protein intake above the range consumed by our subjects reduced rates of bone loss and fracture if those increases were accompanied by appropriate increases in the intake of vegetable protein in the form of high-base-yielding plant foods.

Heaney also takes us to task for teaching the myth that sulfuric acid yields are higher from animal than from vegetable protein. That chastisement reflects an uncritical read of our paper, however, because we neither asserted nor implied such differential acid yields. Indeed, we are on record for taking pains to disabuse followers of that myth, to the extent of even supplying a table showing overlapping values of potential sulfuric acid yields for a wide variety of animal and plant foods (per gram protein) (5). Moreover, any differences in animal compared with vegetable yields of sulfuric acid that might be obtained are only part of the picture. Another part of the picture is the differential animal and vegetable yields of bicarbonate from nonprotein constituents of the food. Many plant foods (eg, roots, tubers, leafy green vegetables, and fruit) are richer per gram protein in such bicarbonate precursors than are animal foods (2). As an index of the NEAP, the ratio of animal to vegetable protein consumed in whole-food diets reflects the differential animal compared with vegetable yields of bicarbonate as well as those of sulfuric acid.

Heaney contends that our findings are at odds with reports of a positive relation between animal protein intake and skeletal health, specifically citing the Framingham Osteoporosis Study by Hannan et al (6). However, the focus of our article was the association of the rate of bone loss (and hip fracture) with the ratio of animal to vegetable protein consumed, whereas Hannan

Appendix 3

The 2 paragraph recall tests (Kaplan Revision), 12 word lists, 8 versions of the Trail Making Test Part A, and 11 versions of the Trail Making Test Part B that were developed by the author of this thesis are shown below.

Paragraph Recall – Kaplan Revision

"DAVID"

David /Simpson/ of Quebec City/, a skilled /astronaut /in the Canadian /aeronautics program/, travelled /on the Space Shuttle/ to the Russian /space station/ on a three-month/ voyage /last March/. During the flight /back to Earth/ an asteroid /hit the aircraft/, causing damage/ to one wing/. The pilot /was forced to land /in the sea /where the crew was rescued /by the Air Force.

David:		Anna (from WMS-R):
Ideas in paragraph:	25	25
Sentences:	3	3
Words/sentence:	30, 16, 18 (mean = 21.3)	37, 17, 13 (mean = 22.3)
Total words:	64	67
Syllables/sentence:	47, 22, 20	52, 19, 18
Total syllables	89	89
Syllables/word:	1.39	1.33

Scoring:

Text	General Rule	Examples of 1-point	Examples of 0-point
David	"David" or variant	Dave; Davey	Doug; Donald
Simpson	"Simpson" is required	----	Simmons
of Quebec City	"Quebec" is required	from Quebec; who lived in Quebec; came from Quebec	----
a skilled	An indication	an experienced;	----

	that he was good at his job	a qualified	
astronaut	"astronaut" is required	-----	aviator; pilot
in the Canadian	"Canadian" or Canada is required	-----	the North
aeronautics program	indication of space or air program	space; aircraft program; organization	automotive; vehicle
travelled	indication that he travelled	flew; went; went up; visits	watched; witnessed
on the Space Shuttle	"Space Shuttle" or variant is required	space ship; rocket; space flight	airplane
to the Russian	any variant of "Russian"	Russia; Soviet; Mir	----
space station	any variant of space station	space ship; space craft; satellite	----
on a three-month	"three-month" is required, or a phrase meaning about three	a few months; two or three months	two-month; two-week
voyage	term meaning voyage	journey; trip flight	----
last March	"March" is required	----	spring; winter; April
During the flight	expression that means during flight	during the mission; on the flight; on the way	----
back to Earth	term meaning back home	back home; home; to our planet; back	moon; Mars
an asteroid	a word implying matter or rock in space	meteorite; meteor; meteoroid; piece of a planet; space rock; satellite	----

hit the aircraft	indication that the aircraft was hit	smashed into the ship; hit or smashed (if clear that aircraft hit)	hit into (or indication that the aircraft hit something)
causing damage	indication that damage was caused by the asteroid	----	----
to one wing	"one wing" or variant is required	a wing; single wing;	one part; both wings
The pilot	"pilot" or variant	flier	----
was forced to land	indication that they were forced to land	had to land; crashed; it went down	landed
in the sea	ocean or variant	ocean; water	----
where the crew was rescued	indication that people on ship were rescued	people were saved; they were rescued; picked up	----
by the Air Force	"Air Force" in any context is required	----	----

Paragraph Recall – Kaplan Revision

"CATHY"

A teacher/ named Cathy/ Davis /was walking/ her bull terrier/ in Johnson park/ in a suburb/ near Vancouver/, when a tall man / tried to take/ her orange/ purse/. Her dog bit the man/ with its powerful jaws/. He realized /that his right/ knee /was bleeding /and began to flee/. The dog chased the man/ into the thorny/ bushes /. Suddenly, a police officer arrived/. He said to the woman/, "you should thank 'Shaggy'".

Cathy:		Robert (from WMS-R):
Ideas in paragraph:	25	25
Sentences:	6	6
Words/sentence:	28, 9, 12, 9, 5, 9 (x =12.0)	25, 9, 10, 11, 7, 6 (x = 11.3)
Total words:	72	68
Syllables/sentence:	39, 11, 16, 12, 11, 11	37, 11, 14, 13, 9, 10
Total syllables	100	94
Syllables/word:	1.39	1.38

Scoring

Text	General Rule	Examples of 1-point	Examples of 0-point
A teacher	"teacher" or variant is required	school teacher; professor	councillor
named Cathy	"Cathy" or variant	Catherine; Kathleen Kate; Katey	Cindy
Davis	"Davis" is required	----	Davidson
was walking	walking or variant	strolling	----
her bull terrier	bull terrier or variant	bulldog; terrier	pit bull; poodle
in Johnson park	"Johnson" and park or variant required	Johnson land, garden; park Johnson's park; Johnson trail	

in a suburb	"suburb" or variant	suburbia; neighbourhood; outlying district	town; city
near Vancouver	"Vancouver" required	----	----
when a tall man	"tall man" or variant	tall male	big; large; a man
tried to take	indication that the man tried to take something	grabbed; tried to grab; tried to steal	attacked her
her orange	orange is required and indication that it is hers	----	yellow; black
purse	"purse" or variant	hand bag; shoulder bag	wallet; bag
Her dog bit the man	"bit" or variant and indication that man was bitten	bit him; took a bite out of him	scratched the man
with its powerful jaws	"powerful jaws" or variant	strong teeth; strong mouth	jaws; teeth
He realized	indication that man realized	he noticed; the man noticed	----
that his right	"right" is required	----	his left; one
knee	"knee" is required	----	leg; arm; foot
was bleeding	"bleeding" or variant	bled; blood	----
and began to flee	"flee" or variant	ran; ran away	----
The dog chased the man	indication that the dog chased the man	ran after the man	----
into the thorny	"thorny" or variant	thorn; prickly; sharp	----
bushes	"bushes" or variant	bush; hedges; shrubs	trees
Suddenly, a police officer arrived	indication that police arrived	cops came; policeman showed up	----

He said to the woman	indication that the police officer spoke	he said	she said
you should thank Shaggy	"Shaggy" in any context	----	Shags; Fluffy

Lists developed for word list recall

List A		List B		List C		List D	
football	38[1]	witness	40	passion	40	merchant	40
muscle	73	platform	77	chairman	77	flower	78
welcome	13	cluster	13	nephew	14	echo	15
mountain	98	duty	95	kitchen	95	title	94
basket	19	button	20	bishop	20	banker	20
gesture	38	debate	36	cotton	36	reply	35
mercy	20	fever	19	assault	18	closet	18
contract	73	appeal	72	disease	72	owner	72
kingdom	27	empire	26	leather	26	lion	26
acre	54	request	54	highway	56	notion	57
rider	28	custom	28	costume	28	current	29
copy	54	heaven	53	purchase	50	victim	50
Average	**44.6**		**44.4**		**44.3**		**44.5**

List E		List F		List G		List H	
temple	41	servant	41	scholar	42	transfer	43
valley	79	message	80	resource	81	winter	82
orange	15	diamond	15	delay	15	consent	15
bottom	93	battle	91	garden	91	daughter	91
needle	21	layer	22	laughter	22	handle	22
liquid	34	wedding	34	carbon	32	barrel	32
doorway	18	flavour	18	instinct	18	carpet	17
motion	72	culture	71	wagon	72	weather	70
organ	26	bubble	25	cottage	25	tribute	25
content	57	conflict	58	pocket	59	commerce	58
ticket	30	rebel	31	cabin	30	pistol	31
drama	49	million	49	prospect	49	surprise	49
Average	**44.6**		**44.6**		**44.7**		**44.6**

