NOTE TO USERS

Université de Sherbrooke

The oxidation of energy substrates during healthy aging.

By

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Department of Physiology and Biophysics

Master's thesis presented to the Faculty of Medicine and Health Sciences

In order to obtain a Master of Science degree (M.Sc.)
In Physiology and Biophysics

December 19, 2007

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List of abbreviations used

^{13}C-β-OHB	carbon-13-labeled beta-hydroxybutyrate
^{13}C-Glu	carbon-13-labeled glucose
AcAc	acetoacetate
ATP	adenosine triphosphate
β-OHB	beta-hydroxybutyrate
CHL	cholesterol
DHA	docosahexanoic acid
ELISA	enzyme-linked immunosorbent assay
EPA	eicosapentanoic acid
GC	gas chromatography
GLUT	glucose transporter
IRMS	isotope ratio mass spectrometry
Kg	kilograms
NEFA	non-esterified fatty acids
MCT	monocarboxylate transporter
PDR	percent dose recovered
TCA cycle	tricarboxylic acid cycle
TG	triacylglycerol

Definitions of selected terms commonly used

Acetyl Coenzyme A (Acetyl CoA) metabolic intermediate that transfers acetyl groups to the Krebs' cycle and other pathways.

Adenosine triphosphate (ATP) the major molecule that transfers energy from metabolism to cell function during its breakdown to ADP.

Energy Substrates molecules used to convert ADP into ATP.

β-oxidation a series of reactions that result in the breakdown of fatty acids into acetyl CoA units for oxidative phosphorylation.

Ketone (ketone body) product of fatty acid metabolism that accumulates in blood during fasting, starvation or severe untreated diabetes mellitus; collectively refers to three molecules acetone, acetoacetate, and β-hydroxybutyrate.

Metabolism a highly regulated system of energy producing and energy utilizing chemical reactions.

Tricarboxylic acid cycle mitochondrial metabolic pathway that utilizes fragments derived from carbohydrate, fat, and protein breakdown, and

produces carbon dioxide, hydrogen (for oxidative phosphorylation) and small amounts of ATP. Also called the citric acid cycle or the Krebs' cycle.

Adapted from Human Physiology: Mechanisms of body function Vander AJ et al 8th ed. McGraw-Hill 2001

Abstract

The oxidation of energy substrates during healthy aging.

Erika Brita Leah Freemantle, Department of Physiology and Biophysics, Université de Sherbrooke.

Introduction: Glucose and ketones are important energy substrates in the human body and brain. Their use is highly regulated depending on energy status which can vary according to multiple factors such as type of cell, fed or fasted state, type of diet, or health state. Use of either substrate is also subject to multiple homeostatic feedback loops. Energy substrate availability has implications in several disorders including declining cognitive function in the elderly. While glucose availability is known to decrease in elderly with cognitive deficits, it is unclear whether this also occurs in healthy elderly, either in the body or brain. Also unknown is whether, in healthy elderly, the use of ketones as energy substrates is affected, and whether ketones could be used as an alternative energy substrate in situations of a decline in glucose availability. A clearer understanding of the use of glucose and ketones in aging is necessary to determine whether declining energy substrate availability that may occur in the elderly is a contributing factor to cognitive deficits, a result of cognitive pathology, or simply a feature of the physiological aging process.

Objective The overall goal of the laboratory where this research was carried out is to ascertain whether alternate energy sources to glucose, i.e. ketones, may help alleviate the risk of declining cognitive function during aging. The specific objective of the research project presented in this thesis was to evaluate the metabolism of glucose and ketones in the healthy elderly compared to young or middle age subjects during mild, short-term ketosis induced by a ketogenic breakfast.

Results Elderly people in relatively good health have a similar capacity to produce ketones and to oxidize ^{13}C-glucose and ^{13}C-β-hydroxybutyrate as middle-aged or young adults.

Discussion The results of this project encourage further exploration of whether ketones could be used as and alternative energy substrate to glucose as, at least in healthy elderly, there is no impedance of raising plasma ketones in response to a ketogenic intervention.

Keywords Energy substrates, glucose, ketones, healthy elderly, carbon-13 stable isotope tracers

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Résumé

L'oxydation des substrats énergétiques au cours du vieillissement sain.

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Introduction Le glucose et les cétones sont des substrats énergétiques importants pour le corps et le cerveau humain. Leur utilisation est spécifiquement régulée selon l'état énergétique qui varie en fonction du type de cellule, de l'état nourrie ou à jeun, du type de diète, de l'état de la santé. L'utilisation est également régulée par des voies de rétrocontrôle homéostatique. La disponibilité des substrats énergétiques est impliquée dans plusieurs désordres dont le déclin des fonctions cognitives chez les personnes âgées, ou une diminution de la disponibilité du glucose est démontrée. Cependant, il n'est pas encore connu si cette diminution est présente chez les personnes âgées en bonne santé; soit dans le corps ou le cerveau. La capacité d'utiliser les cétones comme substrats énergétiques chez les personnes âgées saines et la possibilité d'utiliser les cétones comme substrat énergétique alternatif dans le cas d'un déclin de la disponibilité de glucose sont inconnu. Une meilleure compréhension de l'utilisation du glucose et des cétones sera nécessaire pour clarifier si une diminution de la disponibilité des substrats énergétique contribue au déclin cognitif, se manifeste à la suite des pathologies cognitives, ou encore est simplement une caractéristique du processus physiologique du vieillissement.

Objectif L'objectif principal du laboratoire est de déterminer si les sources d'énergies alternatives au glucose, c'est-à-dire les cétones, pourraient ralentir le déclin cognitif

chez les personnes âgées. L'objectif du projet de recherche de ce mémoire était d'évaluer le métabolisme du glucose et des cétones chez les sujets âgés, d'âge moyen, et jeune après la prise d'un déjeuner induisant une faible cétogenèse de courte durée.

Résultats Les personnes âgées en santé ont une capacité similaire aux sujets d'âge moyen et jeunes à produire des cétones et à oxyder le ^{13}C-glucose et le ^{13}C-β-hydroxybutyrate.

Perspectives Les résultats de ce projet incitent à continuer à explorer si les cétones pourraient être utilisées comme substrats énergétiques afin de contourner le problème d'un déclin de l'utilisation du glucose, car il n'y a aucun obstacle dans la production des cétones suite à une intervention cétonique chez des sujets âgées en bonne santé.

Mots clés substrats énergétiques, glucose, cétones, vieillissement sain, traceur d'isotope stable carbone-13.

Mémoire présenté à la Faculté de médecine, en vue de l'obtention du grade de maîtrise sciences (M.Sc.) en Physiologie et Biophysique 19 décembre 2007

Membres du jury de révision

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Introduction

Bioenergetics

Living cells depend on a complex, highly regulated system of energy producing and energy utilizing chemical reactions referred to as metabolism. The functioning of a cell depends on its ability to extract and use the chemical energy in organic molecules (VANDER *et al.,* 2001). This energy is trapped in the bonds of adenosine tri-phosphate (ATP). ATP is generated by the tricarboxylic acid cycle (TCA cycle) which, coupled with the electron transport chain, will ultimately form ATP from acetyl coenzyme A (acetyl CoA). The TCA cycle is the terminal oxidative pathway for most metabolic fuels. Acetyl CoA can be obtained from different substrates (DEVLIN 2006). In humans, glucose is the primary substrate, mainly made available from the breakdown of dietary carbohydrates. In times of glucose shortage, humans can also utilize either proteins or ketones as an energy substrate, available from the breakdown of fats. The supply of these energy substrates is summarized in Figure 1 (MURRAY *et al.,* 2006).

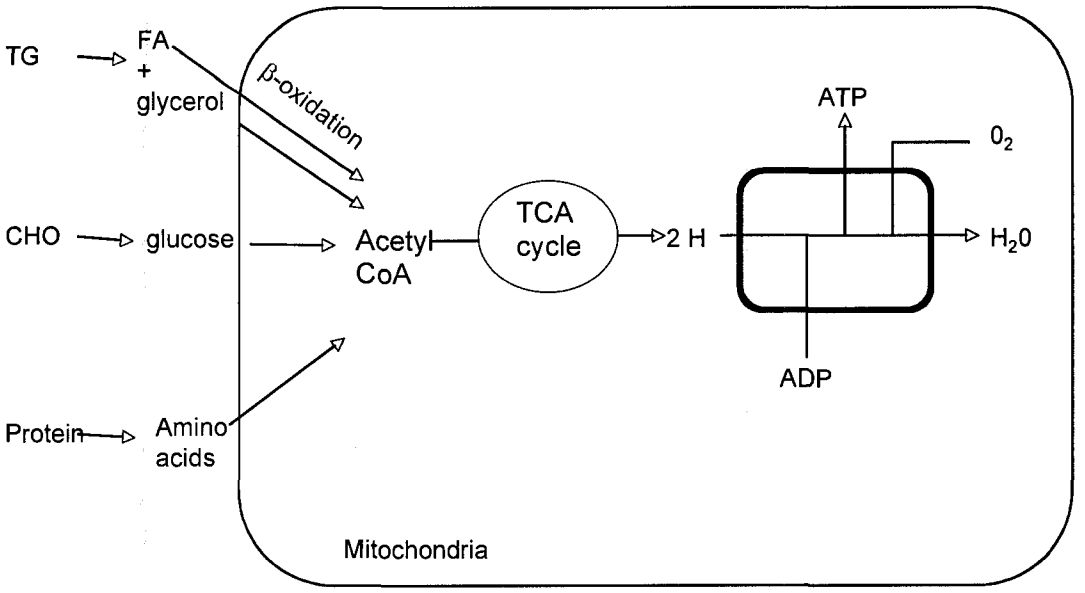


Figure 1 Summary of nutrient supply of energy substrates from fat, proteins, or carbohydrates and subsequent conversion through acetyl CoA, the TCA cycle, and the respiratory chain to ATP.

It should be noted that both glucose and ketones are not only important directly as energy substrates but also as a precursor molecules in glycolysis, glycogenesis and lipogenesis, and that the roles of both depend highly on the energy state of the organism (ie. cell type, fed or fasted, type of diet, healthy or diseased, etc) which is discussed in more detail later. Furthermore these are not the only energy substrates used in humans, as proteins may also be used. However, the focus of this thesis will mainly be the use of glucose and ketones as energy substrates in a non-pathological/physiological situation in liver and brain cells.

Glucose and ketones as energy substrates

Glucose is taken up into the cell by glucose transporters (GLUT). Liver cells use GLUT2, brain cells use GLUT3, and GLUT1 transports glucose across the blood brain barrier. GLUT1 is insulin-dependent while GLUT2 and 3 are insulin-independent. The metabolism of glucose to acetyl CoA upon entry into liver cell is well characterized. It is generally referred to as the glycolytic pathway and begins with the entry of glucose into the cell by GLUT in response to stimulation by insulin. When utilized for energy, glucose is converted first into pyruvate via several steps. Pyruvate is then broken down to acetyl CoA, which enters the TCA cycle to produce ATP (Figure 2) (DEVLIN 2006).

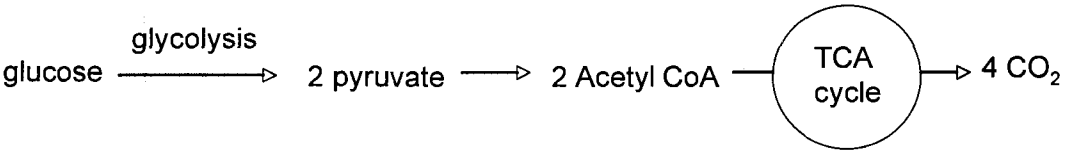


Figure 2 Conversion of glucose to acetyl CoA via the glycolysis pathway during glucose availability.

Ketones are taken up into cells by the monocarboxylate transporter (MCT). The MCT1 isoform is required for ketones to cross the blood-brain barrier (MORRIS 2005). The term -ketone- refers collectively to three molecules: β-hydroxybutyrate (β-OHB), Acetoacetate (AcAc), and Acetone (Ac), shown in Figure 3. Ac is produced mainly from the spontaneous decarboxylation of AcAc, and secondarily from the enzymatic conversion of AcAc by AcAc decarboxylase (KOOREVAAR and VAN STEKELENBURG 1976). Ac is volatile and excreted in the breath. β-OHB is produced from AcAc by the enzyme β-OHB dehydrogenase, and found in measurable levels in the plasma. AcAc is first converted to acetoacetyl CoA and then acetyl CoA, which then enters the TCA cycle (SWINK *et al.,* 1997). AcAc is the only ketone that can be used directly by the TCA cycle.

However, β-OHB can readily be taken up across the blood-brain barrier, subsequently converted into AcAc by β-OHB dehydrogenase, and then used as an energy substrate in brain cells (Figure 4) (MITCHELL *et al.,* 1995).

