EFFECTS OF A MEDIUM CHAIN TRIGLYCERIDE OIL MIXTURE AND ALPHA LIPOIC ACID DIET ON BODY COMPOSITION, ANTIOXIDANT STATUS AND PLASMA LIPID LEVELS IN THE SYRIAN HAMSTER

STEPHANIE WOLLIN

School of Dietetics and Human Nutrition
McGill University
Montréal, Canada

A thesis submitted to the Faculty of Graduate Studies and Research
In partial fulfillment of the requirements of the degree of
Master of Science

March 2003

Your file Votre référence
ISBN: 0-612-88329-9
Our file Notre référence
ISBN: 0-612-88329-9

In loving memory of my Grandfather,
Dr. Delbert Wollin

ABSTRACT

The objective of this study was to examine the effects of a medium chain triglyceride oil mixture (MCTo), designed to increase energy expenditure and improve lipid profiles containing medium chain triglycerides, phytosterols and n-3 fatty acids in the form of flaxseed oil, versus the antioxidant α-lipoic acid (ALA). Forty-eight hamsters were fed (i) hypercholesterolemic (HC) control, (ii) HC MCTo, (iii) HC ALA, (iv) HC MCTo/ALA diets for 4 weeks. No effects on food intake, body weight, total body water, lean body mass, fat mass, and tissue thiobarbituric acid-reactive substances (TBARS) were observed. ALA alone had no effect on total cholesterol (TC); however, MCTo feeding increased TC with ($p<0.03$) and without ($p<0.003$) ALA when compared to control. ALA increased HDL levels compared to control ($p<0.04$) and MCTo/ALA ($p<0.007$) groups. MCTo, with ($p<0.0001$) or without ($p<0.006$) ALA, increased non-HDL cholesterol levels versus control. The non-HDL:HDL ratio was decreased by ALA compared to MCTo (45%) and MCTo/ALA (68%) ($p<0.0001$), a similar trend was seen when compared to the HC control (22%) group ($p<0.14$). Triglyceride levels were not altered by any of the dietary treatments. Liver and heart tissue reduced glutathione (GSH) was increased ($p<0.05$) by all three treatments when compared to control. Both tissues showed an increase ($p<0.05$) in oxidized glutathione (GSSG) when fed ALA compared to all other treatments. Hamsters fed ALA had a lower ($p<0.05$) GSH/GSSG ratio compared to all treatment groups. In conclusion, MCTo feeding does not elicit beneficial effects on circulating plasma lipids and measures of body composition. In addition, our results do not clearly support an improvement in oxidative status through supplementation of ALA. However, our results do support the existence of beneficial effects of ALA on circulating lipoprotein content in the hamster.

RESUME

Un mélange d'huile à base de triglycérides à chaine moyenne (TCM) contenant des phytosterols et des acides gras polyinsaturés n-3 provenant de l'huile de lin concu pour augmenter la dépense énergétique et améliorer le profil lipidique sanguine a été compare à l'antioxydant acide alpha-lipoïque (ALA). Quarante-huit hamsters ont recu un des régimes suivants pendant quatre semaines: (i) regime hypercholestérolémiant contrôle (HC), (ii) hypercholestérolémiant avec TCM (HC TCM), (iii) hypercholestérolémiant avec ALA (HC ALA) et (iv) hypercholestérolémiant contenant un mélange de TCM et ALA (HC TCM/ALA). Aucun effet sur la consommation alimentaire, le gain de poids, la masse d'eau corporelle, la masse maigre, la masse adipeuse et les niveaux de substances réactives à l'acide thiobarbiturique n'a été détecté. Le régime ALA n'a eu aucun effet sur les niveaux de cholesterol total (CT). Par contre, lorsque comparé au groupe contrôle, le régime contenant des TCM a entraîné une augmentation du CT et ce, qu'il soit combiné ($P<0.03$) ou non ($P<0.003$) avec ALA. Le régime ALA a entraîné une augmentation des concentrations de lipoprotéines de densité élevée (HDL) comparativement au groupe contrôle ($P<0.04$) ainsi qu'au groupe TCM/ALA ($P<0.07$). Les concentrations des cholestérol non-HDL (C non-HDL) ont été augmentées par les régimes TCM avec ($P<0.0001$) ou sans ($P<0.006$) ALA comparativement au régime contrôle. Le rapport C non-HDL/C-HDL a été diminué par le régime ALA en comparaison des régimes TCM (45%) et TCM/ALA (68%) ($P<0.0001$). Une tendance semblable a été observé dans le groupe contrôle (22%) ($P<0.14$). Les différents traitements n'ont pas affecté les concentrations de triglycérides. Une augmentation du gluthation réduit (GSH) a été mesurée dans les tissus cardiaques et hépatiques chez les animaux nourris avec les trois régimes expérimentaux comparativement au régime contrôle ($P<0.05$). Par ailleurs, une plus grande concentration de gluthation oxydé (GSSG) a été observée dans les mêmes tissus chez le groupe ALA ($P<0.05$). Le rapport GSH/GSSG était plus faible chez les hamsters nourris au ALA que chez les autres groupes ($P<0.05$). En conclusion, le régime TCM n'a eu aucun effet bénéfique sur les concentrations de lipides plasmatiques ni sur la composition corporelle. Par ailleurs, les résultats portant sur le statut oxydatif du groupe ALA n'étaient pas concluants. Par contre, nos résultats confirment les effets bénéfiques de ALA sur le profil lipoprotéique chez le hamster.

ACKNOWLEDGEMENTS

I would like to extend a heart felt thanks to any and all, whom I met during the pursuit of my Master's degree. It has been a one of a kind journey and all will not be forgotten.

I offer special thanks to Dr Peter J.H. Jones for his support, patience and encouragement over the last 2 years. You have provided me with opportunities I never new were possible, and for this I am truly grateful.

Dr. Stan Kubow and Dr. Jeffrey Cohn your input in preparation of my proposal, presentations and thesis have been priceless. You both have helped me to understand and appreciate the necessity for careful, detailed planning and execution of scientific research. I also acknowledge the sharing of information and laboratory skills received from Dr. Kubow's lab group mainly, Johanne Poirier and Jenny Eng.

I sincerely thank Dr. Yawen Wang and Dr. Mahmoud Raeni-Sarjaz for your incredible advice, patience, and time in helping me learn and understand the statistical analysis. In addition, sharing a lab with you both was an enlightening and incredibly positive experience.

While carrying out the experimental phase of this study I was blessed to have the help and companionship of the following individuals. Chris Vanstone a million thank-you's could not express my gratitude for having your help, support, and your friendship. Gordon Bingham, Mia Zapata, and Lindsay MacKinnon your help looking after my furry creatures was more than appreciated.

To all the faculty and administrative staff, your kind spirits helped make my experience at McGill a memorable one. Of special mention are, Francine Tardif, Lise Grant, Judy Campbell, Katherine Gray-Donald, and David Bissonnette.

Lindi Sibeko, you were my mountain of strength and everlasting encouragement. Your kind words kept me going even in the darkest of hours.

I cannot forget all the smiling and laughing faces with whom I have spent many moments with. Whether we were sharing the trials and tribulations of science, stories, food and/or drink, I remember having a great time. Hats off to all of you..... Dina Spigelski, Catherine Vanstone, Christine Bourque, Marie-Pierre St-Onge, Geoff Hynes, Makiko Yoshida, Naoyuki Ebine, Melanie Journoud, Vivian Lau, Emma Yin, Patrick Owen, Uma Palaniappan, Romaina Iqbal, Tanya Verral, Karine Lipsett, Marisa LeBlanc, Heidi Wilk, and Bonnie Belfer.

Last but not least, I must thank my family! For it is you that enabled me to be where I am today. Mom, Dad, and Taras I share this moment with each of you.

Experience is a hard teacher. She gives you the test first and the lessons afterwards.
Anonymous

TABLE OF CONTENTS

LIST OF FIGURES

LIST OF TABLES

CONTRIBUTION OF AUTHORS

The first manuscript included as part of this thesis is entitled "Alpha-Lipoic Acid in Health and Disease." As first author of this review, I was responsible for selecting appropriate research material to include, writing, formatting and creation of the figure. As my supervisor, Dr. Peter J.H. Jones provided general guidance and editorial assistance.

The second manuscript included as part of the thesis is entitled "Effects of a medium chain triglyceride oil mixture and alpha-lipoic acid diet on body composition, antioxidant status, and plasma lipid levels in the Syrian hamster" and represents the experimental portion of the thesis. As first author, I was the sole researcher responsible for all study requirements including ordering supplies and animals, animal care, sample collection, sample storage, plasma lipid analysis, antioxidant assays, and body composition analysis. Dr. Yawen Wang assisted my statistical analysis. Dr. Stan Kubow provided the protocols for the antioxidant measures and the use of his spectrophotometer. Dr. Kubow and Dr. Wang both offered editorial advice on this manuscript. As my supervisor and principal investigator of the study Dr. Peter J.H. Jones wrote the grant proposal to obtain funding for this project and supervised my work regularly over the course of 2 years. In addition, he provided editorial assistance on the manuscript.