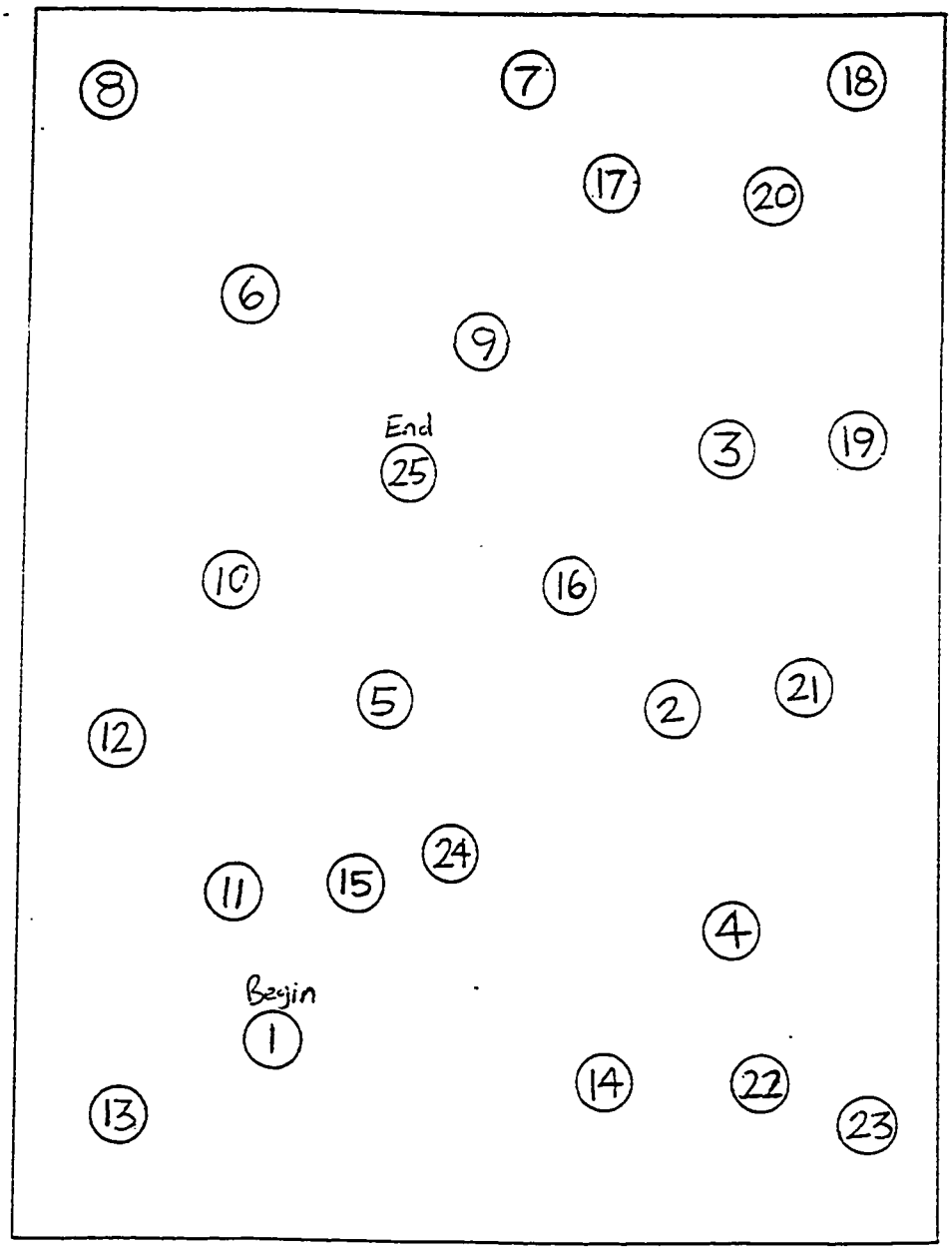
List I		List J		List K		List L	
darkness	43	wisdom	44	habit	44	fabric	44
career	82	murder	83	village	84	agent	84
ankle	15	rabbit	16	oyster	16	distress	16
device	92	desire	88	rifle	87	signal	87
fountain	22	abuse	22	candle	23	marble	23
contest	32	courage	32	devil	32	crystal	31
elbow	17	hunter	17	quarrel	17	stable	17
limit	68	pleasure	67	speaker	67	cousin	65
escape	24	timber	24	treaty	24	triumph	24
neighbour	59	movie	60	dealer	60	concert	64
maker	31	journey	31	lover	31	ideal	31
navy	48	sentence	47	pupil	45	survey	45
Average	**44.4**		**44.3**		**44.2**		**44.3**

[1] Numbers represent word frequencies (Francis & Kucera, 1982).

Versions of the Trail Making Test Parts A and B

The 8 versions of Part A (numbers only) and 11 versions of Part B (numbers and letters) that were developed are shown below.

A



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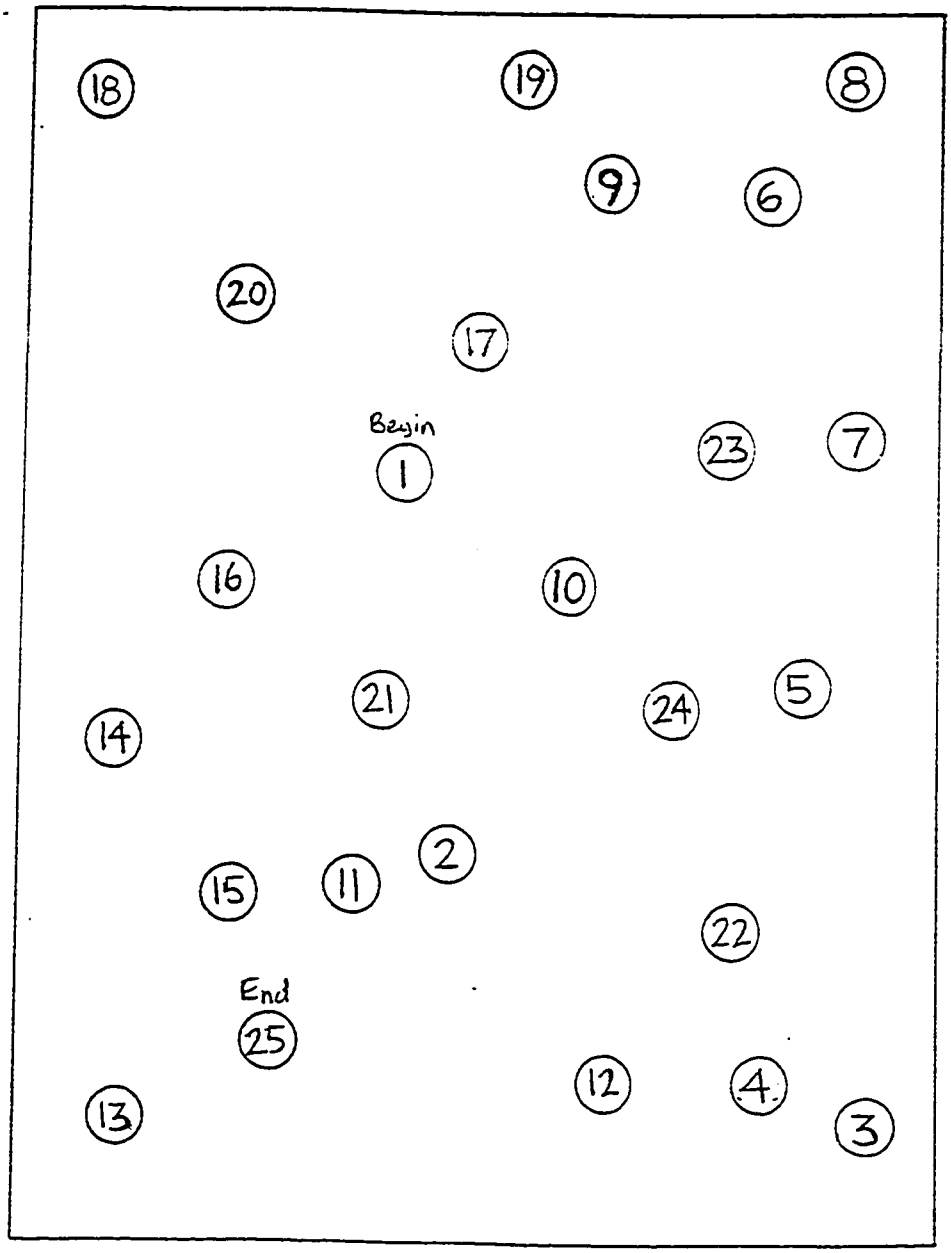

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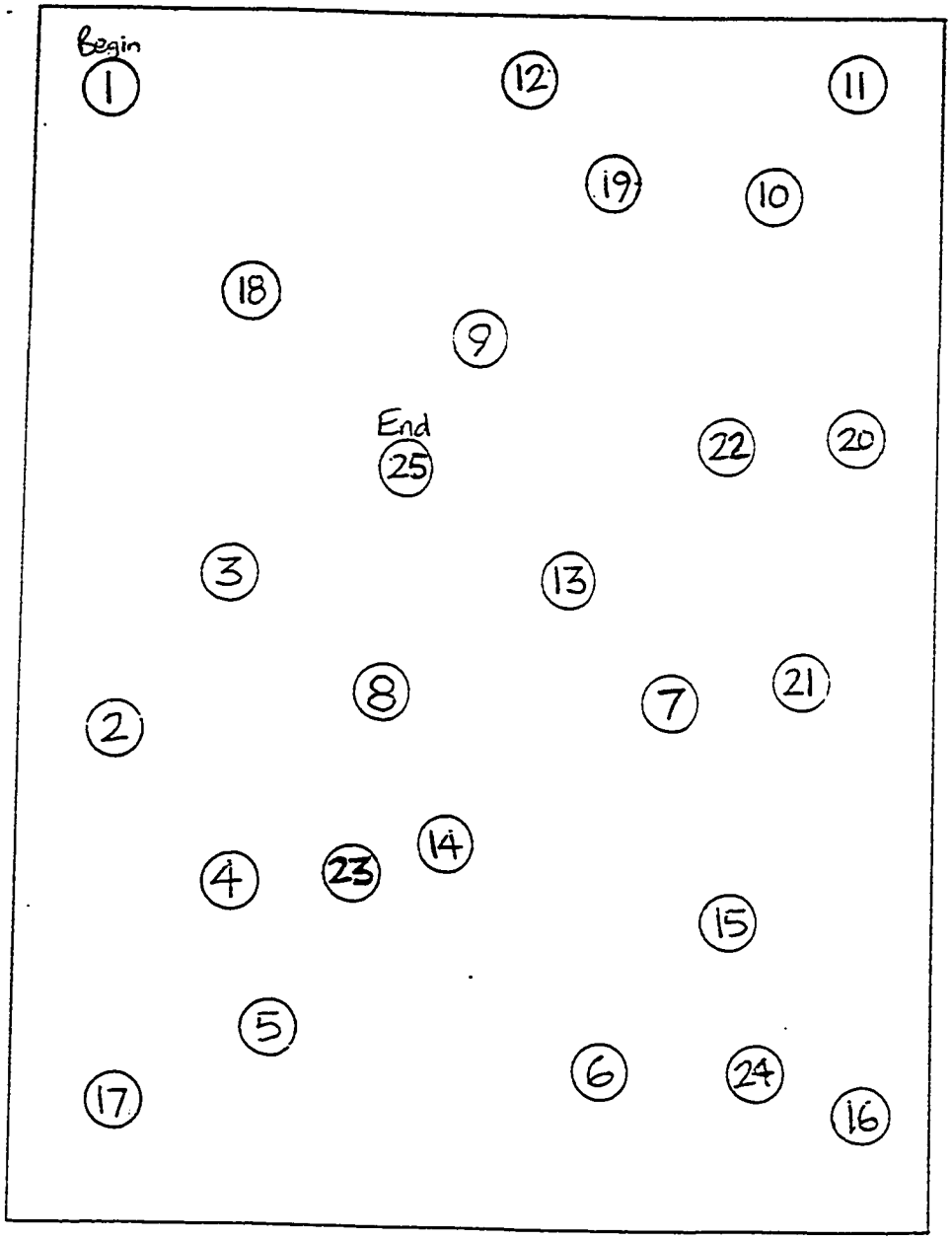