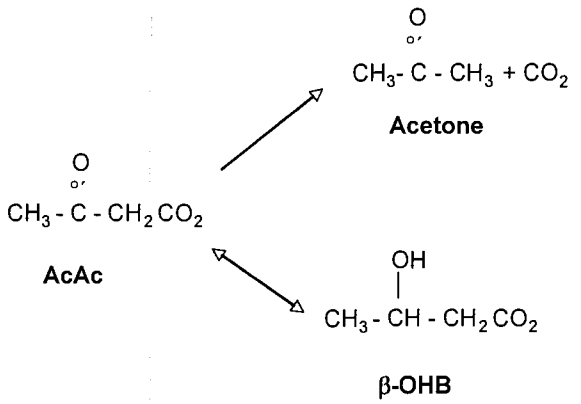


Figure 3 Conversion and interconversion between the three ketones: acetoacetate, acetone and beta- hydroxybutyrate.

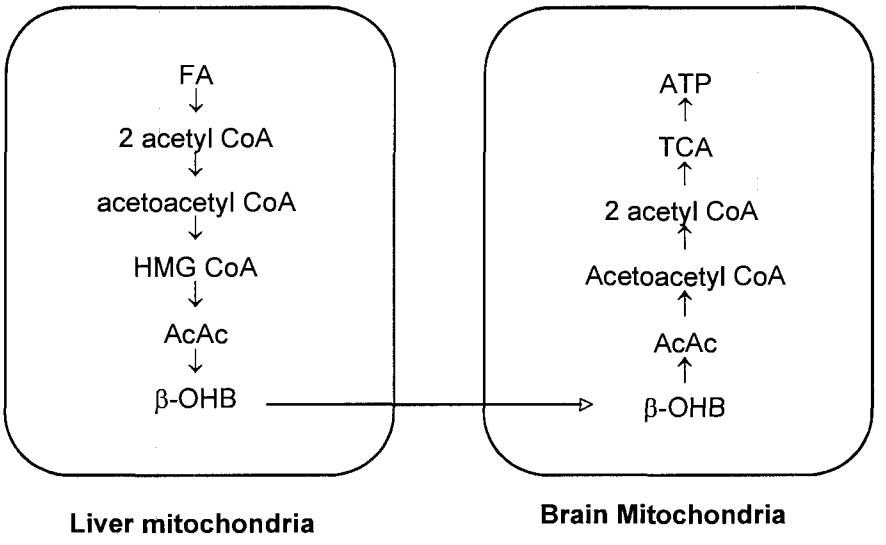
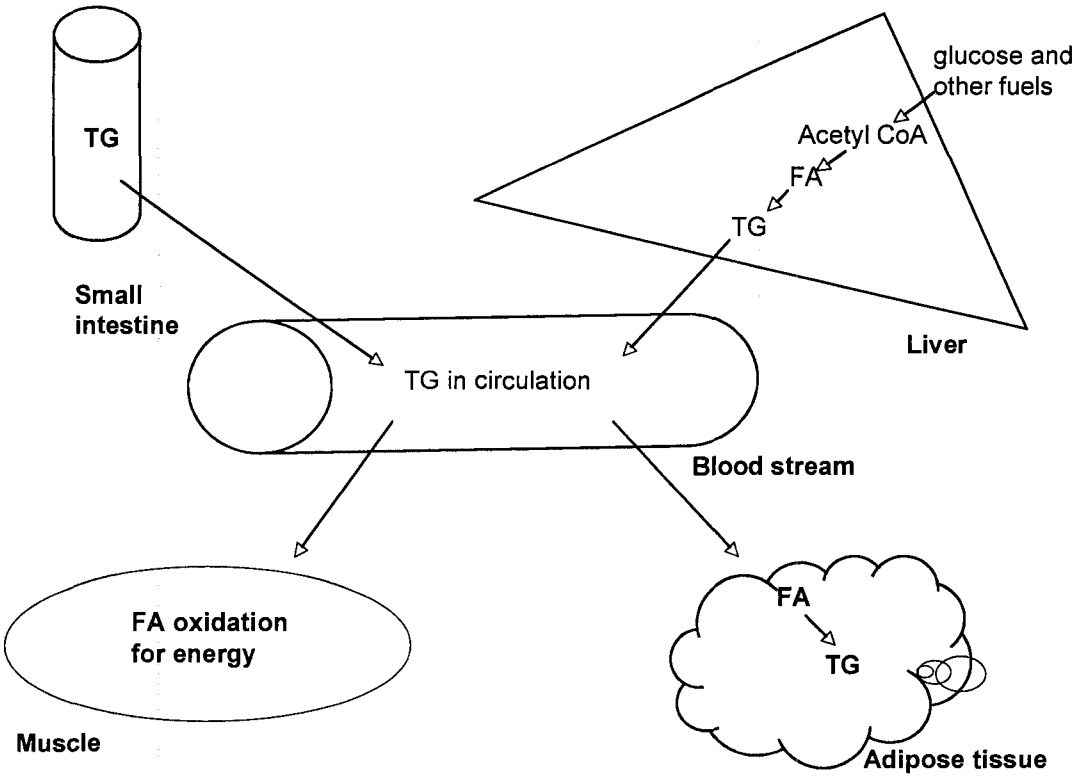


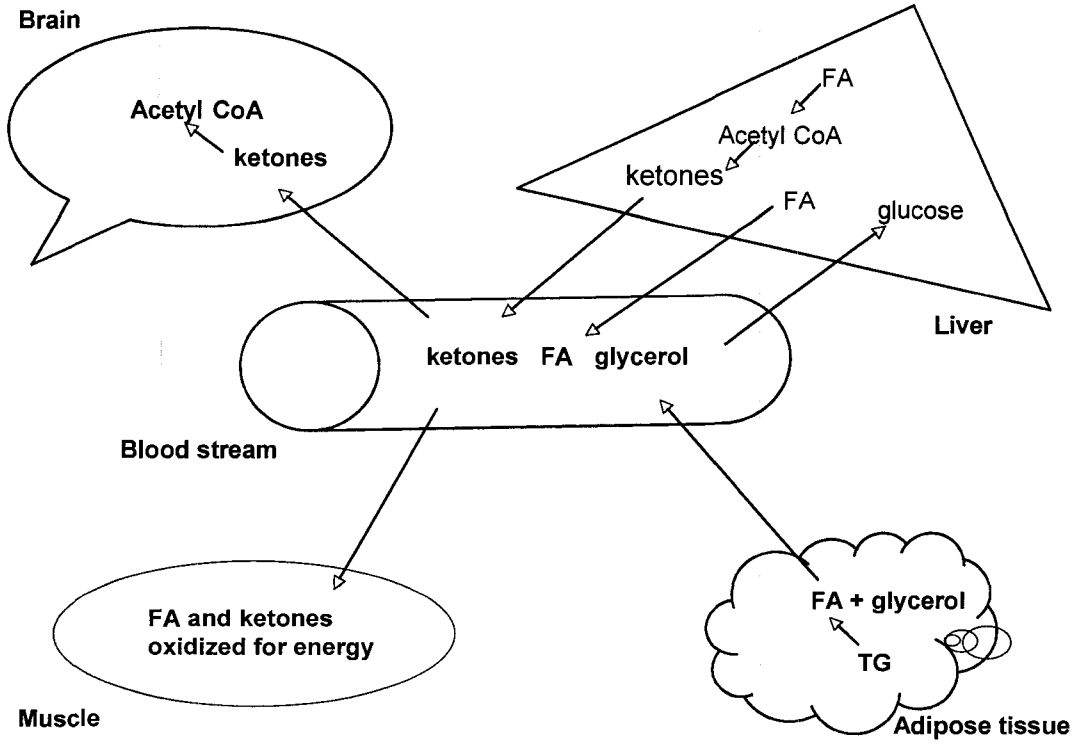
Figure 4 Ketones produced in the liver can be taken up into brain cells and converted to acetyl CoA to be used by the TCA cycle to produce ATP.

Ketones are capable of supplying a large amount of energy. β-OHB oxidation yields a net total of 12.7 kg of ATP per 100 g, while AcAc yield 11.4 kg per 100g, compared to 10.7 kg per 100g of glucose (SWINK *et al.,* 1997). In fuel terms, this energy yield equals 9 kCal per 1 g of fatty acid, though conversion to ketones and via direct oxidation, compared to 4 kCal per 1 g of carbohydrates.

As mentioned, the energetic balance between use, storage or synthesis of glucose and ketones is highly regulated and very complex. It is organ, tissue and cell type dependent, contingent on the presence of necessary enzymes for uptake and utilization of both substrates. It is status-dependent; meaning the use of either substrate can vary with nutritional intake. To further complicate matters, the energy metabolism pathways are subject to regulation by multiple homeostatic feedback loops, shown in Figure 5 (DEVLIN 2006).


TG
Small intestine
glucose and other fuels
Acetyl CoA
FA
TG
Liver
TG in circulation
Blood stream
FA oxidation for energy
Muscle
FA
TG
Adipose tissue

5a


Brain
Acetyl CoA
ketones
FA
Acetyl CoA
ketones
FA
glucose
Liver
ketones FA glycerol
Blood stream
FA and ketones oxidized for energy
Muscle
FA + glycerol
TG
Adipose tissue

5b

Figure 5 a and b During glucose availability (5a) glucose is converted to triacylglycerol circulates to tissues requiring energy. In times of glucose shortage (5b), reserves of fatty acids can be converted to ketones in the liver. Ketones produced in the liver can be taken up into brain cells and converted to acetyl CoA to be used by the TCA cycle to produce ATP.

Implications in aging

The body and brain's capacity to metabolize energy substrates has considerable physiological and pathological ramifications. Glucose availability and use has been implicated in various aging-related diseases. It was first shown several decades ago that there is a decrease in glucose uptake in the brains of Alzheimer's patients compared to controls measured by positron emission tomography (PET) using ^{18}fluorodeoxyglucose, a radioactive molecule to study glucose uptake in tissue (FOSTER *et al.,* 1984). This decrease in glucose uptake in Alzheimer's patients has been replicated in several studies (BOOKHEIMER *et al.,* 2000; DAMASIO *et al.,* 1983; HOYER *et al.,* 1988; KALARIA and HARIK 1989). Furthermore, the decrease in brain glucose uptake occurs decades before the onset of cognitive decline, as shown in subjects genetically at risk for developing Alzheimer's pathology indicated by the presence of the Apolipoprotein E ε4 allele (REIMAN *et al.,* 2004). Poirier et al. (1996) found that as much as 80% of Alzheimer's disease patients are Apolipoprotein E ε4 carriers. The decrease in brain glucose uptake in those at risk for developing Alzheimer's suggests that glucose decline may be a causative factor in development of the pathology (REIMAN *et al.,* 2004).

While glucose is the brain's main energy source, ketones compliment or supplement glucose, by as much as 30% under conditions of fasting (OWEN *et al.,* 1967; SOKOLOFF 1991). In theory, ketones would be a rational alternative to potentially alleviate the pathology that may stem from a decline in the brain's ability to use glucose. An intriguing field of research is emerging to determine if, in fact,

ketones as energy substrates alternative to glucose may be beneficial in helping to alleviate declining cognitive function. There are some indications to encourage this avenue of research. First, it has been shown in humans that raising the ketone concentration in the peripheral circulation by infusion of β-OHB, correspondingly increases the ketone concentration in the brain (HASSELBALCH *et al.,* 1995; HASSELBALCH *et al.,* 1996; PAN *et al.,* 2001). Another study, though in rats, showed that administration of a ketogenic diet for 6 weeks resulted in up-regulation not just of MCT1, the brain ketone transporter, but also in GLUT1 glucose transporters in the brain (LEINO *et al.,* 2001). Pertaining to cognitive function in humans, one study of 20 patients with cognitive impairments, determined by the Mini-Mental State Examination (MMSE) were given in two separate visits, either an emulsion of medium chain triglycerides, which induces ketosis, or long chain triglycerides, as a control. The patients then underwent several cognitive tests including the Alzheimer's disease Assessment scale-Cognitive subscale (ADAS-cog), the MMSE, and the Stroop Color Word Interference, tasks designed to test selective attention and paragraph recall. Results showed that an increase in plasma β-OHB concentrations was related to significantly improved scores on cognitive test parameters, specifically on ADAS-cog scores and paragraph recall (REGER *et al.,* 2004).

Thesis objectives

The first article in this thesis, a review article, provides an introduction to the theory of declining energy substrate use and availability in aging and how this relates to brain function. It describes the role of glucose in brain function and of ketones as an alternative energy substrate. It illustrates the approaches of inducing ketosis safely and effectively in humans, the difficulties associated with it, and the techniques for studying it in terms of brain function. The article provides a summary of the association between cognitive decline, in particular in Alzheimer's disease, and intake of omega-3 fatty acids, and finally the hypothetical link between cognitive function, glucose use, ketones, and omega-3 fatty acids.

Before investigating in depth in clinical research trials whether alternate energy sources may alleviate some of the consequences in the brain of reduced glucose use, there are still many unanswered questions: Is this decline in energy function a feature of a disorder or a part of the physiological process of aging, and does this decrease in energy use extend to other energy substrates such as ketones? It remains quite unclear what occurs to energy metabolism during healthy aging. Thus in order to study age-related disorders, it is first necessary to better understand energy substrate metabolism during healthy aging. This is the purpose of the second article included in this thesis.