1. OVERVIEW

Diseases of the heart and blood vessels, collectively known as cardiovascular disease (CVD), are the leading cause of death in Canada (Health Canada, 1997). Primary risk factors for CVD are obesity, diabetes, hypertension, elevated blood cholesterol levels, and oxidative stress. In an attempt to combat these risk factors, science has turned to the investigation of bioactive substances that may offer protection to the cardiovascular system.

Several studies suggest that oxidative stress plays a significant role in the pathogenesis of atherosclerosis (Westhuysen 1997, Steinberg et al., 1997, Quinn et al. 1987). Therefore, in formulating a combination of bioactive components to combat CVD, a powerful antioxidant, alpha-lipoic acid (ALA) was used. ALA has been shown to protect LDL cholesterol from in vivo oxidation (Packer et al., 1995; Kagan et al., 1992; Marangnon et al., 1999; Lodge 1998). Levels of other functional antioxidants such as, vitamins C and E and glutathione have also been shown to be increased via recycling through supplementation with ALA (Packer et al. 1995, Busse et al. 1992, Hagen et al. 1999). Apart from the antioxidant functions of ALA, effects of ALA on plasma lipid profiles in animals have also been examined yielding inconclusive results. Early studies in the 1970's and 1980's have shown the capacity of ALA to decrease serum total cholesterol in rabbits (Ivanov, 1974) and atherosclerosis in quail (Shih, 1983). In contrast, more recent research has reported no significant effects of ALA supplementation on cholesterol levels (Ford et al., 2001; Segermann et al., 1991; Marangnon et al., 1999)

Medium chain triglycerides (MCT) have been shown to be more easily absorbed in the intestinal lumen compared to long chain triglycerides (LCT) (Caspary, 1992). MCT

also differ from LCT in that they are transported directly to the liver via the portal vein and thus do not pass the adipose tissue prior to hepatic disposal. These characteristics are thought to be responsible for the different rates of fat oxidation for MCT versus LCT. In addition, MCT have been shown to undergo increased oxidation in both animal (Leyton et al., 1987; Johnson et al., 1990) and human studies (Mascioli et al., 1989; Hill et al., 1990; Binnert et al., 1998). These reports of increased oxidative capacity have made MCT appealing as a possible adjunct for the treatment of obesity, however, MCT have also been shown to have deleterious effects on the blood lipid profile causing their use to be less desirable. There is strong evidence in the literature to suggest that MCT increase circulating triglyceride levels (Hill et al., 1990; Swift et al., 1992; Tsai et al., 1999). In addition, MCT have also been shown to increase circulating LDL cholesterol levels (Tsai et al., 1999; Cater et al., 1997). However, some studies have obtained different results demonstrating little effect of MCT on plasma triglycerides (Hainer et al., 1994) and improvements in plasma LDL and total cholesterol (TC) levels (Nicollosi et al., 1998; Geelen et al., 1995; Woollett et al., 1992).

With the existing knowledge of possible negative effects of MCT feeding on blood lipids, the concept of combining MCT with phytosterols and n-3 fatty acids to negate negative effects is provocative. Plant sterols have been shown to decrease both plasma total (Gylling and Miettinen, 1999; Miettinen et al., 1995) and LDL cholesterol (Pelltier et al., 1995; Jones et al., 1999) without significant alterations in plasma HDL cholesterol and triglyceride concentrations. Phytosterols are known to elicit these actions through inhibition of dietary cholesterol absorption from the intestine (Jones et al., 1995). In addition, supplementation with alpha-linolenic acid, in the form of flaxseed oil has

been shown to increase tissue eicosapentanoic (EPA) concentrations in vivo (Mantzioris et al., 1994). EPA is thought to be one of the components responsible for the capacity of fish oils to decrease plasma triglyceride levels (Harris, 1997). Alpha-linolenic acid feeding has been shown to decrease plasma triglyceride levels by 22-24% in humans (Singer, 1992). These results support the rationale for the combined feeding of phytosterols and flaxseed oil in an attempt to temper increases in plasma cholesterol and triglyceride levels caused by MCT feeding.

The MCT oil mixture (MCTo) examined in this thesis combines the antioxidant potential of ALA, the increased energy expenditure of MCT, the cholesterol lowering capabilities of phytosterols, and the hypotriglyceridemic properties of n-3 fatty acids. This oil mixture has been developed to offer protection and improvement of CVD risk factors.

2. LITERATURE REVIEW

2.1 Manuscript 1:

Alpha-Lipoic Acid in Health and Disease

Stephanie D. Wollin, Peter J.H. Jones

School of Dietetics and Human Nutrition

McGill University, Ste-Anne-de-Bellevue, Québec, Canada

Address correspondence to:

Peter J.H. Jones, Ph.D.
School of Dietetics and Human Nutrition
McGill University
21,111 Lakeshore Road
Ste-Anne-de-Bellevue, Québec, Canada, H9X 3V9
Phone: (514) 398-7841
Fax: (514) 398-7739
E-mail: jonesp@macdonald.mcgill.ca

Running head: Alpha-lipoic acid, cardiovascular disease, and diabetes mellitus

To be submitted to the Journal of Nutrition

2.1.1 **Abstract**

Alpha-lipoic acid (ALA) has been identified as a powerful antioxidant that is found naturally in our diet but appears to have increased functional capacity when supplemented as a natural or synthetic isolate. α-Lipoic acid and its active reduced counterpart dihydrolipoic acid (DHLA) have been shown to combat oxidative stress by quenching a wide variety of reactive oxygen species (ROS). Since the molecule is soluble in both aqueous and lipid portions of the cell, its biological functions are not limited solely to one environment over another. In addition to ROS scavenging, ALA has been shown to be involved in the recycling of other antioxidants in the body including vitamins C and E and glutathione. Not only have the antioxidant qualities of this molecule been studied, but there are also several reports pertaining to blood lipid modulating characteristics, protection against LDL oxidation, and modulation of hypertension. Therefore, ALA represents a possible protective agent against some of the key risk factors of cardiovascular disease (CVD). Furthermore, ALA supplementation is already used in Germany as an adjunct to diabetic therapy, because of overwhelming support for its role in modulating blood glucose concentrations and alleviating the symptoms of diabetic neuropathies. The objective of this review is to examine literature pertaining to ALA in relation to the aforementioned chronic disease states and describes the most powerful actions and potential uses of this naturally occurring antioxidant. Despite the numerous studies on ALA, many questions remain relating to ALA supplementation. There remains no consensus on dosage, dose frequency, form of administration, and/or form of ALA. However, collectively the literature aids in the

understanding of potentials uses for ALA supplementation and identifies key areas open to future research.

2.1.2 Introduction

Alpha-lipoic acid (ALA) is a natural compound. Its chemical name is 1, 2-dithiolane-3-pentanoic acid designated: $C_8H_{14}O_2S_2$. It is also commonly referred to as "thioctic acid" (Busby, 1999). In humans ALA is synthesized by the liver and other tissues, where it functions as a cofactor within multi-enzyme dehydrogenases i.e. pyruvate dehydrogenase and alpha keto-glutarate dehydrogenase (Schmidt et al., 1994). More recently ALA has been shown to be required for the oxidative decarboxylation of pyruvate to acetyl-CoA, the critical step bridging the gap between glycolysis and the citric acid cycle (Reed, 1998). α-Lipoic acid is recognized to be both water and fat soluble, and therefore it is distributed widely in plants and animals in both cellular membranes and the cytosol (Wada, 1997). In addition to these co-enzymatic roles, ALA and its reduced dithiol form dihydrolipoic acid (DHLA) are powerful antioxidants whose functions have been described by Biewenga et al. (1997a) to include: 1) quenching of reactive oxygen species, 2) regeneration of exogenous and endogenous antioxidants such as vitamins C and E, and glutathione, 3) chelation of metal ions, and 4) reparation of oxidized proteins. In most cells containing mitochondria, ALA is reduced by an NADH-dependant reaction with lipoamide dehydrogenase to form DHLA. In addition, in cells that lack mitochondria ALA can be reduced to DHLA via NADPH in concert with glutathione and thioredoxin reductases (Jones et al., 2002).

Recent studies suggest that oxidative stress plays a significant role in the etiology of CVD as well as in disease processes related to diabetes mellitus. Related research

investigates approaches that reduce oxidative stress through supplementation with antioxidant compounds, hence the interest in powerful antioxidants such as ALA. The main objective of this review is to examine animal and human research in order to critically define plausible health benefits of ALA supplementation, and assess possible mechanisms of action by which they manifest themselves with regard to CVD risk factors and diabetes mellitus.