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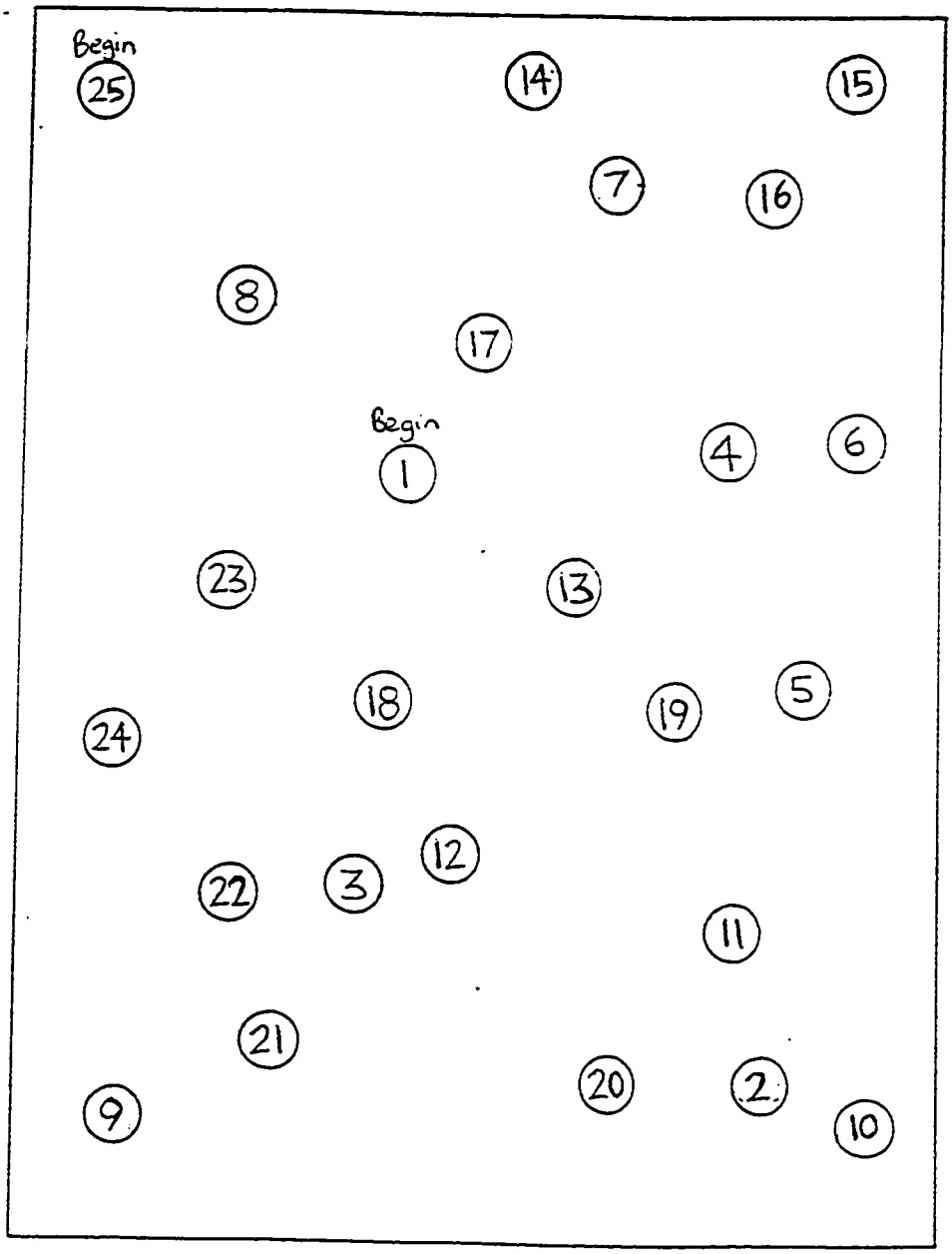
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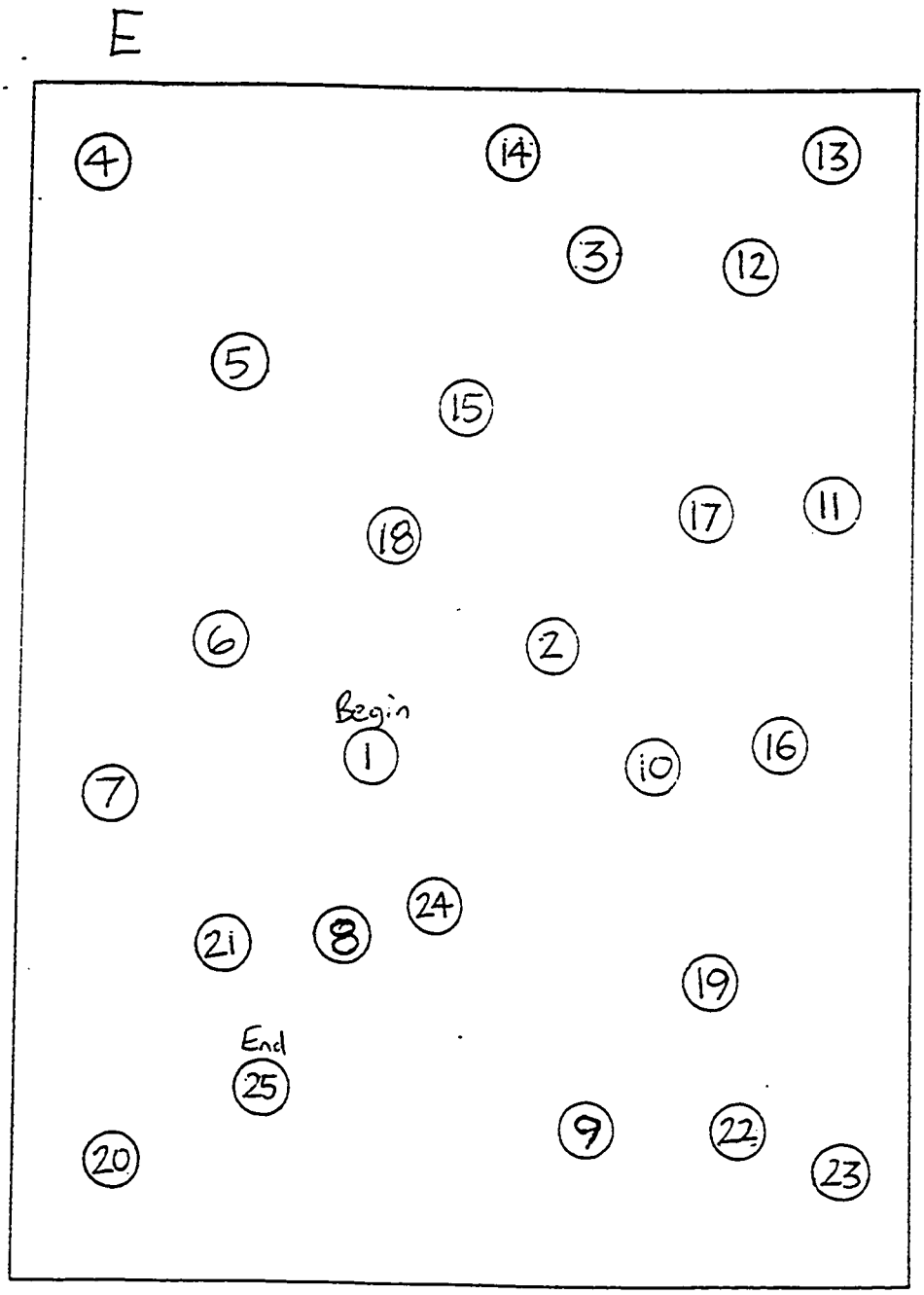
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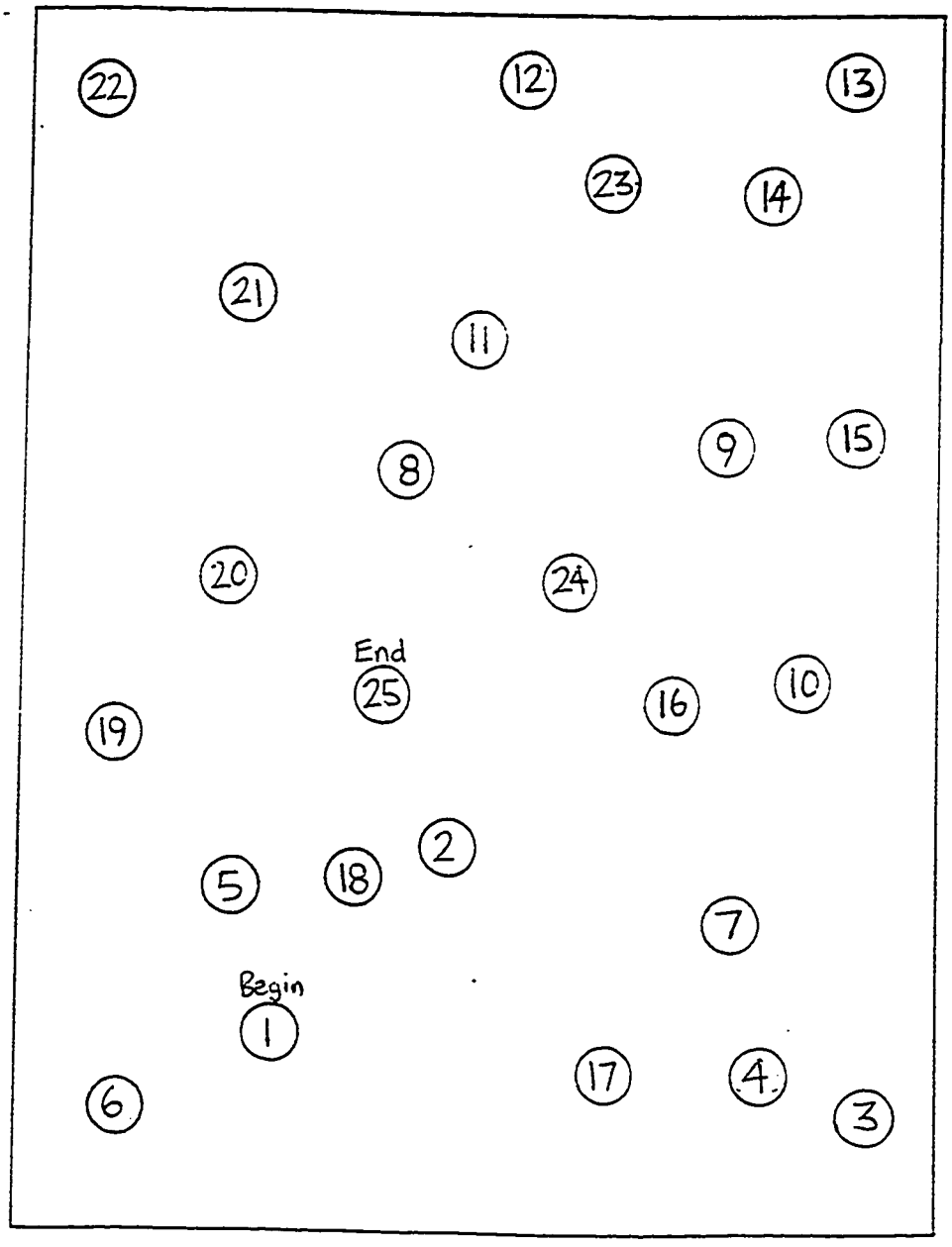