The second article describes a clinical research project carried out at the Research Center on Aging at the Université de Sherbrooke, which was the subject of this master's thesis. Overall, the purpose of the study was to better understand the systemic response in healthy elderly, middle-aged, and young adults to a nutritional intervention designed to induce mild, acute ketosis. Specifically, the objective of this study was to determine if there was any change in ketone production in response to a ketogenic meal in elderly subjects in good health, and to study the oxidation of glucose and β-OHB using carbon-13 stable isotope tracers, a valuable tool in nutritional research given that they allow for the study of metabolism *in vivo* in a non-invasive safe approach to the subject. Following this was to determine if there was any discernible difference in the oxidation of the two energy substrates in healthy subjects of three age groups composed of subjects of 18 to 25 years, 40 to 55 years, and over 70 years of age. The final objective was to discern if parameters relating to the oxidation of these two substrates, specifically, non-esterified fatty acids, insulin, cholesterol, triglycerides, and apolipoprotein E genotype differ in a situation of healthy aging. The intention of this project was to provide a base of knowledge about metabolism in healthy elderly for future studies targeted more to elucidating the contribution of energy substrate use to pathological states and to determine if alternate energy sources, may alleviate some symptoms of aging, including declining cognitive function.

Article 1: Omega-3 fatty acids, energy substrates, and brain function during aging.

Published in *Prostaglandins, Leukotrienes, and Essential Fatty Acids* 75 (2006) 213-220.

By Erika Freemantle, Milène Vandal, Jennifer Tremblay- Mercier, Sébastien Tremblay, Jean-Christophe Blachère, Michel E. Bégin, J. Thomas Brenna, Anthony Windust, and Stephen C. Cunnane

The Students contribution to the first article included in this thesis entitled 'Omega-3 fatty acids, energy substrates, and brain function during aging' was in an editing capacity and contribution to some of the data and ideas described.

Résumé du premier article en Français

Acides gras omega-3, substrats énergétiques et fonctionnement du cerveau pendant le vieillissement

Erika Freemantle, Milène Vandal, Jennifer Tremblay-Mercier, Sébastien Tremblay, Jean-Christophe Blachère, Michel E. Bégin, J. Thomas Brenna, Anthony Windust, Stephen C. Cunnane

Résumé

Lors du vieillissement, le maintien de la cognition est primordial afin de vieillir sainement et de maintenir une autonomie. Cependant, cette période de la vie est accompagnée de perturbations au niveau de la captation du glucose ayant pour effet de détériorer les fonctions cognitives. Les causes de cette détérioration sont peu connues de même que les solutions pour la corriger ou la contourner. Cependant, il semble que les acides gras oméga-3 puissent être reliés au maintient des carburants pour le cerveau et ce, de plusieurs façons. En effet, une consommation d'acides gras oméga-3 élevée, et plus spécifiquement d'acide docosahexaénoïque (DHA), est associée à une moins grande prévalence de déclins cognitifs comme la maladie d'Alzheimer chez les personnes âgées. De plus, les niveaux de DHA dans le cerveau pourraient affecter l'activité de certains transporteurs de glucoses du cerveau mais non pas tous les types de transporteurs de glucose. Ainsi, le DHA pourrait être un régulateur important de la captation du glucose par le cerveau. La synthèse du DHA à partir de l'acide alpha-linolénique (ALA) ou de l'acide eicosapentaénoïque (EPA) est très faible chez l'humain laissant présager que ces précurseurs sont

possiblement impliqués différemment dans le maintient de la cognition lors du vieillissement. Leur rôle ne serait pas liés à leur conversion en DHA mais à des fonctions différentes assurant le maintient de la cognition. Par exemple, l'ALA alimente efficacement la cétogenèse tandis que l'EPA augmenterait la β-oxydation des acides gras. Ainsi, l'ALA et l'EPA pourraient êtres utiles dans la production de substrats énergétiques alternatifs visant à promouvoir la production et l'utilisation des cétones afin de contourner le problème de captation du glucose par le cerveau vieillissant. Ainsi, les différents oméga-3 pourraient avoir des rôles distincts mais complémentaires afin d'assurer le maintient de la cognition lors du vieillissement.

Abstract

The maintenance of optimal cognitive function is a central feature of healthy aging. Impairment in brain glucose uptake is common in aging associated cognitive deterioration, but little is known of how this problem arises or whether it can be corrected or bypassed. Several aspects of the challenge to providing the brain with an adequate supply of fuel during aging seem to relate to omega-3 fatty acids. For instance, low intake of omega-3 fatty acids, especially docosahexaenoic acid (DHA), is becoming increasingly associated with several forms of cognitive decline in the elderly, particularly Alzheimer's disease. Brain DHA level seems to be an important regulator of brain glucose uptake, possibly by affecting the activity of some but not all the glucose transporters. DHA synthesis from either α-linolenic acid (ALA) or eicosapentaenoic acid (EPA) is very low in humans begging the question of whether these DHA precursors are likely to be helpful in maintaining cognition during aging. We speculate that ALA and EPA may well have useful supporting roles in maintaining brain function during aging but not by their conversion to DHA. ALA is an efficient ketogenic fatty acid, while EPA promotes fatty acid oxidation. By helping to produce ketone bodies, the effects of ALA and EPA could well be useful in strategies intended to use ketones to bypass problems of impaired glucose access to the brain during aging. Hence, it may be time to consider whether the main omega-3 fatty acids have distinct but complimentary roles in brain function.

1. Introduction

The proportion of elderly people in most developed countries is increasing and is expected continue to do so for at least 20-30 years. Healthcare costs increase significantly for the elderly, largely as a function of declining health and autonomy. Loss of memory and alterations in behaviour accompany declining brain function associated with aging, and are key symptoms of degenerative brain diseases such as Alzheimer's disease and other forms of dementia.

The dementias are but one of several forms of chronic debilitating brain disorder. One estimate suggests that on a global basis, the burden of illness caused by the full spectrum of brain disorders now matches and may well surpass that of cardiovascular disease and cancer combined [1]. Hence, one of the imperatives of biomedical research over the next 20 years will be to better understand how to maintain optimal brain function and cognition in middle aged and elderly adults.

Several nutrition-related factors heighten the risk of declining cognition, including insulin resistance, Type 2 diabetes, and intentional or non-intentional marked declines in body weight and total body fat. Given the increasing recognition of the important link between energy substrate supply and brain function, research is beginning into the role of nutrition and other lifestyle factors, such as exercise, in at least some forms of cognitive decline associated with aging [2-6]. Our group's research strategy in this field is focused on developing a better understanding of whether deteriorating access of energy substrates to the aging brain contributes to an increased risk of declining cognitive function. Indeed, we wonder if this deterioration may reach the point that it is appropriate to ask whether brain 'energy starvation' could be present in certain conditions like the dementias.

This paper reviews several aspects of the case that unfavourable nutritional status conspires to increase the risk of energy starvation in the aging brain. We also present evidence

suggesting that the three main omega-3 fatty acids may have complimentary yet distinct ways of helping maintaining optimal brain function by their actions on brain energy substrate supply.

2. Brain Glucose Uptake, PET and Brain Function

The brain's principle fuel is glucose, which it consumes at about 25 μmol/100g/min or about 100 g/d [7]. Ketone bodies (or simply ketones) are the brain's principle alternative energy substrate to glucose, especially during fasting or illness. However, glucose always supplies a minimum of 25-30% of the adult human brain's energy requirement, even during prolonged fasting or starvation [7].

Regional changes in brain glucose uptake are readily studied using positron emission tomography (PET), a minimally invasive technique that monitors the presence of positrons produced by a short-lived gamma radiation-emitting nuclide injected into the subject. For brain glucose uptake studies fluorine-18 is the preferred gamma-emitting tracer and is incorporated into a glucose analogue - ^{18}fluorodeoxyglucose. ^{18}Fluorodeoxyglucose is transported into tissues at the same rate as glucose itself but is not metabolized further, so it specifically represents glucose uptake unaffected by its subsequent metabolism.

PET studies have shown for over 20 years now that brain glucose uptake is impaired in Alzheimer's patients [8-10]. The impairment in glucose uptake is most affected in the temporal and parietal association cortices where it may be reduced by up to 20%. This effect is independent of and in addition to the usual age associated decline in brain size and blood flow [11].

Until recently, it was unknown whether decreased brain glucose uptake is caused by or may contribute to the pathology of Alzheimer's disease [12]. Clearly, seriously damaged or dead neurons have low to negligible glucose uptake so a disease-driven decline in cognition

can potentially impair brain glucose utilization. However, long before any decline in brain function can be detected clinically, a mild but significant decrease in brain glucose uptake has recently been reported in individuals genetically susceptible to Alzheimer's disease [13,14]. This patchy 'pre-clinical' deterioration in glucose uptake occurs in the same areas of the temporal and parietal cortex where glucose uptake is most impaired in Alzheimer's disease. Hence, impaired brain glucose uptake now appears to be a potentially significant *contributing factor* to at least some types of declining brain function later in life [12-14].

3. Omega-3 Fatty Acids, Aging and Brain Function

One of the well-established deleterious effects of dietary deficiency of omega-3 fatty acids is on cognitive and behavioural development during infancy. Spurred by the interest in the role of omega-3 fatty acids in brain development, research has begun into the possible implications of low omega-3 fatty acid intake for brain function in the elderly. Thus far, the main observation is that the elderly consuming lower amounts of omega-3 fatty acids, particularly fish [15-23], have an elevated risk of Alzheimer's disease. However, this relationship is not always observed [24]. Post-mortem samples of Alzheimer's brain have decreased age-adjusted docosahexaenoic acid (DHA, 22:6□3) content [25]. Collectively, these studies implicate lower brain DHA in the pathogenesis of Alzheimer's disease. Whether lower brain DHA in Alzheimer's disease is caused by lower DHA intake or by lower DHA synthesis (or by a combination of the two) remains to be determined.

While a relationship between low DHA intake and higher risk of Alzheimer's disease seems plausible based on studies of the role of omega-3 fatty acids in supporting normal brain function in infants and animals, there are as yet only preliminary and inconclusive reports of a therapeutic effect of omega-3 fatty acid supplementation on cognition or memory in elderly people without dementia [23]. Given the long time course before cognitive defects become

clinically detectable and the variable rate of cognitive deterioration, intervention studies using DHA supplementation to prevent or treat cognitive decline will probably need to be large and lengthy if they are to be conclusive. It is also not yet clear whether the connection between low DHA and cognitive decline is causal (higher DHA intake protects against cognitive function) or is an effect of the disease process (neurodegeneration destroys brain DHA).

4. DHA and Glucose Metabolism

Rats made deficient in DHA by severe depletion of total omega-3 fatty acid intake have 80-90% lower brain DHA, as well as 30-40% lower brain uptake of glucose and concomitantly lower cytochrome c oxidase activity in several brain regions [26,27]. Given the key role of glucose in brain function, suboptimal brain function in omega-3 fatty acid deficient animals could be due at least in part to impaired brain glucose uptake linked to lower expression of the glucose transporter, GLUT 1, in blood vessels or astrocytes of the brain. Nevertheless, in these two studies [26,27], expression of GLUT 3 in neurons was unaffected, as was the concentration of GLUT protein and GLUT activity in brain microvessels or astrocytes. Hence, more work needs to be done to ascertain whether the defects in brain glucose transport that have been reported in DHA deficient rats [26,27] can actually account for suboptimal brain function in rats made omega-3 fatty acid deficient. The relevance to cognitive decline associated with aging of this model of impaired glucose supply to the brain in omega-3 deficient rats also remains to be determined.

In several species including humans, glucose tolerance varies directly with the DHA content of skeletal muscle [28-31]. Insulin resistance and Type 2 diabetes develop in part due to less efficient uptake and processing of glucose by skeletal muscle, so these reports, particularly by Borkman et al [28], make it is plausible that low DHA status contributes to a heightened risk of insulin resistance in humans. Since insulin resistance is a common problem

in the elderly and contributes significantly to the risk of Alzheimer's disease [2-6], we speculate that low omega-3 fatty acid intake, low tissue DHA (brain and elsewhere, especially muscle), impaired brain glucose uptake, insulin resistance and risk of Alzheimer's disease are all potentially linked.

5. Marked Differences in ^{13}C-ALA and ^{13}C-DHA Metabolism: Preliminary Data

Whether optimal health in adult humans necessitates intake of pre-formed DHA or whether sufficient DHA can be made endogenously remains controversial in several fields ranging from cardiovascular disease to immunology, neurology, and psychiatry. Biochemically, the complete 'desaturation-chain elongation' pathway converting the parent omega-3 fatty acid - α-linolenic acid (ALA; 18:3ω3) - to DHA exists in humans. However, in comparison to results from animal models or even human infants, adult humans have a very low capacity to convert ALA to DHA. This low capacity is clear in dietary supplementation studies with ALA, in which plasma DHA does not change significantly even in relation to high intakes of ALA (9-21 g/d for up to 6 weeks; reviewed by Cunnane 2003 [32]). The low capacity to convert ALA to DHA in humans is also apparent in stable isotope tracer studies, which generally show that <0.1% of carbon-13 (^{13}C)-ALA is found as ^{13}C-DHA in plasma for periods of up to four weeks after dosing with the tracer [33-35].