2.1.3 Properties of Alpha-Lipoic Acid

Environmental oxygen which fuels biological systems has the capacity to orchestrate biochemical reactions essential for the production of energy, as well as enable the synthesis of biologically essential compounds. However, this powerful molecule also has the capacity to exert toxic effects on aerobic organisms. The name given to these potentially toxic molecules is reactive oxygen species (ROS). Reactive oxygen species have the capacity to attack lipids, sugars, proteins and DNA, which induce their oxidation. Such events can lead to deterioration of food, dysfunction of membranes, protein modification, enzyme inactivation, breaking of DNA strands, and modification of DNA base sequences (Papas, 1998). Current understanding of the actions of ROS suggests a causative role in chronic diseases including heart disease, cancer, diabetes, and more generally aging. Consequently, the role of antioxidants and their ability to protect biological systems from oxidative damage has received increased attention. Hence, ALA has gained much interest over time because it is known to scavenge hydroxyl radicals, singlet oxygen, hydrogen peroxide, hypochlorous acid, peroxynitrite and nitric oxide. In addition, DHLA also quenches peroxyl and superoxide radicals, thus making the

ALA/DHLA redox couple one of the most powerful biological antioxidant systems (Moini et al., 2002). Furthermore, these two molecules exert additional antioxidant actions through chelation of copper, iron, and other transitional metals (Packer, 1995). ALA occurs naturally in the human diet and is found in abundance in animal tissues with high metabolic activity such as heart, liver, kidney, and to a lesser extent in fruits and vegetables (Packer et al., 2001). Vegetable concentrations are from highest to lowest in: spinach, broccoli, tomato, garden pea, brussel sprouts, and rice bran. All ALA supplied by the diet is transported in the bloodstream to tissues and incorporated into cells.

Once part of the cell, ALA must be translocated into the mitochondria where ALA-requiring enzyme complexes exist (Morikawa et al., 2001). ALA of animal origin is absorbed as lipoyl-lysine, due largely to the fact that digestive proteolytic enzymes do not cleave the peptide bond between the two effectively. In addition, ALA can be obtained through de novo synthesis via lipoic acid synthase originating from the fatty acid octanoic acid and cysteine within the mitochondria (Biewenga et al., 1997b; Morikawa et al., 2001; Marquet et al., 2001). Morikawa et al. (2001) comment that in mammals, ALA is not sufficiently supplied by the diet and therefore de novo synthesis takes place in the heart, liver and testis to form ALA needed for purposes such as incorporation into ALA-requiring enzyme complexes. Hence, these sources of ALA are thought to provide very little free ALA into circulation. It is only through supplementation that ALA reaches potentially therapeutic levels within the body.

Studies have reported therapeutic doses in humans ranging from 200-1800 mg of ALA/day (Marangon et al., 1999; Ziegler et al., 1995; Hermann et al., 1996; Rahnau et al., 1999; Teichert et al., 1998; Evans and Goldfine, 2000). In humans, Hermann et al.

(1996) found that the half-life of racemic ALA in plasma was 30 minutes. The authors presumed that the liver was responsible for the elimination of ALA, and that the bioavailability can range from 20-38% depending on the isomer *R* or *S* and the formulation tested. ALA exists as two different enantiomers: the biologically active (R)-isomer, and the (S)-isomer, the latter being part of the synthetic racemic mixture but found minimally in biological tissues (Estrada et al., 1996).

α-Lipoic acid's range in bioavailability leads to differences in detrimental effects after oral dosing when compared to inter-peritoneal (IP), subcutaneous, and intravenous (IV) administration. The oral LD_{50} for rats and mice is 1 130 mg/kg and 502 mg/kg respectively as compared to an IP LD_{50} of 200 mg/kg for rats and 160 mg/kg for mice (Beiwenga et al., 1997a). Extrapolation of these data would lead to the assumption that human beings can tolerate several grams of ALA given orally (Beiwenga et al., 1997a).

After absorption into cells of various tissues, including the brain, ALA is reduced to its dithiol form, DHLA. Dihydrolipoic acid can then be easily transported out of the interior of the cell and function effectively in the extracellular space (Kagan et al., 1992). It is in this form, that the molecule is believed to possess its greatest antioxidant potential. Kagan et al. (1992) reported that DHLA/ALA has a redox potential of –0.32 V compared to GSH/GSSG couple at –0.24 V. This difference establishes that DHLA has greater reducing potential within the cell and therefore could offer more protection from oxidative damage than glutathione, a well-established cellular protector.

Schupke et al. (2001) have identified a series of ALA metabolites which may offer some level of protection within cellular systems. These metabolites of ALA are produced via β-oxidation of ALA's pentanoic side chain. Some of the principal

metabolites are 3-methoxylipoic acid, 3-ketolipoic acid, and bisnorlipoic acid (Schupke et al., 2001). The complete β-oxidation of ALA, has been shown experimentally by Harrison and McCormick (1974). Knowing that CO_2 is a product of substrate metabolism, produced by the degradation of acetyl-CoA via the TCA cycle, these researchers administered ^{14}C-lipoic acid to rats. Results indicated that 25% of the administered dose was found to be exhaled as $^{14}CO_2$ within 2 hours following administration, reaching a total amount of 30% after 24hours. The authors then concluded that approximately 60% of the ALA dose had been metabolized via β-oxidation. Biewenga et al. (1997a) also confirmed β-oxidation in humans by measuring the appearance of the metabolite bisnorlipoic acid in plasma. Peak concentrations appeared approximately 189 minutes after oral administration of 1g of (R)-lipoic acid. To date the exact functions of these metabolites are not clearly understood, however, it is believed that each of these components may contribute to potential the benefits seen during the therapeutic use of ALA (Beiwenga et al., 1997).

2.1.4 The Role of Alpha-Lipoic Acid in Antioxidant Recycling

α-Lipoic acid is also thought to possess the ability to reactivate other antioxidants, which may have been disabled by increased oxidative processes. The reduced form of ALA, DHLA, exhibits this action for exogenous antioxidants such as vitamins C (Jones et al., 2002) and E, as well as for endogenous antioxidants namely, glutathione (Packer, 1995) (**Figure 2.1.4.1**). α-Lipoic acid has a particularly significant influence on the maintenance of intra-cellular glutathione, which is one of the main endogenous antioxidants responsible for free radical scavenging in all cell types. Since glutathione is

not able to cross the cellular membrane it cannot be supplemented therapeutically. However, if it is desirable for glutathione levels to be increased during times of increased stress such as exposure to toxic substances or under disease conditions such as CVD or diabetes, then ALA can be utilized to enhance cellular production of this antioxidant (Busse et al., 1992). In experiments in which ALA was both added to cultured cells and supplemented in animals (Busse et al., 1992; Bustamante et al., 1998; Khanna et al., 1999), researchers demonstrated that it stimulated the increase of glutathione levels by 30-70%. It has been postulated that the movement of DHLA from intracellular to extracellular space (Constantinescu et al., 1995) enables DHLA to reduce cystine to cysteine, which is then taken up by neutral amino acid transporters into the cell and used in glutathione synthesis (Han et al., 1997). This cellular enhancement of glutathione may be of particular importance for cell types such as the eye lens. This tissue must be kept well supplied with glutathione since it does not possess mitochondria and therefore is susceptible to cataract formation due to elevated glucose levels and other agents of oxidative stress (Obrosova et al., 1998). Similarly, Packer & Tritschler (1996) have outlined the profound effect that ALA has on buthionine-sulfoxamine (BSO) induced cataracts. Newborn rats cannot synthesize vitamin C and when they were injected with BSO, which inhibited glutathione synthesis, their antioxidant defenses were weakened causing cataract formation in 100% of BSO treated rats. However, if these same rats were given ALA, only 40% developed cataracts. At the same time there was an increase in lens tissue vitamin C and E, and glutathione after ALA supplementation. The authors commented that the action elicited by ALA on the regeneration of other antioxidants confers uniqueness among the natural antioxidants.

In contrast to these beneficial recycling findings, Jones et al. (2002) conducted an in vitro experiment focusing on the uptake, reduction, and recycling capacity of ALA in cultured human endothelial cells. The authors discovered that intracellular GSH concentrations were unaffected by 15 minute incubation with concentrations of ALA less that 0.5mM, yet concentrations above this value caused a 30% decrease in intracellular GSH. However, Jones et al. (2002) showed that ALA enhanced the ability of human endothelial cells to recycle ascorbate from its oxidized form dehydoascorbate acid with minimal oxidative stress to the cells.

The effect of ALA on the status of lipid peroxidation and antioxidants in aged rats was examined by Arivazhagan et al. (2000). Groups of young (130-160g) and old (380-410g) rats were allocated to controls or fed ALA for 7 or 14 days. Treatment groups received ALA via intraperitoneal injection at 100mg/kg body weight/day, whereas the controls received an injection of physiological saline. The authors found that ALA decreased the level of lipid peroxides in both young and old rats. In addition, baseline antioxidant levels were markedly lower in aged rats, which were subsequently normalized during the 14-day ALA administration. These results imply that ALA improves the enzymatic antioxidant defense system via improvements measured in superoxide dismutase, catalase, and glutathione peroxidase activity (Arivazhagan et al., 2000). A proposed mechanistic explanation for such an effect is through increased ATP production, which may improve overall protein synthesis in cells, thus elevating enzyme synthesis. Non-enzymatic antioxidants such as reduced glutathione, vitamin C and E were also increased by ALA supplementation, thus supporting the idea that ALA acts as

an antioxidant for these compounds enabling them to be recycled (Arivazhagan et al., 2000).