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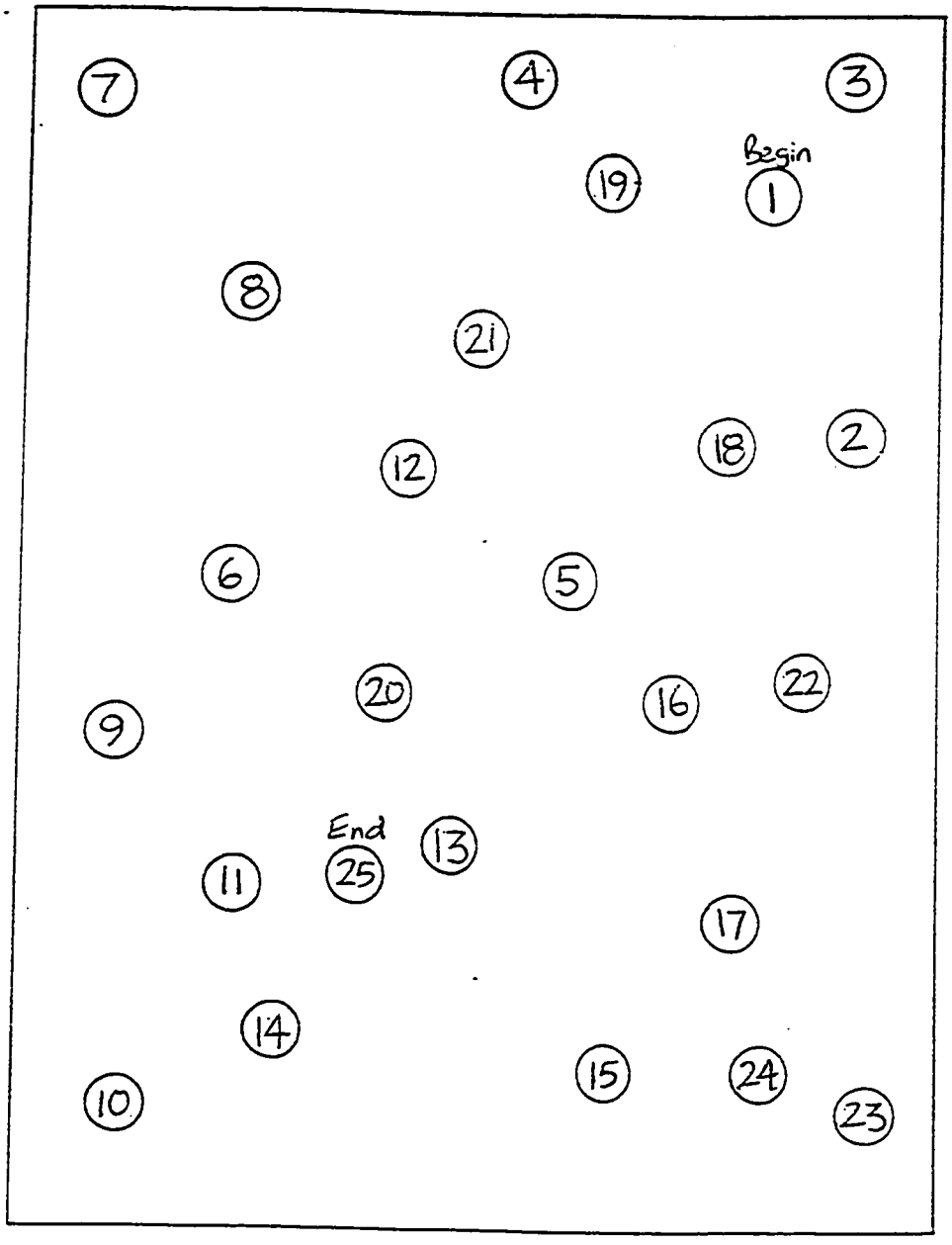

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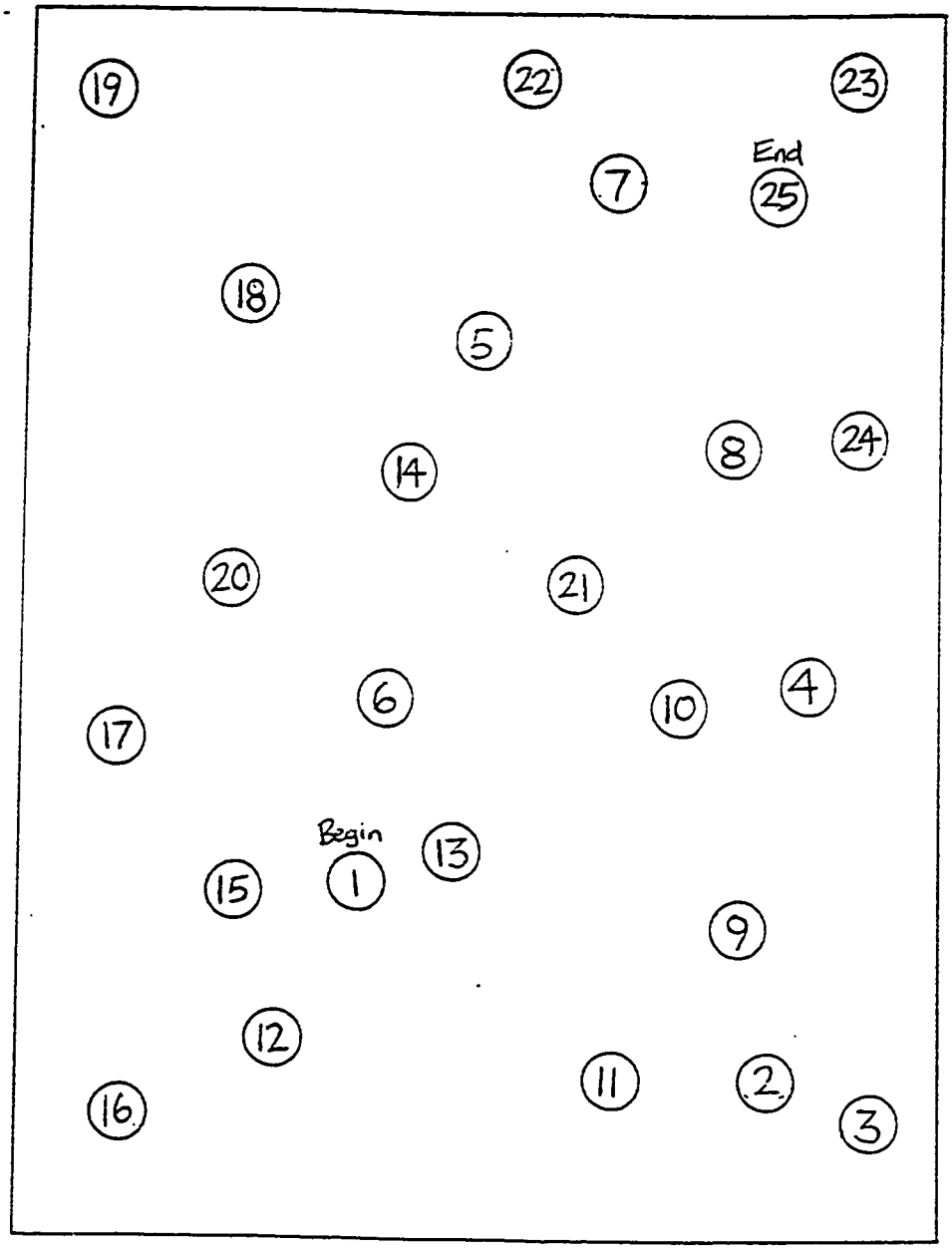