We have published a detailed assessment of the metabolism of ^{13}C-ALA in healthy young women [35] and, as have others [34], we concluded that part of the reason ^{13}C-ALA is so poorly converted to ^{13}C-DHA in humans is because it is readily β-oxidized. Despite inefficient conversion of ALA to DHA, dietary supplementation with ALA nevertheless reduces the risk of cardiovascular disease and cancer [37-39]. This suggests that not all the actions of ALA are necessarily dependent on conversion to DHA and that a more detailed assessment of both ALA and DHA metabolism in the elderly is warranted.

With the exception of one abstract suggesting ^{13}C-ALA conversion to ^{13}C-DHA is even lower in the elderly than in young adults [36], there are no full reports of the metabolism of ^{13}C-ALA in humans as they age. There are also no reports of the metabolism of ^{13}C-DHA given to humans of any age because this tracer has only recently become available. We have begun a comparison of the metabolism of ^{13}C-DHA and ^{13}C-ALA and will soon be extending it to the healthy elderly. Women in their mid-twenties consumed 50 mg of the tracer (^{13}C-DHA or ^{13}C-ALA) in yogurt at breakfast. Subsequent appearance of the tracer in breath $^{13}CO_2$ and in plasma total lipids was monitored over eight days by isotope ratio mass spectrometry (Figure 1). The small quantity of ^{13}C-DHA available to us at that time limited us to two subjects but there were 6 subjects in the ^{13}C-ALA group. Despite the preliminary nature of the ^{13}C-DHA part of this study, it appears that ^{13}C-DHA is much better retained in the plasma and is much less β -oxidized than ^{13}C-ALA, something that has long been assumed but we are now in a position to quantify and compare over different age strata. Results of this study should clarify whether the healthy elderly have altered synthesis or metabolism of DHA. It will then be useful to evaluate whether pathologies associated with aging that impact on cognition, eg. insulin resistance, are associated with changes in DHA synthesis or metabolism.

7. Ketones: Key Alternative Energy Substrates to Glucose

Ketones (β -hydroxybutyrate, acetoacetate and acetone) enter on several levels into the discussion of the link between omega-3 fatty acids, energy substrates and brain function during aging. First and foremost, they are the principal alternative brain fuel to glucose. This is clear both from their ability to supply as much as 65-70% of the adult human brain's fuel needs during prolonged fasting [7,40] and from their key role in supplying both fuel and lipid substrates to the brain during fetal and neonatal development [41-45]. This role of ketones as crucial structural and fuel substrates for the developing brain is supported by the observation

that healthy, well-fed infants are in a constant state of mild ketonemia [46]. Thus, mild ketonemia is physiologically important for normal human development and, unlike in adults, is not necessarily a sign of energy or insulin deficit.

Second, whether given by intravenous infusion or produced endogenously in response to intake of medium chain triglycerides, ketones protect the brain in acute models of experimental stroke [47] and hypoglycaemia [48,49]. They are also associated with very short-term improvement in cognitive function in dementia patients [50]. Medium chain triglycerides were chosen for Reger's study [50] because they readily access mitochondria where they are β -oxidized without the need for transport via carnitine palmitoyl transferase (CPT), thereby becoming effective substrates for ketone production.

Most studies reporting the protective effects of ketones on the brain have been short term (≤1 week), but the efficacy of moderate ketonemia (2-5 mM) lasting over periods of 1-3 years in mitigating refractory epileptic seizures in children supports the view that mild to moderate ketonemia is not only beneficial to the brain but can be well-tolerated and effective in the long-term [51;52]. The potentially beneficial effects of mild to moderate ketosis on the brain do not diminish the challenge of achieving and maintaining mild to moderate ketonemia, a condition almost incompatible with normal 'western' dietary habits that involve near constant stimulation of insulin by dietary carbohydrate.

Medium chain triglycerides are perhaps the most efficient way to produce mild ketonemia in humans but their gastrointestinal side effects in many individuals limit their utility on a large scale. Long chain fatty acids are major fuels in the body but their β -oxidation is dependent on CPT so they are less efficient ketogenic substrates than are medium chain triglycerides. Amongst the most common long chain fatty acids in the diet, one of the omega-3 fatty acids - ALA - is a 5-6 fold better substrate for CPT than is stearate and is a 3 fold better substrate than palmitate (Figure 2) [53]. ALA is also more easily oxidized than

linoleate or oleate [53]. In isolated rat hepatocytes, ALA is preferred by 2-3 fold over either linoleate or oleate as a substrate for ketogenesis [54]. Very high fat diets (~80% fat) enriched in ALA can lead to moderately higher ketonemia in rats than diets based on saturated fats [55]. Collectively, these studies suggest that ALA could be beneficial in producing mild ketonemia aimed at retaining or restoring cognitive function in the elderly. The use of ALA as a mildly ketogenic fatty acid would have the additional beneficial effect of producing a small trickle of DHA, which might help the functionality of neurons now better supported by the additional brain fuel supply.

8. ^{11}C-Acetoacetate: A New PET Tracer

Strategies employing ketogenesis aimed at more efficiently supplying fuel to the aging brain would benefit from imaging methodology that provides a window on brain uptake of ketones. Although in vivo NMR spectroscopy may be applicable for this purpose, PET is probably the technique of choice, its utility having been clearly demonstrated by its widespread clinical use in the assessment of brain glucose uptake. The PET tracer needed for studies of brain ketone uptake is carbon-11, an isotope with a half-life of just 20.5 minutes. Blomqvist and colleagues [56,57] demonstrated the feasibility of making ^{11}C- β - hydroxybutyrate for human studies of brain ketone uptake in diabetes. In planning our own PET studies of brain ketone uptake have followed up the pioneering work of Blomqvist et al [56,57] but have found it easier to make ^{11}C-acetoacetate, the methodology for which we will be publishing soon.

The key question such methodology could answer is whether brain uptake of ketones changes (overall or regionally) either in the healthy elderly with intact cognitive function or in those experiencing cognitive decline. In other words, is brain ketone transport susceptible to the pathology of aging in a manner analogous to the deterioration in brain glucose transport?

At least in a single short term experiment, cognitive function in individuals with Alzheimer's disease is modestly improved by mild ketonemia [50], which implicitly suggests that ketones must still be able to access the Alzheimer's brain or they wouldn't be able to improve cognition. This supposition is testable using PET analysis of brain ketone uptake using either ^{11}C-acetoacetate or ^{11}C- β -hydroxybutyrate. Furthermore, since brain ketone uptake is mediated by a monocarboxylic acid transporter [58], PET methodology would be suitable to quantify the efficacy of nutritional or pharmacological strategies aimed at stimulating this transporter.

9. Insulin Resistance: A Key Factor in Brain Starvation?

The problem of insulin resistance during aging is an implicit part of any discussion of strategies to improve energy substrate availability to the brain. Insulin resistance is common during aging and appears to be a major, if not the main, risk factor for cognitive decline during aging [2-6]. Insulin resistance is the precursor state to Type 2 diabetes mellitus and involves inefficient tissue uptake of glucose, which, in turn, leads to more compensatory insulin production, impaired insulin efficacy, and insulin resistance. Skeletal muscle is the main site of glucose utilization in the body and so declining muscle mass in the elderly appears to be a factor potentially contributing to the increased risk of insulin resistance in the elderly.

Normally, i.e. in the absence of insulin resistance, low carbohydrate intake or fasting decreases plasma insulin, which allows the liberation of free fatty acids from adipose tissue. When insulin is low, tissues capable of using fatty acids as energy substrates (skeletal muscle, heart) do so, while those dependent more on ketones produced by fatty acid β -oxidation (brain) have access to an increased supply produced primarily in the liver. When insulin rises

after a carbohydrate meal, this immediately shuts off free fatty acid mobilization and glucose takes over again as the principle fuel.

In insulin resistance, transport of glucose into peripheral tissues is impaired yet plasma free fatty acids are frequently elevated [3]. Elevated plasma free fatty acids should promote ketonemia but this works only when insulin is low, eg. late in the postprandial period in people with normal insulin sensitivity. However, the chronic hyperinsulinemia of insulin resistance blocks ketone production [59]. Hence, insulin resistance not only impairs tissue uptake of glucose but also impairs production of ketones, which are the main alternative fuels to glucose. It is unclear whether insulin resistance affects free fatty acid uptake but the brain is unable to meet its energy needs from fatty acid β -oxidation, so whether insulin resistance affects free fatty acid uptake elsewhere is somewhat irrelevant to the brain's fuel supply. Thus, when brain glucose uptake is impaired, inhibition of ketogenesis by insulin resistance [59] blocks at least some areas of the brain from getting sufficient amounts either of the primary brain fuels (glucose and ketones). We hypothesize this situation of impaired access of both the brain's major fuels leads to a heightened risk of brain starvation in insulin resistance.

Brain glucose uptake is widely considered to be independent of insulin so insulin resistance should not affect this process. Nevertheless insulin resistance is a key factor in cognitive deterioration so, on the surface, these observations seem inconsistent. Insulin resistance could potentially impair cognition through mechanisms independent of brain glucose transport so this topic still needs further research to clear these uncertainties. We believe stable isotope and PET methodology are well suited to addressing how insulin resistance changes energy substrate metabolism and impacts on cognitive function during aging.

10. Eicosapentaenoic Acid: A Mediator of Ketone Production?

So far, this review into links between energy substrates and brain function during aging implicates two omega-3 fatty acids – ALA and DHA - on several levels: (i) DHA seems important for normal brain glucose uptake and is commonly inadequately consumed by the elderly [15-23]. (ii) DHA also seems important for glucose uptake by skeletal muscle [28] so it may be an important mediator of body's overall ability to use glucose, i.e. it's insulin sensitivity. (iii) DHA is important for optimal neuronal function, a link that probably involves multiple mechanisms including effects on brain glucose uptake. (iv) ALA is a very poor precursor to DHA but nevertheless has a potentially important supporting role in brain function as an efficient and well-tolerated substrate for fatty acid oxidation and ketogenesis.

The intermediate omega-3 fatty acid, eicosapentaenoic acid (EPA, 20:5□3), may also be implicated in brain function during aging. However, the negligible changes in plasma DHA reported in studies with moderately high intakes of EPA [60,61] suggest any beneficial effect of EPA is unlikely to depend exclusively on DHA. Aside from its well-known role as a precursor to the 3 series eicosanoids and related peroxy-fatty acids [62,63], little is known about whether EPA has other functions in the body. However, we speculate that EPA may well facilitate fuel supply to the brain. Evidence is emerging to show that EPA is an activator of one or more of the classes of peroxisome proliferator activated receptors (PPARs) that promote long chain fatty acid oxidation [64,65]. We hypothesize that as a PPAR activator, EPA could stimulate ketogenesis. If so, EPA would facilitate the effect of ALA as an efficient ketone substrate and both would help support the role of DHA as a key structural element in membrane phospholipids.

ALA and EPA both appear to be poor precursors to DHA in adult humans, so it seems imprudent to expect that sufficient DHA can be produced endogenously from either ALA or EPA to meet the brain's needs during aging. Hence, just as thirty years of research has

gradually led to legislation of DHA in infant formula in many countries, it now seems appropriate to rigorously assess whether a dietary source of DHA is advisable in adults, especially the elderly.

12. Omega-3 Fatty Acids: Distinct but Complimentary Roles?

Because of the poor conversion of ALA and EPA to DHA yet beneficial effects of all three omega-3 fatty acids on different aspects of energy metabolism that impinge on the brain, brain function in adults should not be thought of as dependent on DHA alone. We suggest that the distinct roles of omega-3 fatty acids in energy metabolism and brain function are complimentary and that optimal retention of cognitive function in the elderly probably depends on a blend of all three roles (Figure 3). Whether therapeutic avenues exist in which impaired brain function can be corrected by one or more omega-3 fatty acids remains a tantalizing question.

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Figure Legends

Figure 1. Different Metabolism of ^{13}C-ALA and ^{13}C-DHA in Humans.

Preliminary evidence for marked differences in the plasma levels (A) and β-oxidation (B) of ^{13}C-docosahexaenoic acid (^{13}C-DHA; solid line) compared to ^{13}C-α-linolenic acid (^{13}C-ALA; dotted line) in young healthy adults. Oxidation of ^{13}C-glucose is also shown (X---X in Panel B). For each tracer, healthy young women were given a 50 mg oral dose and follow-up was for 168 h (8 days). The β-oxidation data represent a cumulative oxidation of about 24% over 24 h for ^{13}C-ALA, as compared to <5% for ^{13}C-DHA, and about 37% for ^{13}C-glucose. Data are based on n=6 for ^{13}C-ALA, n=6 for ^{13}C-glucose and n=2 for ^{13}C-DHA.