Similar work conducted by Lykkesfeldt et al. (1998) established that liver cells isolated from aged rats are less able to respond to increased oxidative stress caused by mitochondrial decay. These older cells were also observed to contain significantly decreased concentrations of ascorbic acid (AA). When rats were fed a 0.5% wt/wt dietary dose of *R*-lipoic acid for 2 weeks, this age related decline in AA levels was reversed and increased above that of young rats. These data strongly support ALA's role in antioxidant recycling.

The notion of mitochondrial decay and its effects on aging and cognitive function have also been studied (Hagen et al., 2002). These authors supplemented acetyl-L-carnitine (ALCAR) and ALA to young and old rats. Acetyl-L-carnitine has been shown to reverse age-related declines in tissue carnitine levels and improve mitochondrial fatty acid β-oxidation; however, this increased mitochondrial activity heightens the formation of ROS. Therefore, Hagen et al. (2002) tested the combined supplementation of ALCAR at 1.5% wt/vol in drinking water and 0.5% wt/wt ALA in diet on 3-5 month old rats. Animals receiving this combination treatment showed increased metabolism and decreased oxidative stress measured through mitochondrial membrane potential, O_2 consumption, and lipid peroxidation. The results obtained were stronger in the combination treatment when compared to either supplement alone, lending evidence to a synergistic action between ALCAR and ALA.

2.1.5 Alpha-Lipoic Acid and Cardiovascular Disease Risk Factors

2.1.5.1 LDL Oxidation

The risk factors of atherosclerosis are well established, mainly hyperlipidemia, smoking, diabetes mellitus, hyperhomocyste(i)nemia, and hypertension (Hofmann et al., 2000). The mechanisms which each of these factors contributes to the disease process and any interactions that take place between them, are not fully understood. However, one event that is common to these risk factors is the generation of oxidative stress (Hofmann et al., 2000). The hypothesis that oxidative stress constitutes a major causative factor in atherosclerosis is gaining greater acceptance (Westhuyzen, 1997; Tardif & Bourassa, 2000; Lykkesfeldt et al., 1998). Oxidative modifications to LDL cholesterol increase their atherogenicity by altering cell receptor uptake of these particles, particularly cells in the intima of blood vessels (Tardif & Bourassa, 2000). Furthermore, oxidized LDL is taken up by scavenger receptors on monocytes, smooth muscle cells, and macrophages in an uncontrolled process leading to the accumulation of lipid and the formation of foam cells, an early feature of atherosclerotic plaques. It is within this early atherosclerotic lesion that increased oxidative stress evokes inflammatory events, which further generates peroxides, superperoxides, and hydroxyl radicals within the endothelium. The inflammatory events in turn continue the cycle of damage to the vasculature. In light of these mechanisms, current research focusing on the effect of antioxidants exhibiting a protective effect on the oxidation of LDL cholesterol may lead to mitigation of the atherosclerotic process (Forgione & Loscalzo, 2000). Therefore, it is reasonable to believe that the administration of low to moderate amounts of antioxidants would make a substantial contribution towards decreasing the risk to heart disease, stroke, and

hypertension, all of which are associated with the atherosclerotic process (Leaf & Hallaq, 1992, Tardif & Bourassa, 2000).

Knowing that ALA has the capacity to recycle endogenous antioxidants, any attributed cardiovascular benefits established for these recycled entities could possibly be enhanced synergistically through supplementation with ALA. Although not conclusively proven, it has been suggested through epidemiological and clinical evidence (Christen & Hennekens, 2000) that high concentrations of vitamin E may protect against free radical LDL cholesterol oxidation and decrease the risk of coronary heart disease (CHD) (Stampfer et al., 1993; Rimm et al., 1993). Kagen et al. (1992) maintained high concentrations of vitamin E in humans and found that ALA was effective in recycling vitamin E by interacting synergistically with vitamin C. The authors concluded that the recycling of vitamin E and other antioxidants by plasma reductants such as ALA may be an important mechanism for enhanced antioxidant protection of LDL.

Further support of this notion was provided by Marangon et al. (1999), who compared effects of ALA and α-tocopherol (AT) supplementation on measures of oxidative stress. Thirty-one healthy adults participated in the randomized parallel study testing ALA at 600 mg/day or AT 400 IU/day for two months followed by a combination of both supplements for two additional months. Results indicated no significant change in BMI or lipid profile regardless of the supplement taken. When measures of oxidative stress were analyzed, ALA significantly decreased plasma carbonyls while AT had no effect. In addition, both AT and ALA alone decreased urinary F_2-isoprostane levels. In combination, the two supplements further decreased F_2-isoprostane levels. However, there appeared to be a "sequence of addition" rule in play, where adding AT to ALA

induced an effect, but not vice versa. Reasons for such a sequential additive effect have not been defined within the literature. Further, ALA was shown to increase LDL oxidizability lag time to lipid peroxide formation after both copper and 2,2'-azobisamidinopropane hydrochloride (AAPH) catalyzed oxidation. However, oral supplementation with ALA did not affect conjugated diene formation. Marangon et al. (1999) concluded that ALA prevents premature atherosclerosis via its antioxidant effect. Another group of researchers (Lodge et al., 1998) studied the capacity for protection of human LDL peroxidation and thiol chelation of Cu^{2+} by DHLA. Dihydrolipoic acid increased lag time in a concentration dependant manner with treatment concentration ranging from 0 to 10 μM. Similarly, DHLA reduced Cu^{2+} chelation in a dose dependant manner, but when the concentration of DHLA exceeded 5μM this effect was inhibited (Lodge et al., 1998). α-Lipoic acid was documented as not having any effect on the Cu^{2+} induced peroxidation of LDL at any concentration. The reasoning behind this finding is rooted in the evidence that the beneficial effects of DHLA chelation are mediated via the carboxylic and free sulphydral groups of the DHLA molecule, otherwise the same type of effect would have been elicited through the use of ALA in its oxidative form (Lodge et al., 1998).

2.1.5.2 Alpha-Lipoic Acid Effects on Blood Lipid Profile & Plaque Formation

As early as 1958, researchers investigated the lipid lowering capacity of ALA in rabbits (Angelucci & Mascitelli-Coriandoli, 1958; Kritchevsky, 1958). Results of these two studies were contradictory; with Angelucci & Mascitelli-Coriandoli (1958) reporting that ALA decreased plasma cholesterol levels by 50%, yet Kritchevsky (1958) showed

increased aorta atherosclerosis in rabbits and no cholesterol lowering activities with ALA supplementation. In an effort to resolve this discrepancy Ivanov (1974) found that dietary ALA of 1 mg/kg of diet not only reduced the levels of total cholesterol and lipoproteins in the serum and in aortic tissue of rabbits, but also intensified tissue respiration in the heart, liver, and blood vessels. Later, Shih (1983) studied effects of ALA on atherosclerosis in Japanese quail. Birds were fed diets containing 0.25% cholesterol for 12 weeks, while receiving 2.5 mg/wk of ALA from a slow release capsule implanted subcutaneously on the dorsal surface of the neck. Results indicated a decrease in total cholesterol and β-lipoproteins of 40% and 42%, respectively. The author concluded that ALA exhibits a protective effect in the prevention of elevated blood lipids and atherosclerosis in quail. This study by Shih (1983) remains to be one of the only recent in vivo trials testing the primary outcome of ALA on atherosclerotic formation. Although Shih's model of atherosclerosis in the aorta of the quail is thought to be characteristic of human disease, caution in extrapolating these results must be applied until they are further supported by other animal studies.

More recently, Ford et al. (2001) studied the effects of an evening primrose oil supplement and an ALA supplement on a variety of lipid and haemostatic parameters in control and diabetic rats for two weeks. Of interest were findings that supplementation with ALA at 300 mg/kg body weight per day caused a significant decrease in plasma triglyceride concentrations in diabetic rats. However, cholesterol and HDL-cholesterol levels did not differ significantly. The authors concluded that this decrease in plasma triglycerides could possibly facilitate improved endothelial function, which could prove beneficial in CVD, and hence warrant further study (Ford et al., 2001). These results are

consistent with those of a previous study (Segermann et al., 1991), where ALA supplementation given via interperitoneal injection of 7.5 mg/100g body weight per day for 9 days elicited a 45% decrease in triglyceride concentrations.