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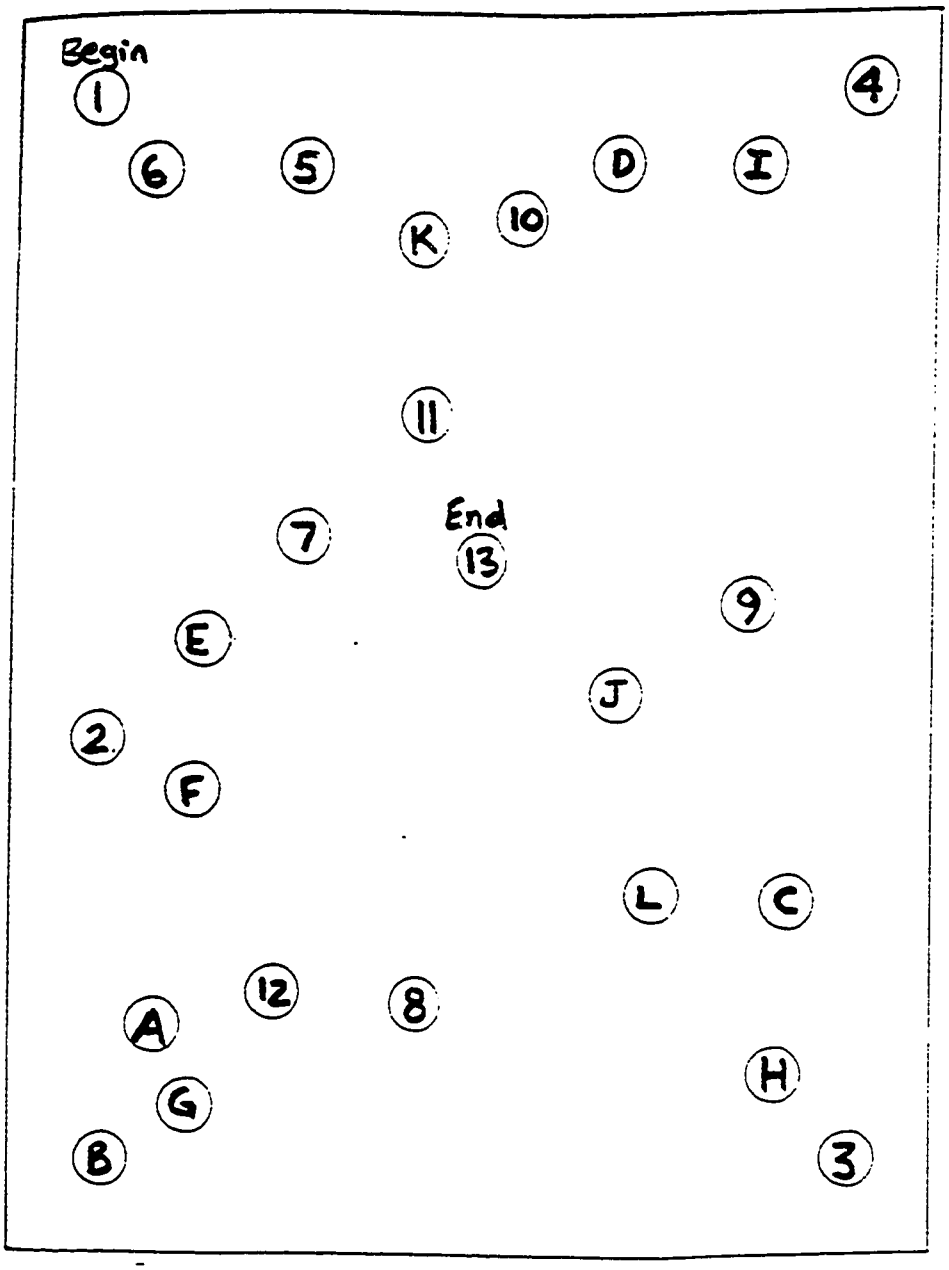

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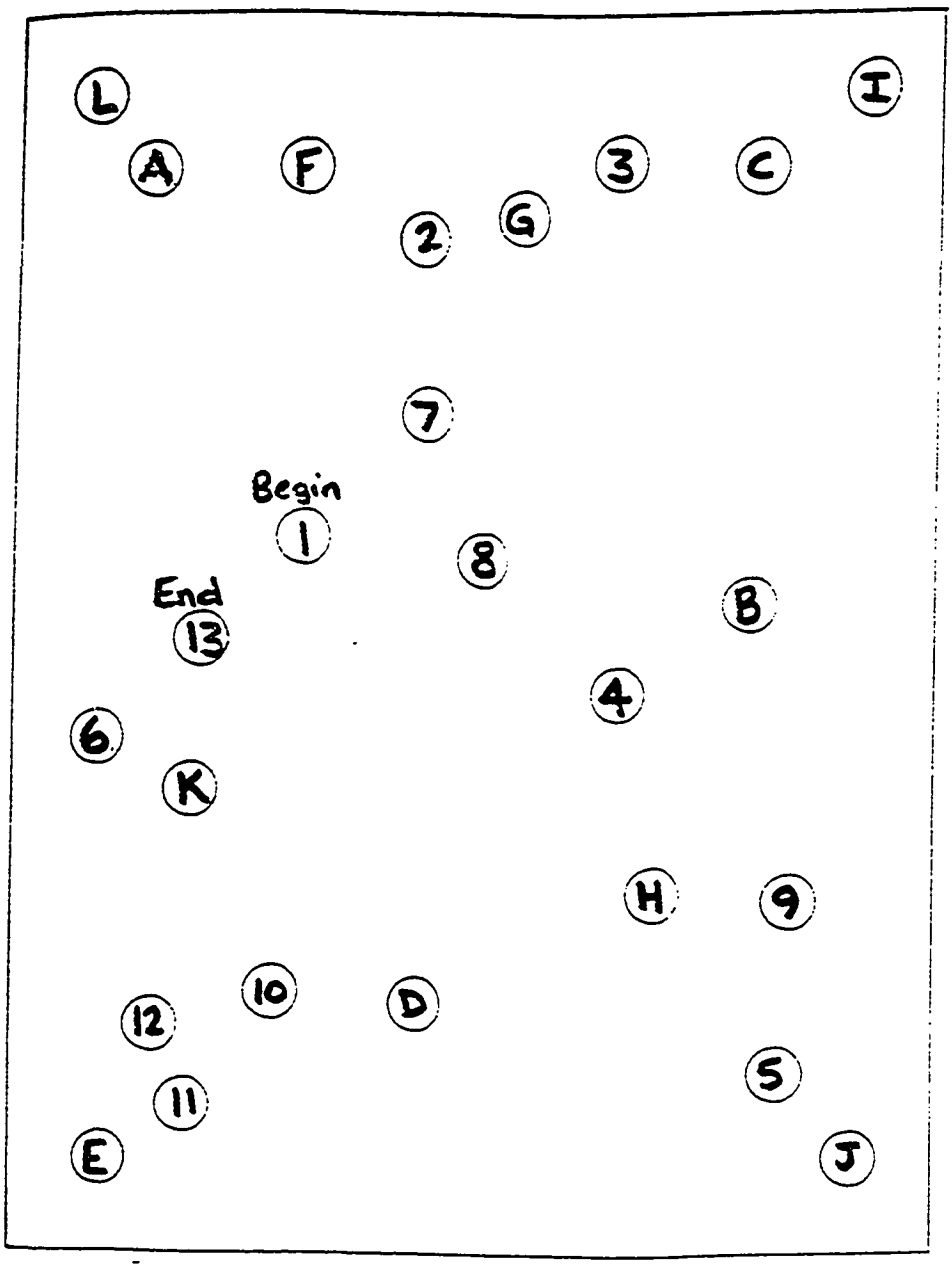

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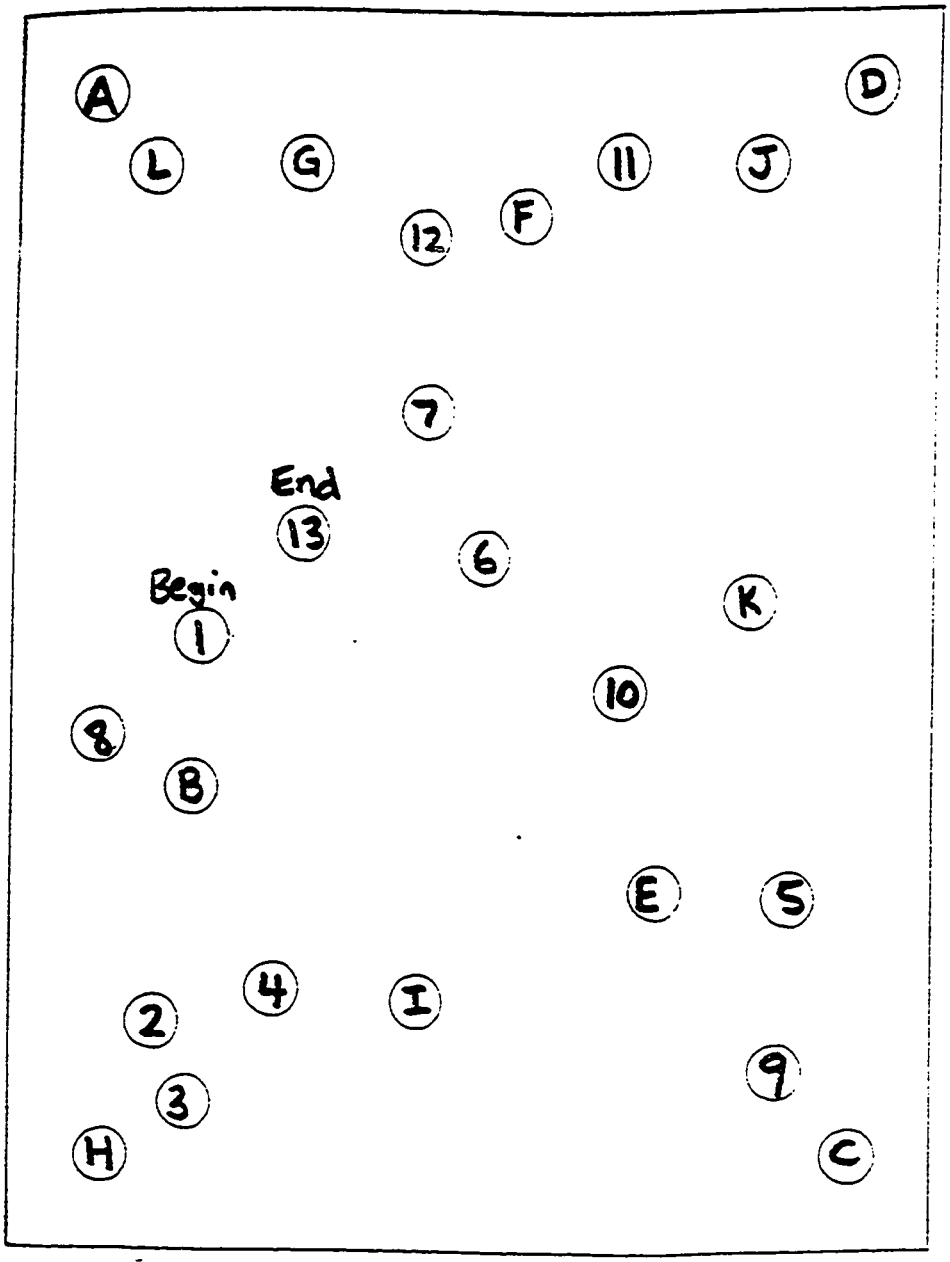