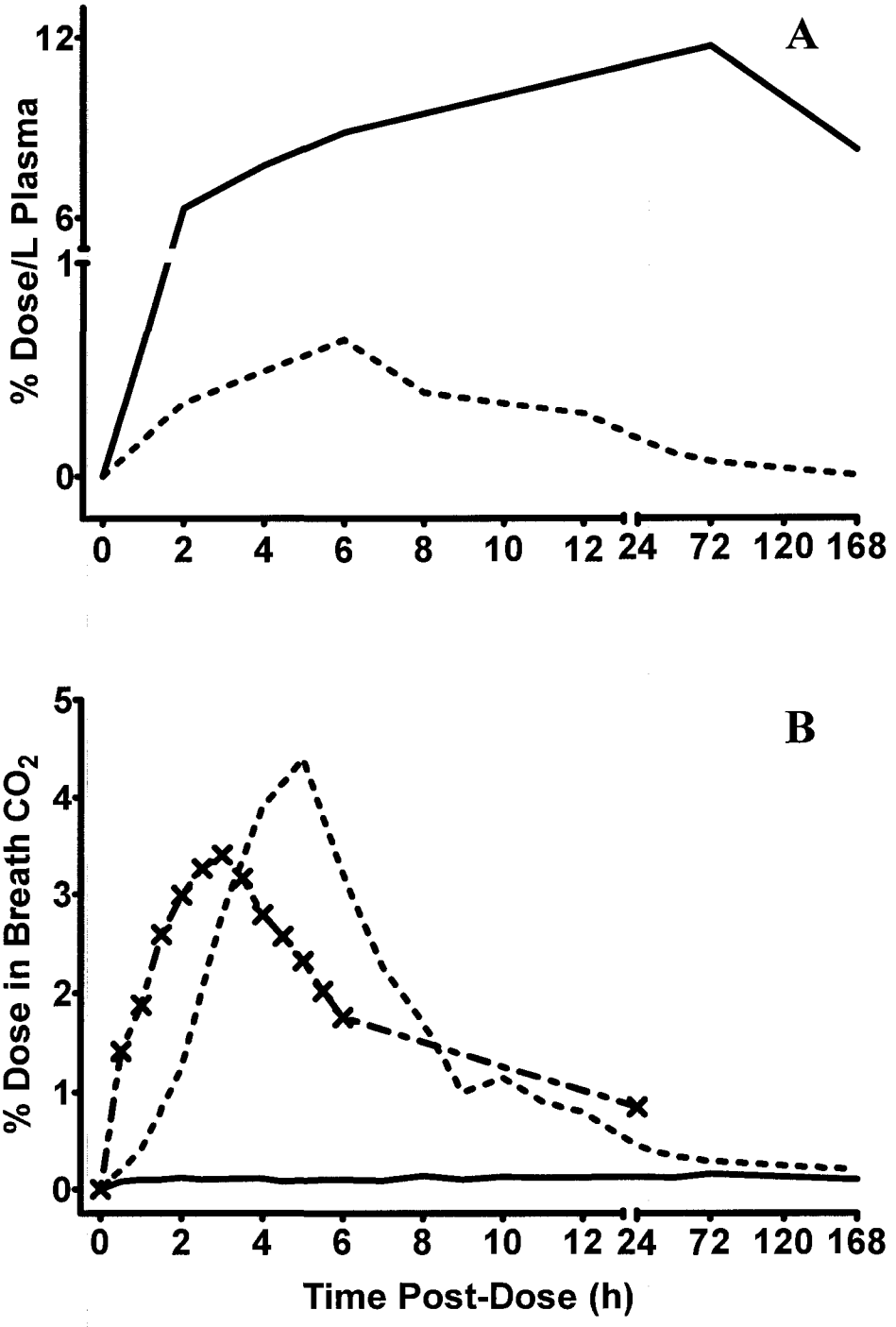
Figure 2.

Differential β-oxidation of long chain fatty acids.

These data normalized to values for linoleic acid (LA; 100%). are summarized from a variety of models reported in the literature and are described in full elsewhere [53]. The comparison is normalized because of the different units used to express fatty acid oxidation in the various studies cited. SA – stearic acid, PA – palmitic acid. OA – oleic acid, EA – elaidic acid, ALA – α-linolenic acid.

Figure 3.

Distinct yet complimentary roles of omega-3 fatty acids in brain function during aging: a proposal. The dotted arrows indicate the weak level of conversion connecting α-linolenate to eicosapentaenoate and docosahexaenoate. The solid arrows indicate ways in which each of these omega-3 fatty acids is proposed to have potentially important effects on brain function distinct from their interconversion.


A
12
6
1
0
% Dose/L Plasma
0 2 4 6 8 10 12 24 72 120 168
B
5
4
3
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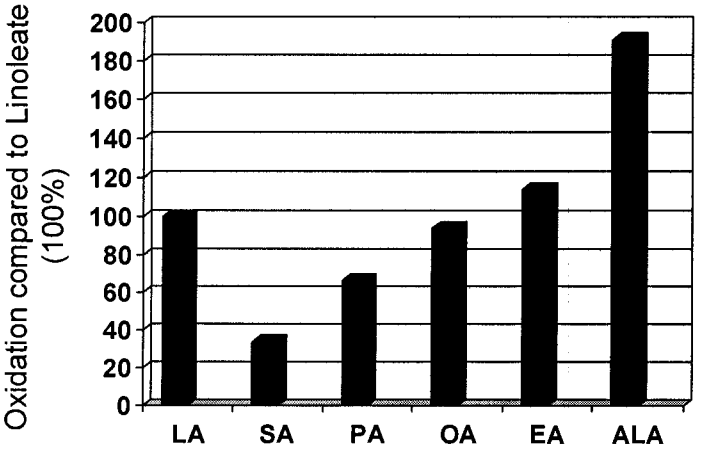
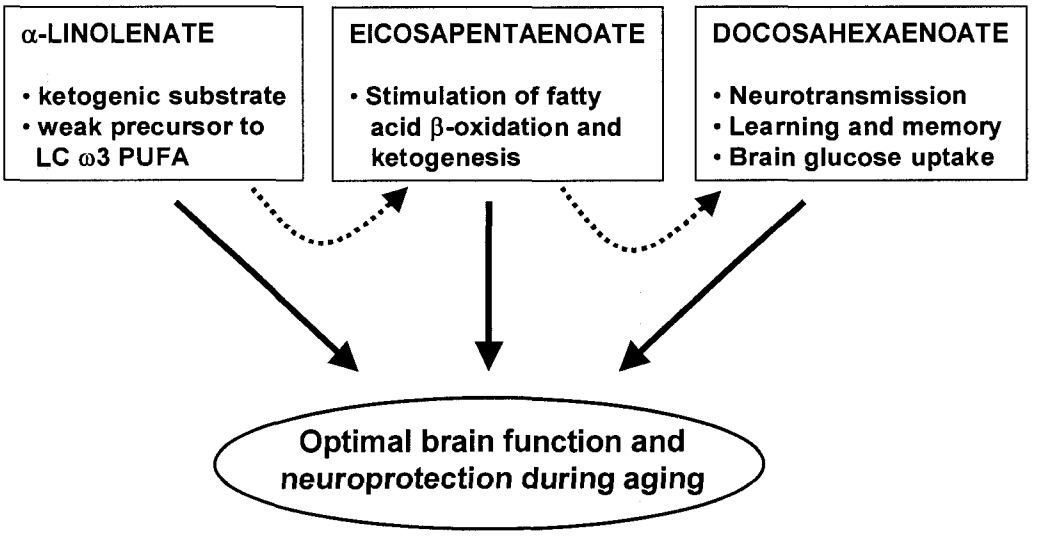
% Dose in Breath CO_2
0 2 4 6 8 10 12 24 72 120 168
Time Post-Dose (h)


Oxidation compared to Linoleate (100%)
200
180
160
140
120
100
80
60
40
20
0
LA
SA
PA
OA
EA
ALA


α-LINOLENATE
• ketogenic substrate
• weak precursor to LC ω3 PUFA
EICOSAPENTAENOATE
• Stimulation of fatty acid β-oxidation and ketogenesis
DOCOSAHEXAENOATE
• Neurotransmission
• Learning and memory
• Brain glucose uptake
Optimal brain function and neuroprotection during aging

Link between the two articles

The two articles are related by the subject matter of glucose and ketones. The first article provides a more solid basis into the rationale for studying and understanding the implications of glucose and ketones in terms of brain function, while the second article is more focused on the peripheral systemic metabolism of glucose and ketones, and includes in more detail the role of other metabolites related to the use of these energy substrates such as insulin, triacylglycerol, cholesterol, and non-esterified fatty acids.

Article 2: Metabolic response to a ketogenic breakfast in the healthy elderly

Submitted to *The American Journal of Clinical Nutrition* December 4, 2007.

By Erika Freemantle, Milène Vandal, Jennifer Tremblay-Mercier, Mélanie Plourde, Judes Poirier, and Stephen C. Cunnane.

The Students contribution to the second article included in this thesis entitled 'Metabolic response to a ketogenic breakfast in the healthy elderly' was collection and analysis of the data presented as part of the Master's research project presented in this thesis, writing, editing, corrections and submission of the article for publication.

Résumé du deuxième article en Français

Réponse métabolique à un déjeuner cétogène chez les personnes âgées en santé.

Erika Freemantle, Milène Vandal, Jennifer Tremblay-Mercier, Mélanie Plourde, Judes Poirier and Stephen C. Cunnane

Résumé

Problématique Le glucose est la source principale d'énergie chez l'humain. Lors d'apports insuffisants de glucose, les cétones compensent le besoin énergétique et ce même pour le cerveau. Chez des personnes âgées atteintes de déclins cognitifs, les cétones pourraient ralentir certains de leurs symptômes. Cependant, le métabolisme des substrats énergétiques lors du vieillissement sain est actuellement inconnu.

Objectif: Déterminer le métabolisme du glucose et des cétones chez les sujets âgés, d'âges moyens ou jeunes après la prise d'un déjeuner induisant une cétogenèse courte et douce.

Protocole: Dix sujets dans chacun des trois groupes d'âge (23 ± 2, 50 ± 4 et 76 ± 5 ans) ont été recrutés et ont consommé un déjeuner cétogène. Le β-hydroxybutyrate, le glucose, l'insuline, les triacylglycéroles, le cholestérol total et les acides gras non estérifiés du plasma ainsi que l'acétone de l'haleine ont été mesurés chez les sujets pendant 6h. Chaque sujet a complété le protocole à deux reprises afin de déterminer la β-oxydation de deux traceurs sur une période de 24 h; soit le glucose et le β-hydroxybutyrate marqués au carbone 13 (^{13}C).

Résultats: Le glucose plasmatique a diminué au cours de la période de 6h de suivi tandis que le β-hydroxybutyrate, l'acétone et l'insuline ont augmenté et ce, dans les trois groupes d'âge. Les niveaux de cholestérol, triacylglycérols et acides gras non estérifiés n'ont pas été modifiés. L'oxydation du ^{13}C-glucose et du ^{13}C-β-hydroxybutyrate a atteint un sommet entre deux et trois heures après la prise de l'un ou l'autre des traceurs dans les trois groupes. L'oxydation cumulative du ^{13}C-glucose pendant 24h était significativement plus élevée chez les sujets âgés comparativement aux sujets d'âge moyen tandis qu'il n'y avait pas de différence pour l'oxydation cumulative pour le ^{13}C-β-hydroxybutyrate. L'Apolipoprotéine E ε4 était associée à des niveaux de cholestérol plus élevés sans toutefois affecter les autres paramètres métaboliques.

Conclusion: Les personnes âgées en santé ont une capacité similaire aux sujets d'âge moyen et jeune à produire des cétones et à oxyder le ^{13}C-glucose et le ^{13}C-β-hydroxybutyrate

Abstract

Objective: To determine whether the metabolism of glucose or ketones differs in the healthy elderly compared to young or middle-aged adults during mild, short-term ketosis induced by a ketogenic breakfast.

Design and participants: Healthy subjects in three age groups (23±1, 50±1 and 76±2 y old) were given a ketogenic meal and plasma β-hydroxybutyrate, glucose, insulin, triacylglycerols, total cholesterol, non-esterified fatty acids and breath acetone were measured over the subsequent 6 h. Each subject completed the protocol twice in order to determine the oxidation of a tracer dose of both carbon-13 (^{13}C) glucose and ^{13}C-β-hydroxybutyrate. The tracers were given separately in random order. Apolipoprotein E genotype was also determined in all subjects.