2.1.5.3 Hypertension

Another aspect of the cardiovascular risk profile that has been studied in relation to ALA is hypertension. Vasdev et al. (2000) determined if dietary supplementation of ALA could lower blood pressure in spontaneously hypertensive rats (SHR). Non-hypertensive, SHR control, and SHR supplemented rats were fed ALA at 500 mg/kg feed for 9 weeks. Results showed no significant effect of ALA on body weight during supplementation; however, supplementation did attenuate hypertension measured using tail cuff methodology. The authors speculated that ALA increased the free sulphydral groups of calcium channels, leading to a decrease in cystolic free calcium, vascular tone and hypertension (Vasdev et al., 2000). Similar results have been obtained by Midaoui & Champlain (2002) where male rats were used to investigate whether a chronic dietary supplementation with ALA could prevent blood pressure elevation. Hypertension was induced through the addition of a 10% D-glucose drink to either the control chow diet or the ALA supplemented diet (500 mg/kg feed). Results indicated that feeding with the glucose solution alone increased systolic blood pressure on average of 166 mm Hg after 3 weeks (Midaoui & Champlain, 2002). However, supplementation with ALA was able to attenuate this rise, enabling ALA animals to maintain blood pressure values the same as animals not receiving the induced glucose hypertension. The authors postulated that the antihypertensive effects of ALA are associated with an attenuation of oxidative stress in

the aortic vessel and by preservation of glutathione peroxidase activity in the plasma of glucose-treated rats.

2.1.6 Alpha-Lipoic Acid and Diabetes Mellitus

Excessive oscillations in blood glucose concentrations cause various cellular functions to deteriorate at an accelerated pace, which is the basis for many of the pathologies associated with disorders of glucose utilization (Evans et al., 2000). It has been postulated that ALA may be useful in ameliorating these oscillations. Several studies have established that ALA is the most powerful, natural compound that is available for stimulating glucose uptake, lowering elevated blood glucose values, and improving insulin sensitivity in Type II diabetics (Evans et al., 2000; Ziegler et al., 1999; Jacob et al., 1996; Estrada et al., 1996; Moini et al., 2002). The use of ALA is attractive for the treatment of diabetes because it is proposed to work synergistically with other treatment modalities. In addition, as a natural compound in foods and supplements, there are no serious established side effects or contraindications when used in combination with current treatment regime for diabetics (Evans et al., 2000). It has been reported (Roberts, 2001) that at the highest doses given orally to diabetics some individuals suffer from mild stomach and intestinal side effects. In addition, Shapiro & Gong (2002) noted that some diabetics treated with i.v. ALA suffered headache and GI upset.

2.1.6.1 Effects of Alpha-Lipoic Acid on Glucose Metabolism

Jacob et al. (1996) conducted a small pilot study on 20 well-controlled Type II diabetics. The subjects were treated for ten days with 500 mg daily IV infusions of

thioctic acid. Whole body glucose disposal was measured using glucose clamp methodology, and these results were presented as a function of glucose metabolic clearance rates. After the 10 day treatment period the metabolic clearance rate of glucose was significantly improved, by 30%. An in vitro study carried out by Estrada et al. (1996) using L6 myotube and 3T3-L1 adipocytes tested the effects of medium infusion with the (R)-isomer, (S)-isomer, or a racemic mixture of ALA. The results indicated a clear functional difference between the isomers, with (R)-ALA increasing glucose uptake with the same effectiveness of that seen with insulin alone. These similarities led the authors to investigate if ALA functions through phosphatidylinositol 3-kinase, a regulatory subunit in the insulin-signaling pathway. To test this capacity, L6 myotubes and 3T3-L1 adipocytes were incubated with wortmannin, known to inhibit the stimulatory effects of insulin on glucose uptake. Results showed that wortmannin completely prevented the rise in glucose uptake seen as a response to ALA incubation. Therefore, these authors lend evidence to one possible mechanism through which ALA exerts it effects on glucose uptake at the cellular level. Similarly, Moini et al. (2002) investigated (R)-ALA action on glucose uptake in 3T3-L1 adipocytes. However, these authors wanted to investigate a different mechanistic action of ALA on the insulin signal transduction pathway, mainly an "oxidant-mimetic system". Results indicated that adipocyte cells were not capable of reducing ALA into its potent antioxidant form DHLA. The authors presume that acting as a pro-oxidant, (R)-ALA oxidizes critical thiol groups present in the β-subunit of the receptor thus initiating insulin signal transduction via receptor autophosphorylation. Therefore, it may be these oxidative protein modifications that contribute to ALA/DHLA's versatile effects. Although the two

aforementioned studies (Estrada et al., 1996; Moini et al., 2002) agree that ALA has the capacity to improve glucose metabolism in muscle and fat cells, the mechanism of action still remains to be elucidated.

The notion of a coenzyme deficiency in Type II diabetics was suggested by Konrad et al. (1999). This group postulated such an idea after finding that ALA treatment decreased serum lactate and pyruvate concentrations and improved glucose effectiveness in lean and obese Type II diabetics. Oral glucose tolerance tests (OGTT) and modified frequently sampled intravenous glucose tolerance tests (FSIGTT) were conducted on 10 lean and 10 obese controls in addition to 10 lean and 10 obese Type II diabetics. These diabetic subjects were treated with 600 mg of ALA twice per day for 4 weeks prior to the above-mentioned tests. After 4 weeks of ALA treatment fasting lactate and pyruvate concentrations were reduced in both lean and obese diabetics, and increments of pyruvate and lactate after glucose loading were prevented. The authors (Konrad et al., 1999) noted no mean incremental change in insulin levels in either lean or obese subjects. Moreover, the BMI and triglyceride levels of the study subjects were not significantly different after ALA treatment. The authors suggested that the most probable cause for the decreased levels of lactate and pyruvate lay in a defect in glucose oxidation at the level of pyruvate dehydrogenase. It can thus be concluded that oral treatment with ALA seems to improve intracellular glucose utilization, probably by stimulating pyruvate dehydrogenase, and increasing glucose disposal in patients with Type II diabetes (Konrad et al., 1999).

2.1.6.2 Alpha-Lipoic Acid and Diabetic Neuropathy

Diabetic polyneuropathy is encountered by one third of all diabetics, and is associated with increased mortality in relation to its severity (Ziegler et al., 1999). Diabetic neuropathies are a common consequence of hyperglycemia and may occur early in the disease state. The conditions involve abnormal sensations in the extremities including numbness, prickling, tingling, heightened sensitivity, and pain. The complication itself is responsive to the normalization of serum glucose levels and hence the relationship to investigating ALA. Several mechanisms through which ALA has been reported to contribute to neuroprotection in diabetes have been discussed in the literature. Hermann et al. (1996) outline that ALA has been shown to enhance nerve blood flow, protect against protein glycation, and exert strong metal chelating properties, all of which offer relief from diabetic neuropathies.

Kishi et al. (1999) studied effects of ALA on glucose uptake, sorbitol pathway, and energy metabolism in experimental diabetic neuropathy. A randomized parallel design animal study tested a control diet versus a supplemented diet containing varying concentrations of ALA from 10 mg/kg to 100 mg/kg on both normal and diabetic rats. Results indicated that ALA supplementation did not affect the body weights, blood glucose, or glycosylated hemoglobin of either the control or diabetic rats. However, an effect on glucose uptake was seen in nerves plagued by experimental diabetic neuropathy (EDN). α-Lipoic acid was able to correct the deficit in glucose uptake within EDN, in a dose dependant fashion with 100 mg/kg of ALA completely correcting the condition in the sciatic nerve (Kishi et al., 1999).

2.1.7 Alpha-Lipoic Acid: The Questions Remaining

The literature surrounding ALA showcases a powerful natural compound that elicits a variety of health benefits in animals and humans. However, a major problem that presents is that clear congruent findings within the existing literature are absent, the questions that remain are several. Firstly, there exists the issue of what form of ALA is supplemented, R, S, or a racemic mixture of the two. No conclusive evidence supports use of one formulation over another. Second, discrepancy remains concerning a therapeutic dose. The studies reviewed here cover a possible dosing range of 200-1800 mg/day in humans alone with no consensus as to optimum dose. In addition, from this review it is difficult to pinpoint a dose that will reach the functional threshold necessary to produce beneficial effects most of the time. Lastly, methods of supplementation themselves should not go unnoticed. Reviewed studies have shown ALA supplementation using tablets, powder, or liquid solutions given orally, IV infusions, IP injections, and subcutaneous implant. Therefore, to provide optimal beneficial effects for the treatment of oxidative stress and/or chronic disease it becomes important to consider the type of ALA supplemented, the dose provided, and supplementation method used to obtain the desired effects.

2.1.8 Summary

One of the first non-traditional ideas about the relationship between food and health to gain scientific respectability was the hypothesis that antioxidant nutrients might protect against chronic diseases. The literature has clearly established the antioxidant properties of ALA and demonstrated important areas of medicine where it can be

employed. It is through understanding the health benefits of a natural compound, that science can inevitably uncover the desire to try to put such a compound to good use within a population. The data presented here elucidate the ways by which ALA may impact the CVD risk profile through beneficial actions on LDL oxidation, blood lipid profiles, plaque formation, and hypertension. In addition, functionality for ALA is well supported by its current use in Germany as an effective adjunct to diabetic therapy, mediated through its effects on the improvement of glucose metabolism and relief of the symptoms of diabetic neuropathy. All of the aforementioned benefits must be weighed carefully against the lack of consensus regarding the most appropriate form, dose, and supplementation method for ALA.