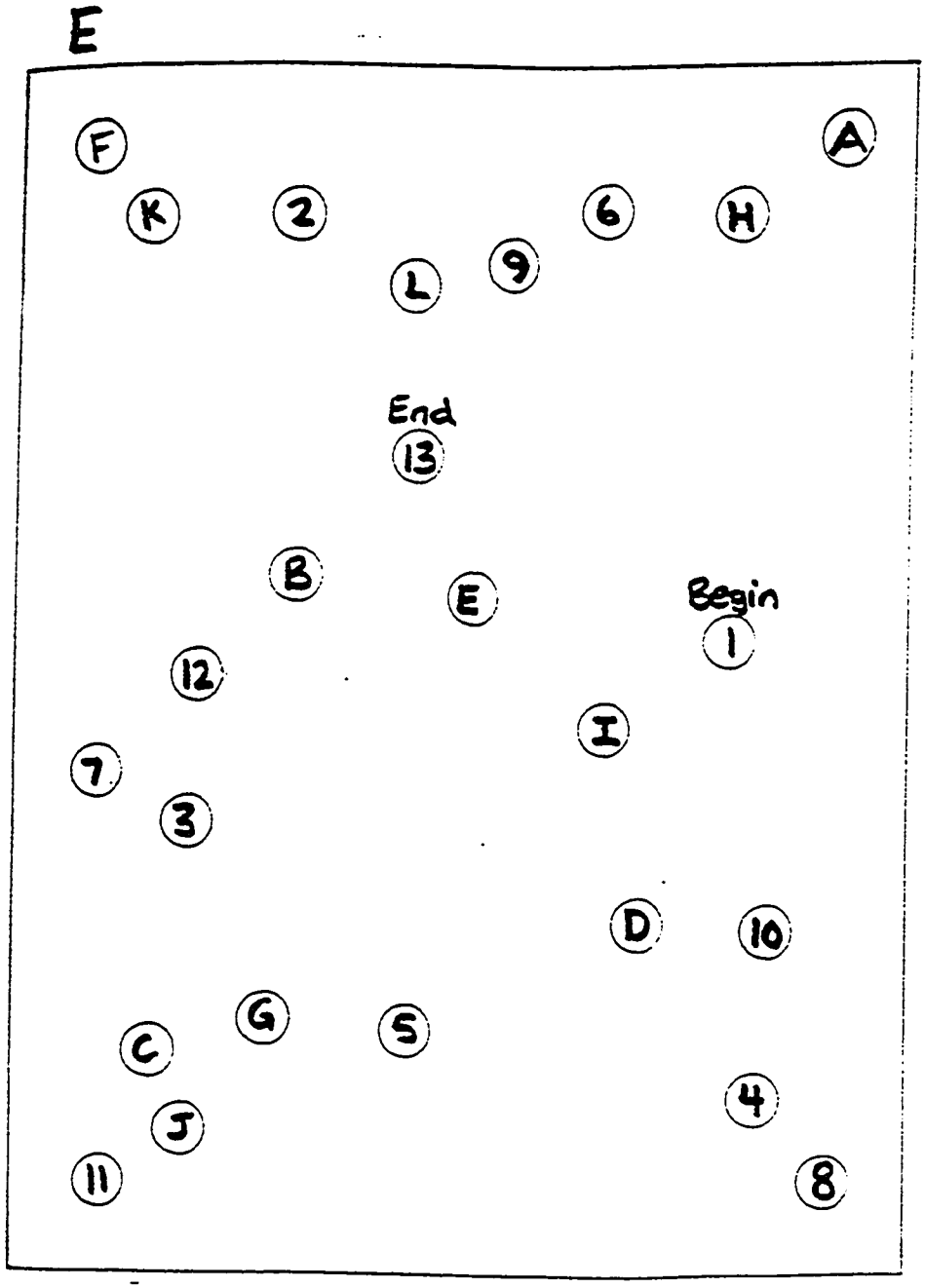
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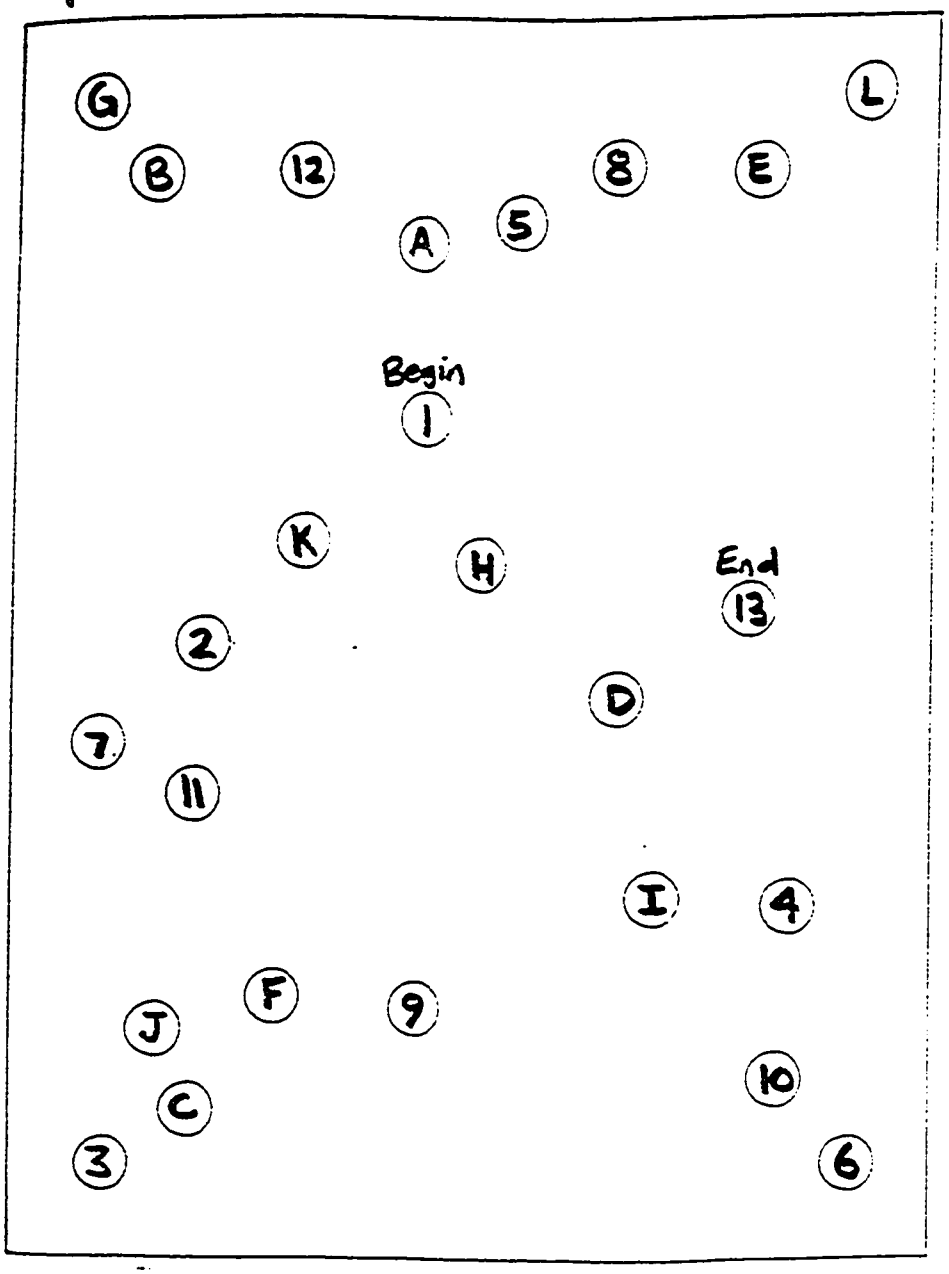

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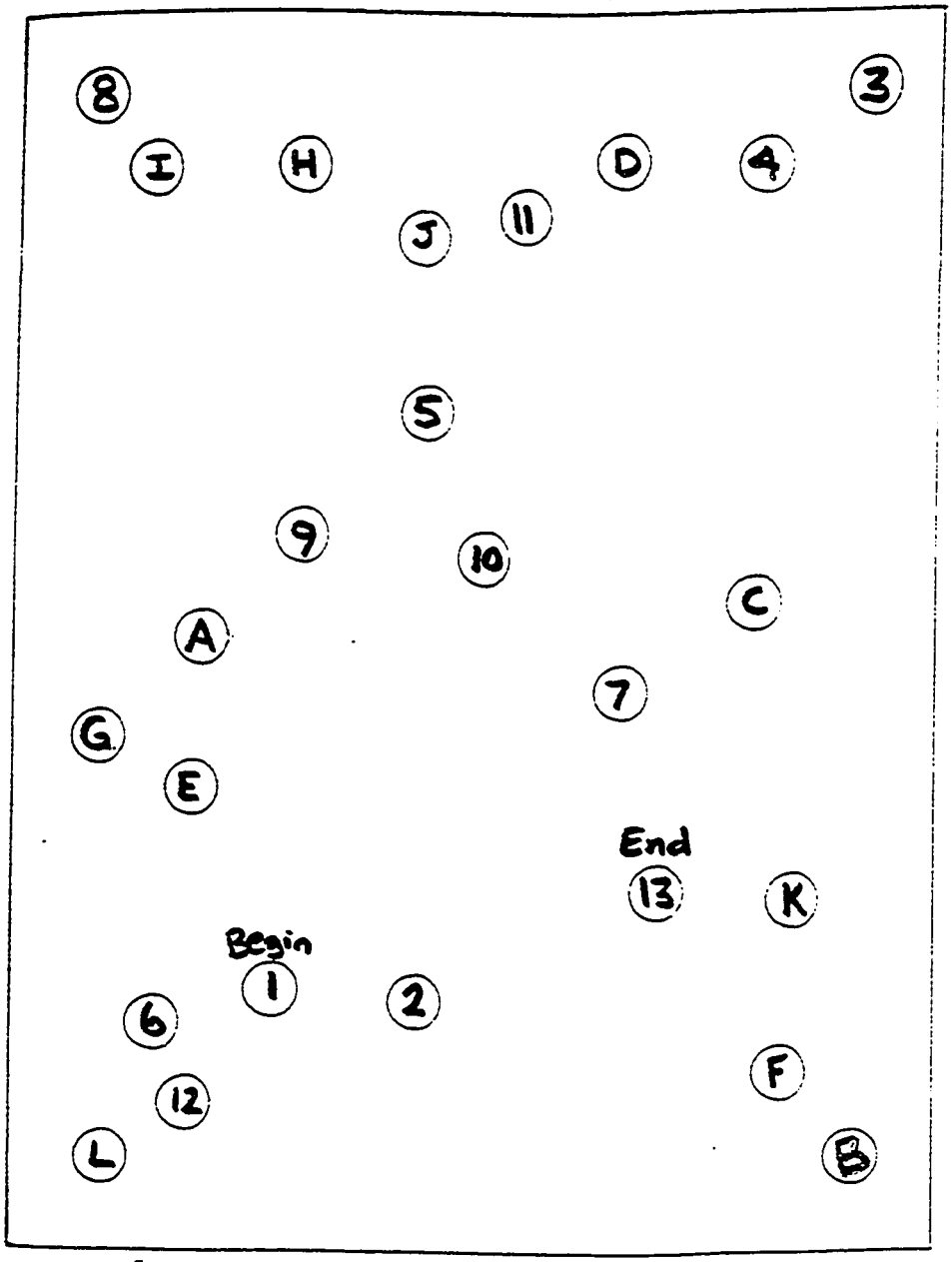