Results: Plasma glucose decreased and β-hydroxybutyrate, acetone and insulin increased similarly over 6 h in all three groups after the ketogenic meal. There was no significant change in cholesterol, triacylglycerols or non-esterified fatty acids over the 6 h. ^{13}C-glucose and ^{13}C-β-hydroxybutyrate oxidation peaked at 2-3 h post-dose for all age groups. Cumulative ^{13}C-glucose oxidation over 24 h was significantly higher in the elderly but only versus the middle-aged group. There was no difference in cumulative ^{13}C-β-hydroxybutyrate oxidation between the three groups. Apolipoprotein E (ε4) was associated with elevated fasting cholesterol but was unrelated to the other plasma metabolites.

Conclusion: Elderly people in good health have a similar capacity to produce ketones and to oxidize ^{13}C-β-hydroxybutyrate as middle-aged or young adults, but oxidize ^{13}C-glucose a little more rapidly than healthy middle-aged adults.

Keywords: ketones, glucose, healthy elderly, ^{13}C stable isotope tracers.

Introduction

In humans, glucose is the brain's primary energy substrate and ketone bodies (ketones) are it's primary replacement fuel during fasting or low carbohydrate intake (1). Ketones refers collectively to three molecules: acetoacetate (AcAc), β-hydroxybutyrate (β-OHB), and acetone (2). During ketogenesis, AcAc is formed first and is the only ketone metabolized by the tricarboxylic acid cycle as an energy substrate. After being converted back to AcAc by β-OHB dehydrogenase, β-OHB can also serve as an energy substrate (3). Acetone is produced by decarboxylation of AcAc and is exhaled in the breath in proportion to plasma ketone concentrations (2).

Impaired availability of energy substrates to the brain may be implicated in the progression towards Alzheimer's disease (4, 5). Raising blood ketones with a ketogenic meal shows preliminary potential to alleviate some features of the cognitive deficit in Alzheimer's disease (6). Given this potentially important clinical application, but the relative scarcity of information about how energy substrates are utilized during healthy aging, i.e. during aging minimally confounded by symptomatic degenerative disease, our primary objective was to evaluate glucose and ketone utilization in the healthy elderly compared to young and middle-aged adults.

Insulin inhibits ketone production so to achieve short-term ketogenesis subjects were given a very low carbohydrate breakfast composed of medium chain triacylglycerol (MCT), heavy cream, protein powder and water. MCT efficiently induce mild to moderate ketosis in humans (7) because they are rapidly absorbed and pass directly via the hepatic portal venous circulation to the liver where they are β-oxidized with some of the resulting acetyl CoA being captured in ketones. MCT do not require a carnitine-dependent transport system to enter the inner mitochondrial space, and are thus more readily available for oxidation and at a lower energetic cost than long

chain triacylglycerol (LCT) (8). Although the present study was not designed or powered for analysis of the effect of genotype, apolipoprotein E genotype of our subjects was determined since it affects both post-prandial fat metabolism (9) and risk of Alzheimer's disease (10, 11).

Materials and Methods

Subjects: Subjects were recruited in three age groups: 18-25 y old (young: Y), 40-55 y old (middle-aged: M), and 70-85 y old (elderly: E). This distribution maintained a minimum 15 y gap between age groups and also avoided the increasing impact of frailty beyond 85 y old (12). All subjects were non-smokers and determined to be in relatively good health by a medical evaluation and blood screening done after a 12 h overnight fast. Fasting glucose and hemoglobin HbA_{1c} were used to rule out the presence of overt diabetes. A complete blood cell count was used for blood disorders; electrolyte profile; AST and ALT for renal and liver function; HDL and LDL cholesterol; triglycerides; albumin for nutritional status; C-reactive protein as a marker of inflammatory processes; and TSH for thyroid function. Anthropometric parameters such as height, weight, body mass index (BMI), and fasting plasma metabolites did not differ significantly between age groups (**Table 1**). Approval for the study was obtained from the Research Ethics Committee of the Health and Social Services Center – Sherbrooke University Geriatrics Institute, which oversees all human research done at the Research Center on Aging.

Tracer protocol and sample collection: Subjects arrived at 7:30 a.m. after having fasted overnight for 12 h. An intravenous forearm catheter was installed and baseline blood samples taken. The catheter was kept patent by flushing hourly with non-heparinized saline. The stable isotope tracer was then consumed (^{13}C-glucose or ^{13}C-β-OHB), followed immediately by the ketogenic breakfast drink, which was consumed within approximately 30 mins. After consuming the ketogenic breakfast, blood samples were taken hourly over 6 h using a 5 ml latex-free syringe (Becton Dickinson, Franklin Lakes, NJ) and transferred immediately to a 5 mL K_2-EDTA-

coated tube (Becton Dickinson, Franklin Lakes, NJ). Tubes were stored on ice at 4°C until the conclusion of the study period at which point they were all centrifuged at 3500 rpm for 18 min at 4°C. The separated plasma was stored at -20°C until further analyzed. During the 6 h study period, water was available *ad libitum* and subjects were asked to remain in a resting position as much as possible with short walks if necessary.

Each subject participated in two identical metabolic study days, one to test ^{13}C-glucose metabolism and the other to test ^{13}C-β-OHB metabolism. The tracers were U-$^{13}C_6$ D-glucose or 2,4-$^{13}C_2$ sodium D-3-hydroxybutyrate (50 mg each; Cambridge Isotope Laboratories, Andover, MA) were consumed in 15 mL nanopure water and in randomized order. The two study days were separated by one to three weeks. Breath samples for $^{13}CO_2$ and acetone analysis were collected in triplicate at baseline and every 30 min afterwards using a breath collection device (Easysampler, Quintron Instrument Company, Milwaukee, WI) and 10 mL evacuated glass tubes (Exetainer, Labco Ltd, Buckinghamshire, UK). The first ~150 mL of exhaled air is dead space (13), so to collect a true alveolar breath sample, the subjects exhaled for 3 sec before breath sample collection. For acetone analysis, 1 mL of breath was transferred from one of the three Exetainer tubes to a glass gas-tight syringe (Hamilton Company, Reno, NV).

Ketogenic breakfast drink: The ketogenic breakfast drink consisted of a blend of MCT (Mead Johnson, Ottawa, ONT, CA), 35% heavy cream (Québon Ultra Crème, Longueuil, QC, CA), raspberry-flavored milk protein powder (Davisco Foods International, Inc., Eden Prairie, MN, courtesy of Agropur Cooperative, Granby, QC, CA) and water (**Table 2).** The fatty acid composition of the ketogenic breakfast is

shown in **Table 3**. This ketogenic breakfast was designed to give a ratio of total fat to protein plus carbohydrate of 4.5:1, which is sufficient to induce mild, short-term ketosis in young adults (2). The total carbohydrate content of the drink was limited to the carbohydrate already in the cream (3.2%). Total protein content was calculated to be 1/3 of the subject's daily protein requirement as determined by the Harris-Benedict equation and the Canada Food Guide (Health Canada, Ottawa, ON, CA). Total fat was then adjusted to be equivalent to 4.5 times the protein plus carbohydrate content. Subjects received an average of 1104 kCal, 90% of which was fat. In the breakfast drink, the amount of total fat (g), MCT (g), fat/body weight (g/kg), or fat/BMI ($g/kg/m^2$) did not differ significantly across the three study groups.

Isotope ratio mass spectrometry: Enrichment of ^{13}C in breath CO_2 following the ingestion of the ^{13}C tracer was analyzed by isotope ratio mass spectrometry (Europa 20-20, Sercon Ltd, Crewe, Cheshire, UK) as previously described (14). 5% CO_2/N_2 was the reference gas and He was the carrier gas (Praxair Canada Inc. Mississauga, ON, Canada). Atom percent (AP) is the relative abundance of ^{13}C in the sample calculated by the following equation:

(1)

$$AP = \frac{100}{1/[(\delta/1000 + 1)\ ^{13}C_{ref} + 1]}$$

^{13}C data in delta notation (δ) is the ratio of ^{13}C to ^{12}C calibrated against the reference gas and the international standard, Peedee Belemnite (15). The percent dose recovered (PDR) of the tracer administered to the subjects was calculated as in equation (2),

(2)

$$PDR = \frac{APE \times VCO_2}{\text{mmol } ^{13}\text{C-tracer administered}} \times 100\%$$

In which atom percent excess (APE) is calculated using of the value obtained in equation (1) for time t minus the value obtained at time 0. Taking into account the chemical purity, the isotopic enrichment of the tracer, and the natural abundance of ^{13}C, the quantity of ^{13}C excreted on breath (mmol) was calculated as shown in equation (3):

(3) $$\text{mmol } ^{13}C = \frac{\text{mg tracer}}{\text{molecular weight}} \times \text{chemical purity} \times ([99\% \#^{13}C] + [1\% \text{ total } \# C])$$

The chemical purity of both tracers was 98% and their isotopic purity was 99%. The CO_2 production constant of 300 mmol/h was used as determined by Schofield (16) and previously validated for healthy adults (17). V_{CO2} was then calculated by multiplying the CO_2 production constant (300 mmol/h) by body surface area, calculated according to Gehan and George (18).

Gas chromatographic analysis of acetone: Triplicate 0.3 ml samples of breath collected into gastight syringes were injected directly on to a capillary gas chromatograph equipped with a flame ionization detector (Agilent model 6890, Palo Alto, CA) and 30 m DB-WAX column (0.25 mm i.d.; Agilent J&W Scientific Santa Clara, CA). The temperature of the oven was set at 30°C and held for one minute and then increased at a rate of 5°C/min to 60°C where it was held for 2 min. The carrier gas was He and the flow rate was 7 mL/min. The injector temperature was 150°C and the detector temperature was 250°C. Acetone peak areas were calibrated against an aqueous acetone standard. A 0.2 mL of the aqueous standard was then injected into the gas chromatograph.

Other analyses: Plasma glucose, β-OHB, cholesterol, triacylglycerols (TG), and non-esterified fatty acids (NEFA) were measured by colorimetric assay using an automated clinical chemistry analyzer (Dimension XPand Plus, Dade Behring Inc., Newark, DE) and commercially available reagent kits from the same company, except for β-OHB (RX Daytona kit; Randox Laboratories Ltd., Antrim, UK), and NEFA (Wako Diagnostics, Richmond, VA). Insulin was analyzed by ELISA (Mercodia, Upssala, Sweden) and a microplate reader (model 3550, BioRad, Hercules, CA). ApoE genotype was analyzed at the McGill University Center for Studies in Aging (19).

Fatty acid composition of the ketogenic breakfast, MCT, and cream was analyzed by extraction of the total lipids into 2:1 chloroform/methanol with 0.02% BHT, using triheptadecanoin as the internal standard (20). The total lipids were then saponified with 1 M methanolic KOH followed by derivitization of the fatty acids to fatty acid methyl esters using 14% BF_3 methanol. Fatty acid methyl esters were analyzed using a gas chromatograph (Agilent model 6890) equipped with a 50 m BPX-70 fused capillary column (0.25 mm i.d. x 0.25 µm film thickness; J&W Scientific, Folsom, CA). Splitless injection and flame ionization detection were performed at 250°C. The oven temperature program was 50°C for 2 min, increasing to 170°C at a rate of 20°C/min, held for 15 min, increased to 210°C at a rate of 5°C/min and held there for 7 min. The inlet pressure of the carrier gas (He) was 233 kPa at 50°C. The identity of individual fatty acids was determined by comparing retention times with standard mixtures of fatty acids (NuChek 68A, 411, 455; NuChek Prep, Inc., Elysian, MN) and a custom mixture of saturated fatty acid standards.

Statistical analysis: Results are given as mean ± SEM. Comparisons during the metabolic study period are shown from baseline (time 0 h; T_0) up to 6 h later (T_6), and again 24 h later (T_{24}) for tracer oxidation. To determine if tracer oxidation differed over time or between age groups, a repeated measures two-way ANOVA was performed followed by a Bonferroni *post-hoc* test to determine where significant differences existed. The Pearson test was used to test the significance of correlations between plasma and breath metabolites. Ketogenic breakfast composition was analyzed by one-way ANOVA. Statistical analysis of tracer oxidation data, differences in ketogenic meals composition and fatty acid profile between groups, and correlations were performed with Prism software (version 4.0, GraphPad Prism, San Diego, CA). An independent variables ANOVA test for time and age was performed to determine if any of the plasma metabolites differed between age groups or by ApoE ε4 genotype. Statistical analysis of plasma metabolites was performed with SPSS software (version 12.0, SPSS Inc, Chicago, IL). Significance was set at $p \leq 0.05$.

Results

Plasma and breath metabolites: From baseline (T_0) to 6 h after taking the ketogenic breakfast drink and tracer (T_6), plasma glucose was mostly stable in all three groups but between T_3 and T_6, glucose was 12% higher in the E compared to the Y group ($p< 0.05$; **Figure 1**). In all three groups, plasma insulin peaked at 90-105 pmol/L at T_1 to T_2. Except at T_2 in the M group, the M and E groups had a similar post-prandial insulin response to the Y group. Between T_0 and T_6 and in all three groups, plasma β-OHB rose from ~0.1 to ~1.3 mmol/L and breath acetone rose from ~13 to ~87 nmol/L (**Figure 1**). Breath acetone was higher at T_6 in the M and E groups versus the Y group ($p< 0.05$). For all subjects, there was a significant positive correlation between plasma β-OHB and breath acetone at T_0 and T_6 ($p< 0.001$; **Figure 2**).