What remains now is for implementation of reasonable and appropriate guidelines for the public to follow regarding supplementation with ALA. For instance, there is the need to answer the questions how much is beneficial as an adjunct to healthy living and how much is too much? Such guidelines are required in that ALA is currently available within neutraceutical and natural health product marketplaces, therefore it warrants health professionals' attention in order to protect the health and well being of society.

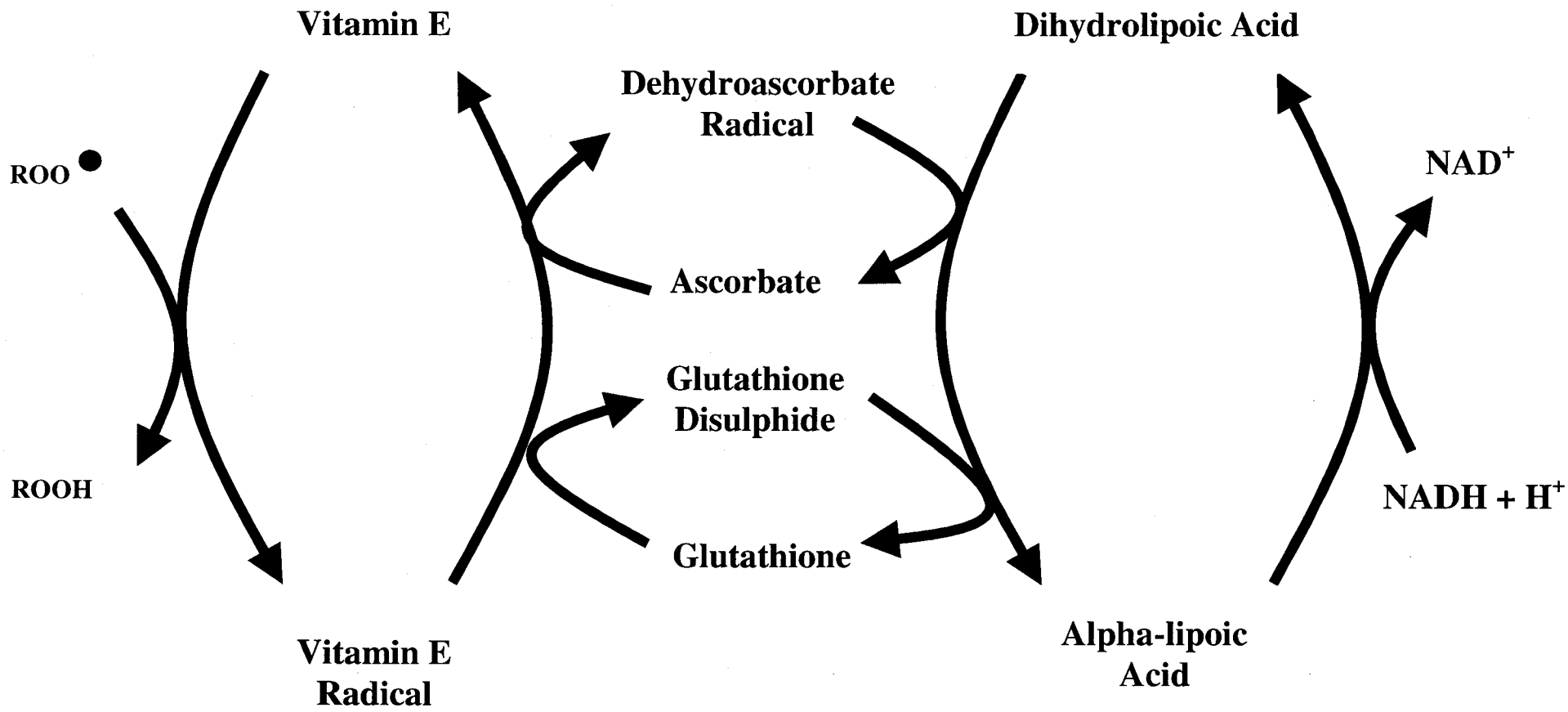


Figure 2.1.4.1: The role of α-lipoic acid in the recycling of other antioxidant systems. Adapted from Packer et al., 1995.

3. LITERATURE REVIEW: FORMULATION OF A HEALTHY MEDIUM-CHAIN TRIGLYCERIDE OIL MIXTURE

3.1 Differences of Dietary Fatty Acids: During Digestion, Absorption, and Transport

Structural differences exist between long (LCT), medium (MCT) and short chain triglycerides (SCT). These differences influence their metabolism during digestion, absorption, and transport. Shorter fatty acids undergo faster and more complete hydrolysis due to their smaller molecular weight and capacity to activate pancreatic lipase, a key digestive enzyme (Clement, 1976; Mascicoli et al., 1989). Short chain triglycerides and MCT are therefore absorbed more rapidly into the intestinal lumen compared to LCT (Caspary, 1992). Once these components are absorbed, the mode of transport for the fatty acids to the liver and other organs is also influenced by chain length. Long chain triglycerides are packaged into chylomicrons, which are secreted into the lymphatic system and then into the blood circulation, thus passing through the body before reaching the liver (McDonald et al., 1980; Vallot et al., 1985). Long chain triglycerides are more likely to be taken up by adipose tissue prior to reaching the liver. Conversely, SCT and MCT travel from the intestine directly to the liver through the portal vein, and therefore do not pass by adipose tissue prior to hepatic disposal. In addition, MCT have little tendency to be elongated or deposited as body fat (Bell et al., 1997). Hence, SCT and MCT arrive at the liver (a major site of fatty acid oxidation), more quickly compared to LCT following a meal. Thus, ketonemia may be more pronounced when feeding MCT versus LCT, however, this is reported to occur mainly when fed in large quantities, >40 g/day (Bell et al., 1997).

3.2 Differences of Dietary Fatty Acids: Effects on Fatty Acid Oxidation

Several methods have been used to examine the rate of oxidation of fatty acids differing in chain length in both animal and human models. As respiratory carbon dioxide (CO_2) is the product of nutrient oxidation, measurement of CO_2 production offers a means of assessing substrate oxidation. By labeling dietary fatty acids with isotopes, which result in isotopically labeled $^{13}CO_2$, the extent of fatty acid oxidation can be determined.

In vitro investigation of isolated hepatocytes incubated with various dietary fatty acids has shown greater utilization of 1-^{14}C ocatanoate compared to 1-^{14}C oleate, thus implying that short chain length increases oxidation (Pegorier, 1988; Crozier, 1988). Animal experiments have provided similar results. Using oral fatty acids labeled with ^{14}C, increased oxidation in rats was related to decreasing chain length (Leyton et al., 1987). Similar results were observed when rats were injected with lipid emulsions of ^{14}C-labelled MCT versus ^{14}C-labelled LCT. More complete oxidation of the MCT (90%) occurred relative to the LCT (45%) after 24 hours (Johnson et al., 1990). In human studies measuring substrate oxidation, intravenous administration of MCT or LCT emulsions resulted in significantly increased oxidation of MCFA over a 10-hour period, while oxidation of LCFA was similar to basal levels (Mascicoli et al., 1991). An explanation for this increase in oxidative capacity has been given by Hill et al. (1990), stating that ingesting large amounts of MCT can promote substantial synthesis of LCFA by the liver. This synthesis from acetate or the elongation of C8:0 and C10:0 fatty acids are energetically expensive, and could account for the large differences seen in energy substrate oxidation.

More recently, Binnert et al. (1998) conducted a fatty acid tracer study which demonstrated that compared to lean controls, LCT oxidation was impaired in obese individuals, while MCT oxidation was both higher than LCT and equivalent to that seen in controls. In this study, the metabolic fate of both an oral MCT and LCT load was followed for 6 hours in 8 control and 8 obese individuals using indirect calorimetry and stable isotope-labeled fatty acids. Results demonstrated that LCT were less rapidly oxidized in obese compared to controls (3.2% compared with 6.0% respectively) over 6 hours. In contrast, MCT oxidation was not altered in obese subjects compared with controls and the proportion of MCT oxidized was 3-fold greater than that of the LCT. These results suggest that consumption of SCT and MCT in place of LCT may result in an increase of a dietary fat-induced difference in fatty acid oxidation in obese versus non-obese individuals. Thus, in terms of promoting energy balance, obese individuals may profit to a greater extent, from the consumption of SCT and MCT rather than LCT, than non-obese individuals.

3.3 Effects of Medium Chain Triglycerides on Body Weight in Animals

Many of these animal studies compare diets composed of MCT and LCT with an endpoint of interest in examining differences in body fat deposition. In 1982, Baba et al. fed 15 rats through oral intubation with an MCT vs LCT oil rich diet for 6 weeks. The diet contained 50% of its calories from fat. The results indicated that the animals fed the MCT diet had significantly lower levels of dissectible fat. The authors attributed this difference to higher resting and maximal oxygen consumption, as well as an overall higher metabolic rate. A similar study by Geleibter et al. (1983) showed that feeding rats

via oral intubation a diet of 50% kcal from MCT versus LCT caused 20% less weight gain in the MCT group. In addition, the MCT group exhibited fat depots weighing 23% less than LCT fed rats. These authors provided increased rigor to their results by monitoring total spontaneous physical activity over a 24h period, and noted no significant differences between groups. Further support of these findings were seen by Chanez et al. (1991), when rats were fed LCT or MCT containing diets for 45 days containing 32% of kcal as dietary fat. The data showed that rats fed the MCT diet had 26% less weight gain when compared to animals fed LCT.