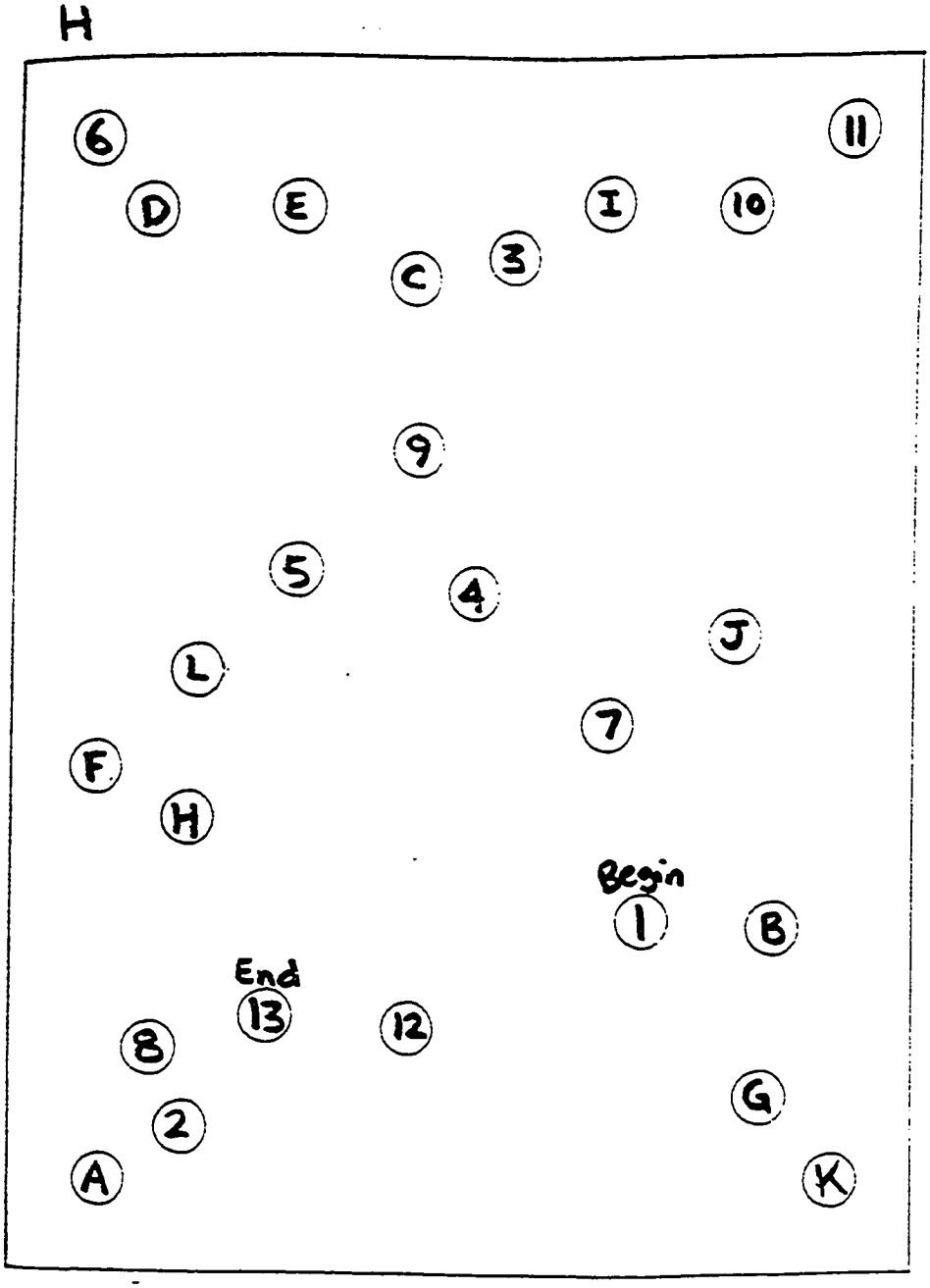
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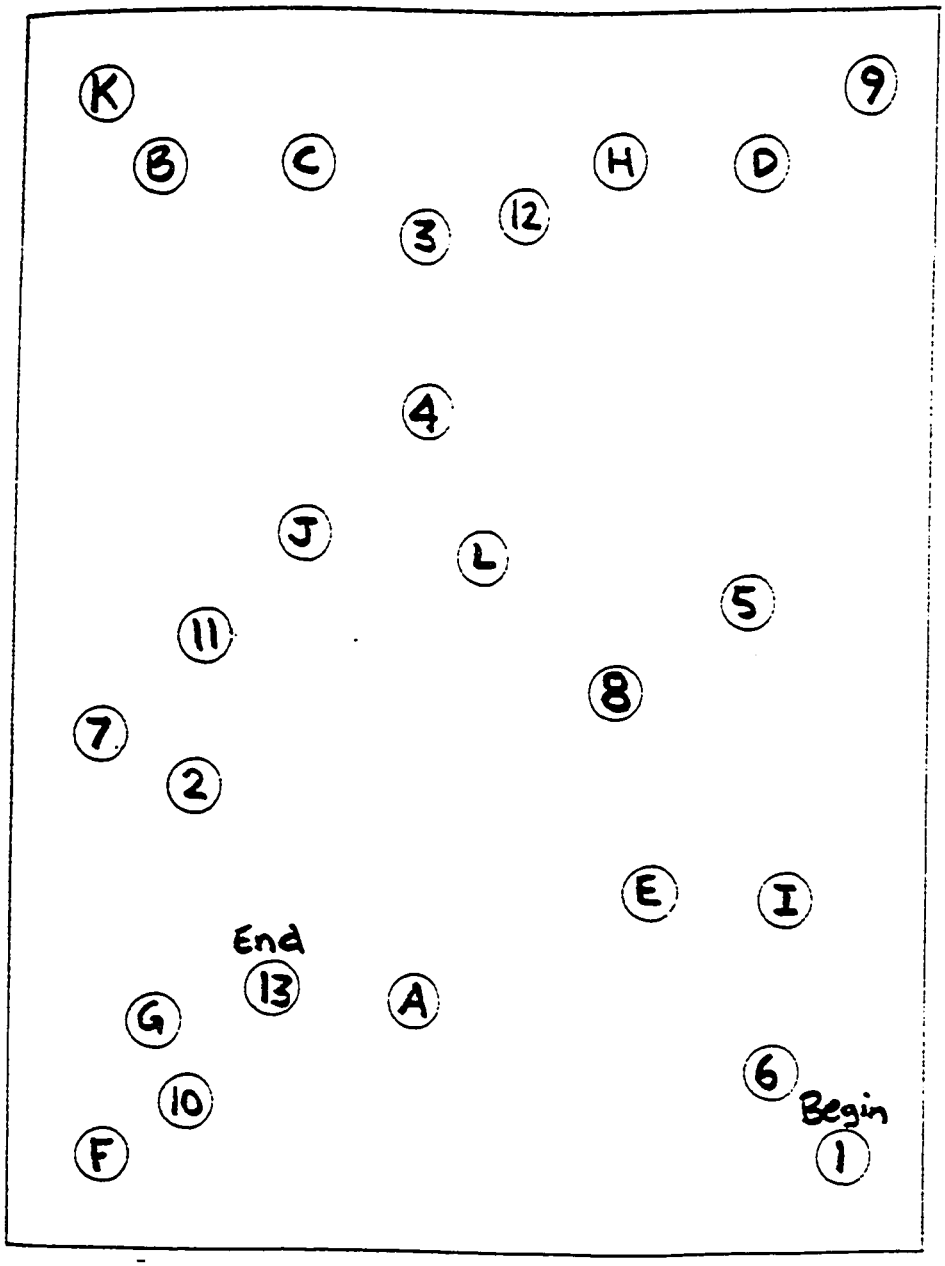