^{13}C Tracer oxidation: In all subjects and with both tracers, $^{13}CO_2$ excretion on breath peaked at 2-4 h post-dose and returned close to baseline within 24 h of tracer administration. In all three age groups, ^{13}C-glucose oxidation peaked at 6.4 to 7.4 % dose/h between $T_{2.5}$ and T_3 (**Figure 3**). At $T_{4.5}$, T_5 and T_6, ^{13}C glucose oxidation was significantly higher in the E compared to the M group ($p< 0.05$). Cumulative ^{13}C glucose oxidation 24 h after dosing was 72%, 62%, and 77% of dose for Y, M and E subjects, respectively (**Figure 3**). From T_5 to T_{24}, cumulative oxidation of ^{13}C glucose was significantly higher in the E versus M group ($p<0.05$), but not compared to the Y group. In all three groups, ^{13}C β-OHB oxidation peaked at ~7.5 % dose/h at T_2. Cumulative 24 h ^{13}C β-OHB oxidation was 65%, 74%, and 77% of the dose administered in Y, M and E subjects, respectively, with no significant differences between groups (**Figure 3**).

Other measurements: There was no significant effect of the ketogenic breakfast on plasma TG, NEFA, or total cholesterol over the 6 h study period (**Figure 4**). However, from T_3 to T_6, plasma TG and total cholesterol were significantly elevated in the E group compared to the Y group ($p< 0.05$).

Genotype distribution could only be determined for 27 of the 31 subjects (**Table 4).** For statistical comparisons, genotypes were grouped according to presence or not of the ApoE ε4 allele. As expected, ε4 carriers had significantly elevated plasma cholesterol, but had no significant differences in other metabolites (data not shown).

Discussion

Overall, we found that for 6 h after consuming a ketogenic breakfast drink, elderly, middle-aged and young adults in good health had comparable changes in plasma β-OHB and breath acetone. To our knowledge, previously published studies of ketone levels in the elderly have not reported their production after a ketogenic meal. For instance, higher plasma β-OHB was reported for the elderly, but only after an 18 h fast (21). Our study confirms the previously reported short term ketogenic effect of a very low carbohydrate breakfast (2), and shows that the healthy elderly achieve a level of ketosis (plasma β-OHB and breath acetone) and 24 h oxidation of β-OHB that is comparable to or slightly above what is observed in healthy young and middle-aged subjects. In the absence of differences in plasma β-OHB or β-OHB oxidation, whether the doubling of breath acetone at the end of the 6 h metabolic study day is physiologically meaningful remains to be determined.

Our elderly group had statistically significant but very modest differences in glucose metabolism compared to the middle-aged our young adults. Although fasting glucose was not statistically different between the three groups, plasma glucose (but not insulin) was statistically higher in the elderly towards the end of the metabolic study period. Cumulative glucose oxidation over 24 h was 24% higher in the elderly but only versus the middle-aged group; the glucose oxidation did not differ significantly between the elderly and young groups. Without further experimentation, these data are difficult to interpret because although higher plasma glucose could be due to various mechanisms related to emerging insulin resistance, one would not expect a concomitant rise in glucose oxidation (Figure 3) if, in fact, glucose metabolism was impaired.

Statistically significant differences between age groups in cholesterol and TG also emerged 3-6 h after taking the breakfast meal. Issa et al. have also reported somewhat slower TG clearance after consuming a meal containing 40 g of fat (22). Several studies have suggested that slower post-prandial clearance of an oral fat load may contribute to aging-associated pathology such as coronary heart disease (23, 24) and may be influenced by declining insulin sensitivity (25-27). Post-prandially, the plasma cholesterol response of both the M and the E groups was elevated compared to the Y group. This could be attributed to the presence of four subjects in the M group who were ApoE ε4 carriers, as this polymorphism is known to elevate cholesterol levels (28). In fact, when the ε4 carriers were removed, cholesterol data for the M group fell between the Y and E groups (data not shown).

Although baseline plasma TG was non-significantly higher in the elderly, none of the subjects showed a significant post-prandial TG response between T_0 - T_6. Given that the ketogenic breakfast contained approximately 50% LCT (**Table 3**), a post-prandial increase in plasma TG would have been anticipated. Seaton et al. found that in comparison with LCT, there was no significant change in plasma TG and even a slight decrease during the first hour after a single dose of 48 g of MCT (29). Hill et al. observed an increase in fasting TG but no change over 6 h after giving a single dose of MCT following a 6 day diet in which MCT represented 40% of daily energy requirements (30). MCT are clearly absorbed differently from LCT but, in our study, it is still not clear whether MCT or the low carbohydrate content of the meal could have suppressed the plasma TG response to the LCT in the cream.

By design, the ketogenic breakfast given to our subjects was not strictly isoenergetic across groups. Rather, using the Harris-Benedict equation, the energy content of the ketogenic breakfast was calculated in terms of percentage of basal

energy needs, which takes into account several parameters including gender, age, and anthropometric parameters. Other methods to match meals across groups with different anthropometry include normalizing to only one parameter such as fat in the meal to body weight, BMI, or hip-to-waist ratio. Recent studies suggest a stronger relation of parameters such as insulin resistance to body fat mass rather than to age itself (31, 32). As such, determining % body fat distribution, fat mass, or indirect calorimetry for energy use might have helped us more accurately compare subjects. Regardless, neither the calculated values for basal energy expenditure nor the total fat content (g), MCT content (g), fat content/body weight (g/kg), or fat content/BMI ($g/kg/m^2$) differed significantly between the three age groups ($P>0.05$).

Another possible limitation to this study was the number of subjects included. Due to insufficient published data implicating the parameters under study, power calculation was not performed. Hence our data must be considered preliminary. However, in similar published research evaluating metabolism in the healthy elderly (12), we anticipated that 8-13 subjects per group was likely to be sufficient to determine whether significant differences would be likely to be observed.

Our main objective was to assess the short-term ketone response to a ketogenic breakfast during healthy aging and we conclude that the ability to produce ketones appears to be fully functional during healthy aging. Hence, these results support emerging strategies aiming to use physiological levels of ketones to correct or bypass deteriorating brain glucose uptake in the elderly.

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Table 1

Anthropometric characteristics and fasting plasma constituents.

	Young (n = 11)	Middle-aged (n = 12)	Elderly (n = 9)
Anthropometry:			
Age (y)	23 ± 1	50 ± 1	76 ± 2
Height (m)	1.74 ± 0.03	1.65 ± 0.03	1.67 ± 0.08
Weight (kg)	77.4 ± 4.9	74.2 ± 4.6	72.3 ± 3.7
BMI (kg/m^2)	25.3 ± 1.1	27.2 ± 1.6	25.7 ± 1.3
Fasting plasma measures:			
β-Hydroxybutyrate (mmol/L)	0.07 ± 0.10	0.09 ± 0.13	0.07 ± 0.04
Glucose (mmol/L)	5.4 ± 0.6	5.3 ± 0.4	5.7 ± 0.7
Insulin (mUI/L)	6.8 ± 4.4	4.5 ± 3.9	4.0 ± 2.6
Triacylglycerol (mmol/L)	0.9 ± 0.3	1.1 ± 0.5	1.5 ± 0.5
Non-esterified fatty acids (mmol/L)	0.6 ± 0.3	0.5 ± 0.1	0.6 ± 0.2
Cholesterol (mmol/L)	4.2 ± 0.4	5.3 ± 1.1	5.3 ± 0.7

Mean ± SEM. No significant difference in any parameter except age ($P<0.0001$).

Table 2

Ketogenic breakfast meal composition[1]

	(g)	(%)
Components:		
protein powder	25 ± 1	10
cream	100 ± 0	41
medium chain triacylglycerol	71 ± 4	29
water	46 ± 2	20
Macronutrients:		
protein	25 ± 1	18
carbohydrate	3 ± 0	2
fat	110 ± 4	80

[1] Calculated to give a ratio of 4.5:1 parts fat to protein plus carbohydrates based on 1/3 of the subject's daily protein requirements according to basal energy expenditure. Meal components and macronutrients are given as mean ± SEM (n = 32). Meal content did not differ significantly between age groups.

Table 3

Fatty acid composition (%) of the ketogenic breakfast and its fat components[1]

	Breakfast n = 32	MCT n = 3	Cream n = 3
8:0	14.4 ± 1.5	39.8 ± 0.4	N/D
10:0	31.3 ± 0.8	58.6 ± 0.3	5.9 ± 0.1
12:0	4.0 ± 0.1	1.6 ± 0.1	8.9 ± 0.1
14:0	9.8 ± 0.4	N/D	21.9 ± 0.2
16:0	20.4 ± 0.7	N/D	31.9 ± 0.1
18:0	4.9 ± 0.3	N/D	6.5 ± 0.2
Total Saturates	**84.7 ± 1.3**	**100.0 ± 0**	**75.0 ± 0.2**
14:1n-5	1.3 ± 0.6	N/D	2.3 ± 0.0
16:1n-7	1.0 ± 0.1	N/D	2.4 ± 0.1
18:1n-9	11.0 ± 0.6	N/D	18.1 ± 0.2
Total Monounsaturates	**14.0 ± 0.6**	**N/D**	**22.8 ± 0.2**
18:2n-6	1.1 ± 0.2	N/D	2.3 ± 0.1
Total Polyunsaturates	**1.1 ± 0.2**	**N/D**	**2.3 ± 0.1**

[1] Meal composition, given as mean ± SEM. Meal energy content did not differ significantly between age groups. N/D = not detected.

Table 4.

Apolipoprotein E genotype of the subjects.

	2/2	3/2	3/3	4/3	4/4	4/2	total
Young	0	4	5	0	0	0	9
Middle-aged	1	3	3	3	0	1	11
Elderly	0	0	6	1	0	0	7
% Frequency	4	26	51	15	0	4	100

Apolipoprotein E genotype is shown as the combinations of Apolipoprotein E ε 2, 3, or 4 variant alleles.

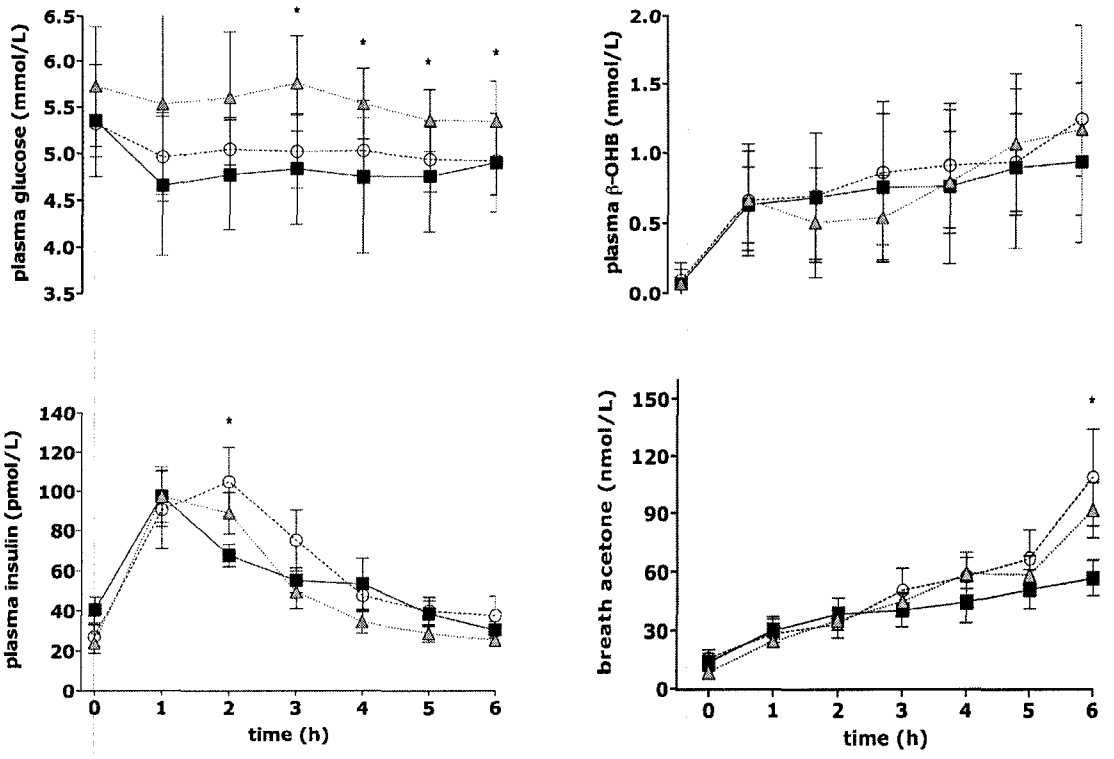
plasma glucose (mmol/L)
6.5
6.0
5.5
5.0
4.5
4.0
3.5
plasma β-OHB (mmol/L)
2.0
1.5
1.0
0.5
0.0
plasma insulin (pmol/L)
140
120
100
80
60
40
20
0
breath acetone (nmol/L)
150
120
90
60
30
0
0 1 2 3 4 5 6
time (h)
0 1 2 3 4 5 6
time (h)

Figure 1.