3.4 Lipid Modulating Effects of a Medium Chain Triglyceride Oil Mixture

3.4.1 Effects of Medium Chain Triglyceride Feeding in Animals

Nicollosi et al. (1998) tested five diets with differing fatty acid composition on 100 Golden Syrian hamsters over an eight-week period. The treatment diets were composed primarily of the following five fatty acid components: C8:0, C14:0, C18:0, *cis*-C18:1, *trans*-C18:1. Of interest here are the results obtained from feeding with the hamsters the 20% wt/wt MCT diet. Results indicated that animals fed C8:0, *cis*-C18:1, or *trans*-C18:1 diets had lower plasma total, LDL, and HDL-cholesterol compared to the C14:0 group. Similarly, Geelen et al. (1995) compared diets composed of corn oil or MCT in rats and observed a significant decrease in total cholesterol within MCT fed animals. However, these same animals exhibited an increase in plasma triglyceride concentrations.

Another study using hamsters (Woollett et al., 1992) discussed the idea that in hamster plasma, LDL-cholesterol levels are elevated three fold when saturated

triglycerides are fed in conjunction with small quantities of pure cholesterol. This LDL elevation is noted to be caused by significant suppression of the LDL receptor activity and an almost doubling of hepatic cholesterol production rate (Woollett et al., 1992). These researchers wanted to investigate precisely which saturated fatty acids were responsible for such changes. Woollett et al. (1992) tested experimental diets comprised of 0.12% cholesterol, 10% olive oil, plus 10% triacylglycerol made up of a single saturated fatty acids varying in chain length from C6:0 to C18:0. The authors found no significant changes in plasma LDL-cholesterol concentrations, suppression of hepatic LDL receptor activity, or elevation in the rate of LDL-cholesterol synthesis when feeding fatty acids less than or equal to C10:0. Thus, the conclusion drawn is that only C12:0, C16:0, and C18:0 have detrimental effects on plasma LDL-cholesterol concentrations when fed in concert with pure cholesterol (Woollett et al., 1992). In contrast, Meijer et al. (2001) found that hamsters fed MCT or palmitic acid showed a trend towards increased total cholesterol levels when compared to animals fed an oleic acid containing diet.

3.4.2 Effects of Medium Chain Triglyceride Feeding in Humans

Hill et al. (1990) conducted a randomized double-blind inpatient human trial with men, in order to examine changes in blood lipids during six days of overfeeding with MCT or LCT. Subjects were provided with liquid formulas and were overfed by 150% of energy requirements for 5 days in a crossover design experiment. Test formulas were composed of either; MCT oil (60% C8:0, 30% C10:0) or soybean oil (30% C18: 1 n-9, 50% C18: 2 n-6), where fat made up 40% of energy intake. Treatments were separated by a one-week washout period. Results showed that on MCT liquid formula, total

cholesterol levels did not change, however, triglyceride levels increased 3-fold. Furthermore, on the LCT formula total cholesterol levels decreased by 20% and triglyceride levels remained the same as pre-treatment levels.

Another study conducted by Swift et al. (1992) was designed to examine plasma lipids and lipoproteins during 6 days of maintenance feeding with long chain, medium chain and mixed chain triglycerides. Ten healthy males participated in a randomized, inpatient trial consuming a liquid formula diet. The experimental oils were given as 40% of total daily caloric energy for 6 days in a crossover design. The MCT oil contained 60% C8:0, 32% C10:0 while the LCT was in the form of soybean oil and contained 30% C18:1n-9, 50% C18:2n-6. The subjects were given a one-week washout period between treatments. The MCT feeding was found to increase triglyceride levels by 42%, as well as decrease HDL cholesterol levels by 15%. In contrast, the soybean formula appeared to decrease triglyceride levels by 20%, and mildly increase HDL cholesterol levels.

In 1997, Cater et al. conducted a randomized, crossover, inpatient study that compared the effects of MCT, palm oil, and high oleic acid sunflower oil on plasma triglyceride, lipid, and lipoprotein concentrations in nine middle aged men with mild hypercholesterolemia. Each treatment diet was administered for 3 weeks and separated by a one-week washout period. The overall energy composition of the diet was 53% fat, 35% carbohydrate, 12% protein. Of the 53% fat, 43% was treatment fat, either MCT oil (70% C8:0, 30% C10:0), palm oil (50% C16:0, 35% C18:1 n-9), or sunflower oil (90% C18:1 n-9). The authors found that with regards to total cholesterol and LDL cholesterol measures, palm oil and MCT oil were virtually indistinguishable, both significantly increasing these parameters. Meanwhile, the MCT diet also increased triglycerides and

VLDL-cholesterol concentrations significantly more than the palm and sunflower oil diets. Finally, no significant change in HDL cholesterol levels was detected on either of the three diet treatments.

More recently Tsai et al. (1999) examined the mechanisms mediating lipoprotein responses to diets with MCT and lauric acid. Eighteen healthy premenopausal women participated in the randomized, crossover, outpatient trial. Subjects consumed controlled diets consisting of 40% fat with a constant level of monounsaturated fatty acids (MUFA) and cholesterol. The baseline diet was rich in polyunsaturated fatty acids (PUFA) and was consumed for 1 week prior to the experimental phase, followed by 4 weeks of treatment with either 14% substituted as MCT (C8:0 and C10:0) or 14% substituted as lauric acid (C12:0). The washout period between treatments was seven weeks. Results indicated that when substituted for PUFA, MCT had 2/3 the cholesterol-raising potency of lauric acid. However, MCT also increased triglyceride levels by 5%.

In contrast to the above, when investigating the role of oils containing triglycerides and medium chain fatty acids in the dietary treatment of obesity, Hainer et al (1994) measured MCT effects on resting energy expenditure and serum lipids. The authors investigated 60 obese patients for a four-week period that underwent diet, exercise, and behaviour therapy during hospitalization. A subset of 11 subjects was then supplemented with 15 ml/day of MCT oil for the final two weeks in adjunct to their diet therapy. Results suggested that both treatments decreased total cholesterol and triglyceride levels. However, diet therapy alone was found to decrease HDL-cholesterol levels whereas, the MCT supplemented group exhibited no change in HDL-cholesterol levels. In addition, Calabresse et al. (1999) conducted a randomized cross-over trial in

twenty healthy men. After a fasting blood draw subjects ingested 71g of either canola or MCT oil, repeat blood draws were then taken at hours 1–5 post-ingestion. Results indicated that MCT bolus feeding decreased triglyceride levels by 15% between baseline and three hours after ingestion.

Despite the efficacy of MCT on increasing fat oxidation rates, negative affects on plasma triglyceride and cholesterol levels compromise their use for weight maintenance. Therefore, in order to continue investigating MCT as a possible weight maintenance regulator these negative aspects must be addressed. Hence, the addition of cholesterol lowering phytosterols and triglyceride modulating n-3 fatty acids may serve to improve the negative cardiovascular risks of using MCT for weight maintenance.

3.4.3 The Cholesterol Lowering Effects of Phytosterols

Plant sterols produce a wide spectrum of biological activities in animals and humans (Ling & Jones, 1995). In particular, phytosterols have been considered as an efficacious cholesterol-lowering agent since the early 1950's. In this regard, they have been widely studied over the last few decades and currently provide an alternative to drug therapy for people whom suffer from hypercholesterolemia.

Miettinen et al. (1995) conducted a one-year, randomized, double blind study in 153 subjects with mild hypercholesterolemia. Fifty-one subjects consumed control margarine without sitostanol-esters while 102 consumed margarine containing 1.8 to 2.6 g of sitostanol-esters/day. The authors found that sitostanol esters decreased total cholesterol by 10.2% after one year. Pelletier et al. (1995) studied the effects soybean phytosterols on blood cholesterol levels in 12 normolipidemic healthy males using a

randomized, crossover study design. The subjects were fed controlled diets under supervision and underwent 1 week of adaptation prior to treatment commencement. The dietary treatments lasted for 4 weeks and were made up of a control diet containing 29 mg of phytosterols/day and an experimental diet containing 740 mg of soybean phytosterols/day. The two experimental periods were not separated by a washout period. However, the authors reported that the HDL:LDL ratio increased by 25% during the experimental phase, with a 10% decrease in total cholesterol and a concomitant 15% decrease in LDL-cholesterol. These results provide evidence that modest intakes of phytosterols can lead to an improvement of the CVD risk profile.