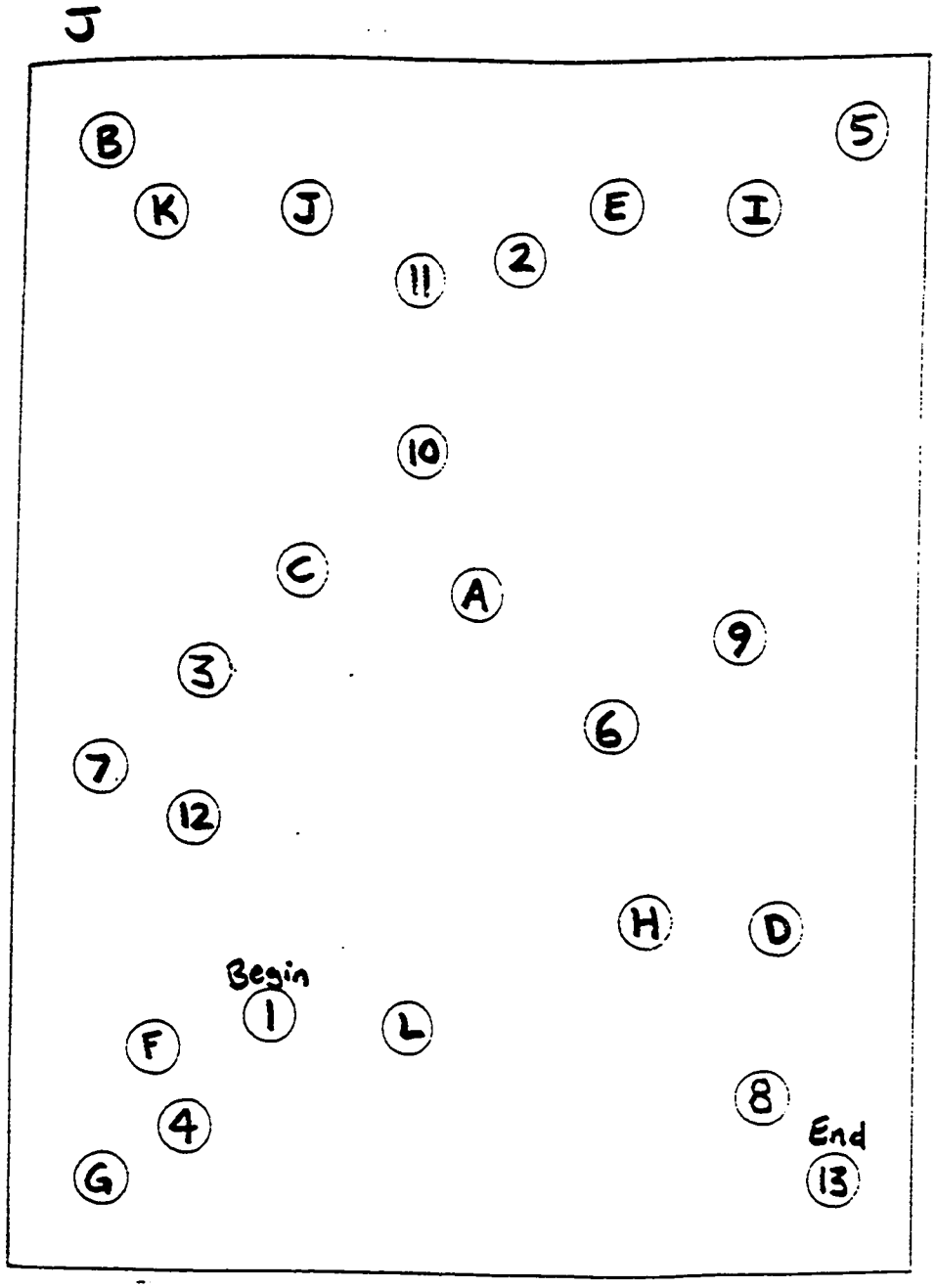
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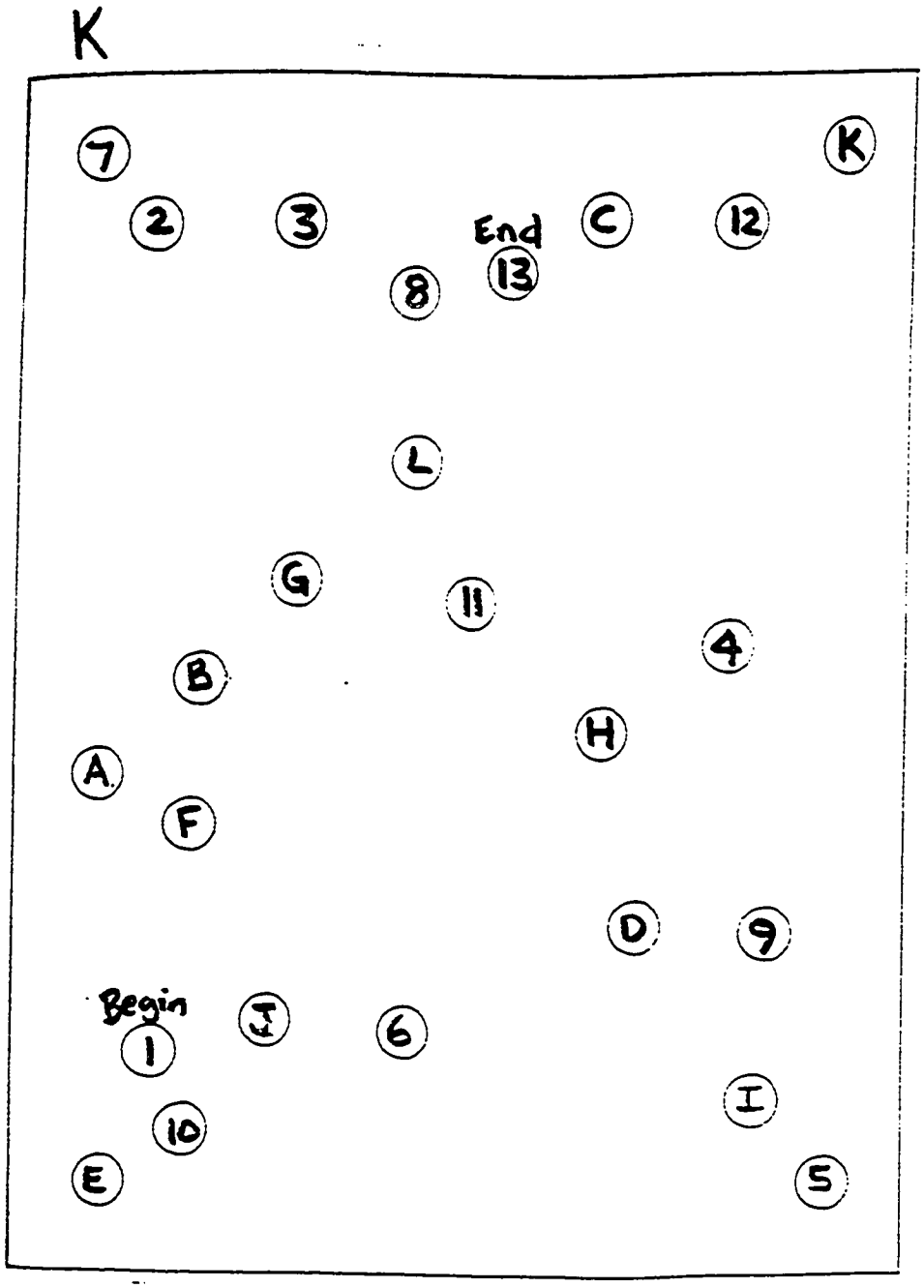
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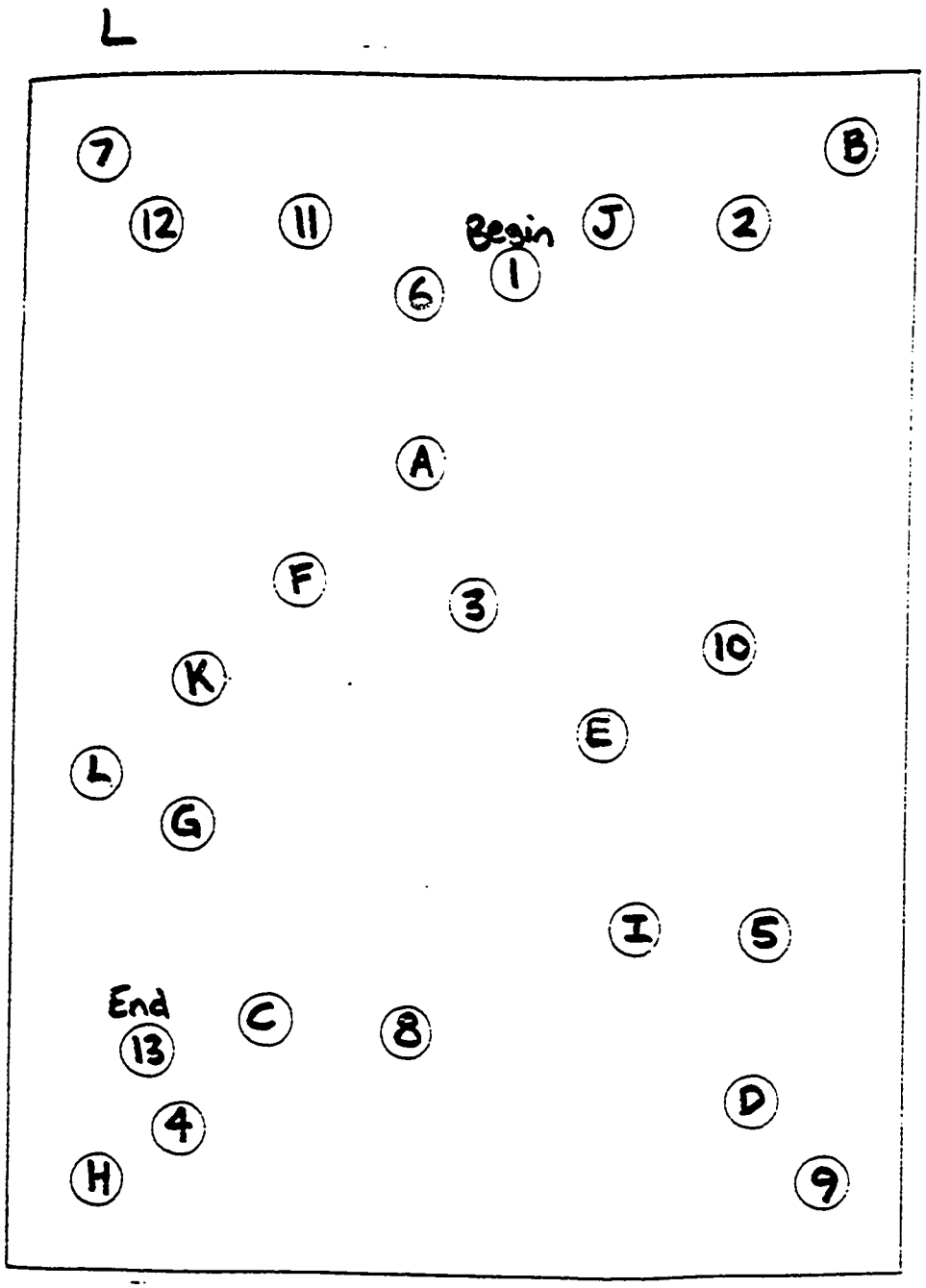
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