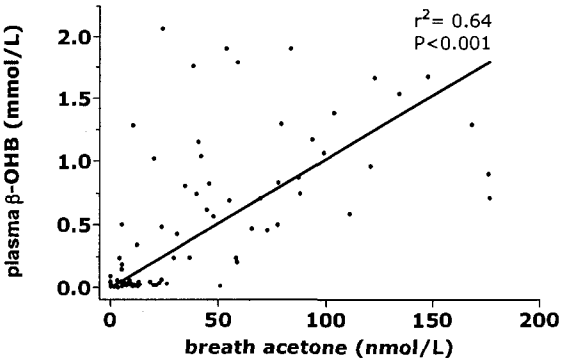
r^2= 0.64
P<0.001
plasma β-OHB (mmol/L)
2.0
1.5
1.0
0.5
0.0
0
50
100
150
200
breath acetone (nmol/L)

Figure 2.

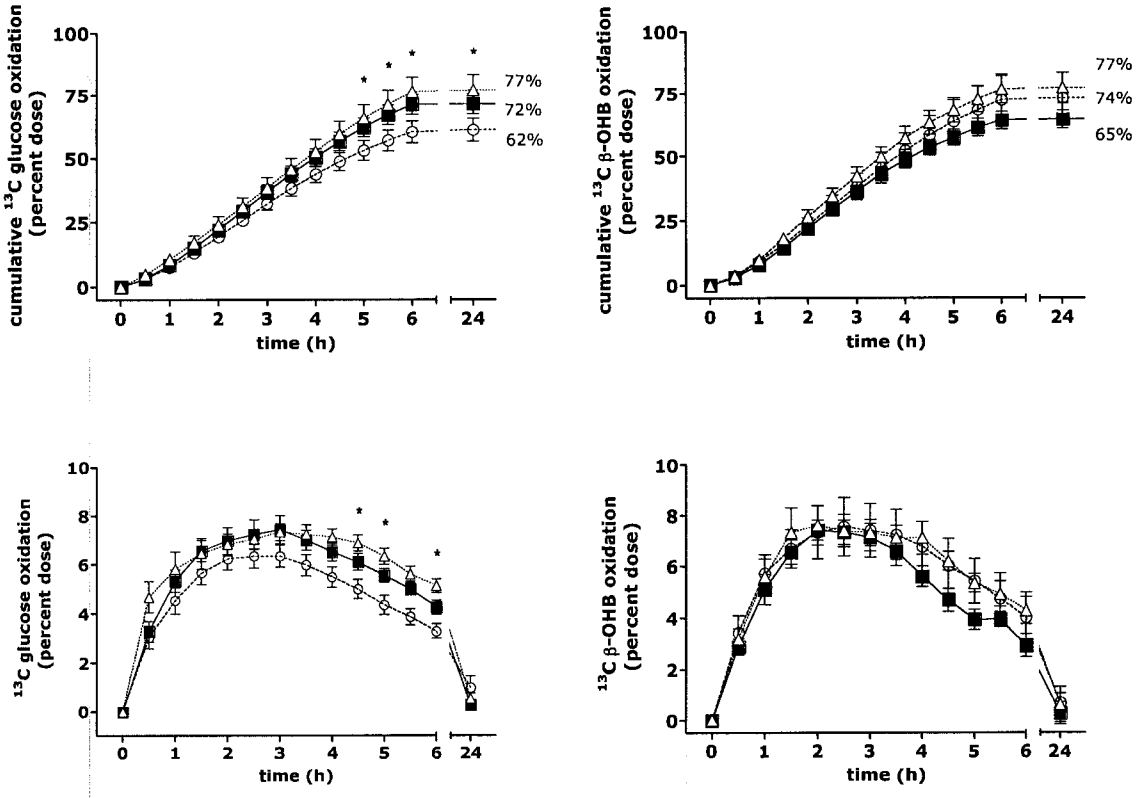
cumulative ^{13}C glucose oxidation (percent dose)
100
75
50
25
0
0 1 2 3 4 5 6 24
time (h)
77%
72%
62%
cumulative ^{13}C β-OHB oxidation (percent dose)
77%
74%
65%
^{13}C glucose oxidation (percent dose)
10
8
6
4
2
0
^{13}C β-OHB oxidation (percent dose)

Figure 3.

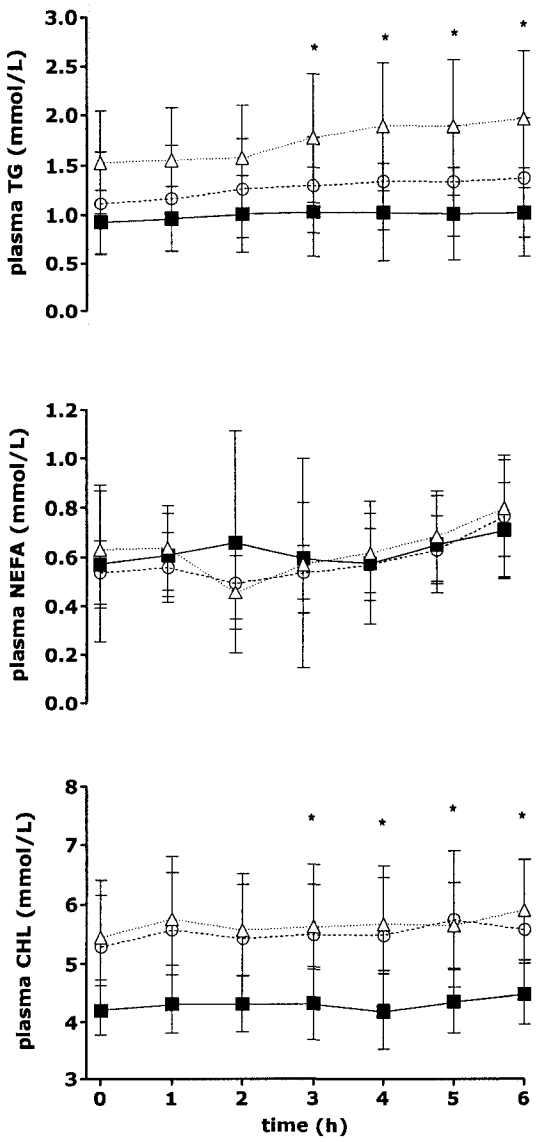
plasma TG (mmol/L)
3.0
2.5
2.0
1.5
1.0
0.5
0.0
plasma NEFA (mmol/L)
1.2
1.0
0.8
0.6
0.4
0.2
0.0
plasma CHL (mmol/L)
8
7
6
5
4
3
0
1
2
3
4
5
6
time (h)

Figure 4.

Figure Legends

Figure 1.

Plasma glucose (upper left), insulin (lower left), β-hydroxybutyrate (upper right), and breath acetone (lower right) over 6 h following consumption of a ketogenic breakfast at time 0 (mean ± SEM; *P<0.05). Symbols represent young (■), middle-aged (○) and elderly (▲) subjects.

Figure 2.

Correlation between breath acetone and plasma β-hydroxybutyrate before and 6 h after consuming a ketogenic breakfast.

Figure 3.

Oxidation of ^{13}C glucose (lower left - % dose/h; upper left – cumulative oxidation/24 h) and ^{13}C β-hydroxybutyrate (lower right - % dose/h; upper right – cumulative oxidation/24 h) following consumption of a ketogenic breakfast and the respective tracer at time 0 (mean ± SEM; *P<0.05). Symbols represent young (■), middle-aged (○) and elderly (▲) subjects.

Figure 4.

Plasma triacylglycerols (TG), non-esterified fatty acids (NEFA), and cholesterol (CHL) over 6 h following consumption of a ketogenic breakfast at

time 0. Symbols represent young (■), middle-aged (○) and elderly (▲) subjects (mean ± SEM; *P<0.05).

Discussion and conclusions

Brain energy substrate use is a very important part of the functioning of the brain as an organ. This is evident given how many regulatory feedback controls over cellular energy supply and alternative pathways exist in the brain, and the preferential diversion of energy substrates to brain tissue by the body during environmental stressors. Glucose and ketones account for the majority of the brain energy supply, and are also very important in energy reserves. The oxidation of glucose has been implicated in age-related disorders, in particular in cognitive disorders, such as Alzheimer's disease.

Aging is characterized by a decrease in the number of cells in the body due to a combination of increased cell death and decreased cell division. There are several cellular theories on aging. The telomerase hypothesis states that cells have a finite predetermined lifespan and that with every round of cell division, the telomeres (segments on the ends of DNA) get shortened until it begins to cut into the genetic code and can no longer replicate, cell division cannot occur. The free radical hypothesis posits that the free radicals produced during oxidative metabolism and other reactive oxygen species damage macromolecules in the cell, that accumulate and either impair cell functioning or trigger apoptosis (VANDER *et al.,* 2001).

The physiological manifestations of aging are a general gradual deterioration in the function of tissues and organ systems and in the capacity of the body's homeostatic control systems to respond to environmental stressors (VANDER *et al.,* 2001). Aging, though, is not a disease and must be studied distinct from disease states. It is an element of the physiological process of human development.

The overall purpose of this thesis was to better understand the utilization of glucose and ketones as energy substrates. Glucose and ketones can be used as an energy substrate in both the body and the brain. While ketones show potential to alleviate deficits seen in a decline in glucose availability, such as cognitive deficits, the efficacy of inducing mild ketosis to increase brain energy availability requires further exploration. Specific objectives of this research project were to evaluate the oxidation of glucose and ketones in the human body, under conditions of mild, acute ketosis in healthy subjects. This was achieved by administering in 30 healthy young, middle-aged, and elderly subjects a nutritional intervention designed to induce ketosis and then evaluating the oxidation of glucose and ketones using carbon-13 stable isotope tracers for glucose and β-OHB in two separate 6 hour study days. Levels of glucose, acetone, β-OHB, cholesterol, triacylglycerol, insulin and non-esterified fatty acids were also measured. The results of the research project in this thesis indicate that healthy elderly are as

capable as of producing and oxidizing ketones as healthy young subjects. This illustrates the fact that aging must be studied not viewed in terms as pathology but as a physiological process. Furthermore, when studying age-related disorders and trying to understand age-related pathology, it is recommended to assess pathology in comparison to an elderly population of healthy subjects, it may not be adequate to use a control group of adult subjects.

The question of energy substrate use and production in the brain is far from answered. However, the results of this project are very encouraging. They indicate that if ketones could be used as an energy substrate to offset declining glucose use in the brain, there is no peripheral systemic impediment in the elderly to suggest that they cannot produce the ketones necessary to be available to the brain.

From this, it will be important to determine if there are any differences in brain uptake of ketones in elderly. This is now possible due to the development by our laboratory of a new carbon-11 tracer for acetoacetic acid (TREMBLAY *et al.,* 2007). Research is now underway to determine using this tracer if there is any change in brain uptake of ketones using positron emission tomography. Preliminary experiments will focus on brain uptake of ketones in rats given a ketogenic diet. This will help elucidate if there are

changes in ketone uptake during ketosis. Subsequent research will involve studying brain ketone uptake in persons with cognitive deficits.

Also, there will be a need to examine methods and techniques of inducing ketosis in a safe and efficient manner. Several projects are in development or in progress to help address the issue of inducing ketosis. These include supplements and metabolism of eicosapentaenoic acid (EPA) as a ketogenic substrate in both young and elderly subjects. Preliminary results of a supplement study shows that healthy elderly have a similar response of plasma EPA and Docosahexaenoic acid (DHA) to omega-3 supplementation, indicating no physiological change in omega-3 fatty acid metabolism during healthy aging (VANDAL *et al.,* unpublished data). Although the role of omega-3 in cognitive disorders is still controversial (PLOURDE *et al.,* 2007), evidence indicates that EPA may be an important constituent in augmenting ketone production, likely by activation of the peroxisome-proliferator-activated receptor α (PPAR α) (CHAMBRIER *et al.,* 2002). Further research is in progress to determine if direct stimulation of PPAR production by a class of pharmaceutical agents known as fibrates may also result in increased ketone levels.

These studies will provide, in the coming years, clarification of this very exciting and promising line of research into alternative brain energy substrates and hopefully will ultimately help develop a better treatment for the devastating problem of cognitive dysfunction in elderly.

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Appendix

I Authorization form to include Freemantle E, et al. Omega-3 fatty acids, energy substrates, and brain function during aging. Prostaglandins Leukot Essent Fatty Acids 2006;75:213-20.

II Authorization form to include Freemantle E, et al. Metabolic response to a ketogenic breakfast in the healthy elderly. Submitted to Am J Clin Nutr. *4 Dec 2007.*