In 1999, Jones et al. conducted a parallel study examining the cholesterol-lowering efficacy of a sitostanol-containing phytosterol mixture with a precisely controlled diet in hyperlipidemic men. Thirty-two men were fed either a control diet consisting of prepared foods alone or a diet containing 1.7 g of tall oil phytosterols/ day for 30 days. The authors reported that total cholesterol concentrations were lower on day 30 after supplementation with phytosterols. More importantly, they discovered that LDL-cholesterol concentrations on day 30 had decreased 8.9% on the control diet and 24.4% on the phytosterol-enriched diets. The authors noted that there were no significant changes in HDL-cholesterol and triglyceride concentrations in either group. An interesting point is made by Law (2000), stating that the 2g addition of phytosterols to an average daily portion of margarine reduces serum LDL cholesterol by an average of 0.54 mmol/l. This translates into a reduction of risk of heart disease of about 25%, which is larger than the expected impact of people reducing their saturated fat intake (Law, 2000).

The efficacy for the utilization of plant sterols in the diet is now well established. The launch of margarines containing plant stanols that lower total and LDL cholesterol without affecting HDL cholesterol and triglyceride levels is the first step in what may become an important innovation in the primary prevention of heart disease. Hence, it is important to study these active compounds and their effects in combination with other agents thought to be beneficial to human health.

3.4.4 The Effect of n-3 Fatty Acids on Hypertriglyceridemia

The importance of n-3 fatty acids in health and disease has been well documented. A recent review by Connor (2000) provides a partial list of diseases that may be prevented or ameliorated with n-3 fatty acids including coronary heart disease and stroke, retinal and brain development in infancy, autoimmune disorders, Crohn's disease, cancers of the breast, colon, and prostate, mild hypertension, and rheumatoid arthritis. Such an extensive list represents the magnitude of interest which still surrounds supplementing the diet with n-3 fatty acids in an effort to combat the aforementioned ailments.

Early epidemiological research revealed that fish-eating populations exhibited a decreased incidence of cardiovascular disease. Since then science has uncovered evidence that marine n-3 polyunsaturated fatty acids can decrease triglyceride levels, VLDL levels, blood clotting function, and homocysteine levels (Harris et al., 1997). Alpha-linolenic acid (α-LA) contained in flaxseed oil is also a good dietary source of n-3 PUFAs. Hence, understanding the blood lipid modulation properties of α-LA from plant sources is of importance, not merely the properties of fish oils. A study by Simon et al.

(1995) concluded that serum α-LA concentrations were negatively correlated with the risk of stroke. Similarly, another group (Ascherio et al., 1996) found that supplementing α-LA at 0.8-1.5 g/day significantly decreased risk of myocardial infarction. Furthermore, Singer et al. (1992) reported that that α-LA supplementation with vegetable based oils significantly decreased triglyceride levels, however, Mantzioris et al. (1994) and Abbey et al. (1990) reported no significant changes in triglycerides with α-LA administration.

In a review by Harris (1997), the point is raised that the triglyceride lowering effects of fish oils are not seen in the hamster model in all cases. This conclusion however, is specific to fish oil and does not make any conclusions regarding the effects of n-3 fatty acids in the form of flaxseed oil in the hamster and thus this question remains. Despite this established trend, Kubow et al. (1996) found that supplementation with vitamin E, inhibited fish-oil induced hyperlipidemia and tissue lipid peroxidation in the hamster. After a 27-day feeding trial, the serum triglyceride concentrations were higher in animals fed fish oils (high-cholesterol) relative to other groups fed vitamin E (2.5 mg/g oil). In addition this same group exhibited lower tissue lipid peroxidation. The authors present an interesting possibility that a depletion of tissue vitamin E and lipid peroxidation could be involved in the hyperlipidemic response of the hamster to fish oils. From these results it would appear that further studies examining the hyperlipidemic response of hamsters fed a moderately atherogenic diet in combination with antioxidant supplementation are warranted.

4. RATIONALE

Prolonged and controlled MCT versus LCT feeding has been shown to have effects on body energy balance measured through changes in body composition in animals. Preliminary investigations in our laboratory examining the addition of n-3 fatty acids and phytosterols have shown suppression of any rise in triglyceride levels associated with MCT feeding and to reduce LDL cholesterol by up to 15% in humans. Correspondingly, alpha lipoic acid has been demonstrated to possess a wide array of antioxidant actions, which may directly or indirectly impact on circulating lipid levels and antioxidant state of tissues. However, whether a synergism exists between ALA and other beneficial substances in combination has not yet been investigated.

5. NULL HYPOTHESIS AND OBJECTIVE

5.1 Null Hypothesis

Feeding male hamsters a moderately high cholesterol diet containing a MCT oil mixture alone and in combination with ALA versus a moderately hypercholesterolemic control diet for 30 days will have no effect on:

1. Plasma triglyceride, or total-, HDL, and non-HDL cholesterol levels.
2. Lean body mass and fat mass body compartments as measured through tracer deuterium and calculated using total body water estimation.
3. Antioxidant status measures including liver and heart reduced glutathione (GSH), oxidized glutathione (GSSG), GSH: GSSG ratio and thiobarbituric acid substances (TBARS).

5.2 Main Objective

To examine the efficacy and mechanism of action of an orally-administered dietary oil mixture and alpha-lipoic acid, given independently and in combination, on body weight and composition, cholesterol and lipoprotein profiles, and antioxidant status in the hamster.

6. MANUSCRIPT 2

Effects of a Medium Chain Triglyceride Oil Mixture and Alpha-lipoic Acid Diet on Body Composition, Antioxidant Status, and Plasma Lipid Levels in the Syrian Hamster

Stephanie D. Wollin, Yanwen Wang, Stan Kubow, Peter J.H. Jones

School of Dietetics and Human Nutrition

McGill University, Ste-Anne-de-Bellevue, Québec, Canada

Address correspondence to:

Peter J.H. Jones, Ph.D.
School of Dietetics and Human Nutrition
McGill University
21,111 Lakeshore Road
Ste-Anne-de-Bellevue, Québec, Canada, H9X 3V9
Phone: (514) 398-7841
Fax: (514) 398-7739
e-mail: jonesp@macdonald.mcgill.ca

Supported by the Natural Sciences and Engineering Research Council of Canada (NSERC) and Forbes Medi-Tech Inc. Vancouver, BC, Canada

Parts of these data have been presented at the Experimental Biology Conference (FASEB), New Orleans, LA, April 20-24, 2002.

Running head: Alpha-lipoic acid, MCT, lipid metabolism, antioxidant

To be submitted to the Journal of Nutrition

6.1 Abstract

The objective of this study was to examine the effects of a medium chain triglyceride oil mixture (MCTo), designed to increase energy expenditure and improve lipid profiles containing medium chain triglycerides, phytosterols and omega-3 fatty acids in the form of flaxseed oil, versus the antioxidant α-lipoic acid (ALA). Forty-eight hamsters were fed (i) hypercholesterolemic (HC) control, (ii) HC MCTo, (iii) HC ALA, (iv) HC MCTo/ALA diets for 4 weeks. No differences were observed on food intake, body weight, total body water, lean and fat mass, and tissue thiobarbituric acid-reactive substances (TBARS). ALA alone had no effect on total cholesterol (TC); however, MCTo feeding increased TC with ($p<0.03$) and without ($p<0.003$) ALA when compared to control. ALA increased HDL levels compared to control ($p<0.04$) and MCTo/ALA ($p<0.007$) groups. MCTo, with ($p<0.0001$) or without ($p<0.006$) ALA, increased non-HDL cholesterol levels versus control. The non-HDL:HDL cholesterol ratio was decreased by ALA compared to MCTo (45%) and MCTo/ALA (68%) ($p<0.0001$), a similar trend was seen when compared to the HC control (22%) group ($p<0.14$). Triglyceride levels were not altered by any dietary treatment. Liver and heart tissue reduced glutathione (GSH) was increased ($p<0.05$) by all three treatments when compared to control. Both tissues showed an increase ($p<0.05$) in oxidized glutathione (GSSG) when fed ALA as compared to all treatments. Hamsters fed ALA had a lower ($p<0.05$) GSH/GSSG ratio compared to all treatment groups. In conclusion, MCTo feeding does not elicit beneficial effects on circulating plasma lipids and measures of body composition. In addition, our results do not clearly support an improvement in

oxidative status through supplementation of ALA. However, our results do support the existence of beneficial effects of ALA on circulating lipoprotein content in the hamster.

6.2 Introduction

Diseases of the heart and blood vessels, collectively known as cardiovascular disease (CVD), are the leading cause of death in Canada (Health Canada, 1997). Primary risk factors for CVD are obesity, diabetes, hypertension, elevated blood cholesterol levels, and oxidative stress. In an attempt to combat these risk factors, science has turned to the investigation of bioactive substances that may offer protection to the cardiovascular system.

Several studies suggest that oxidative stress plays a significant role in the pathogenesis of atherosclerosis (Westhuysen, 1997; Steinberg et al., 1997; Quinn et al. 1987). Therefore, in formulating a combination of bioactive components to combat CVD, a powerful antioxidant, alpha-lipoic acid (ALA) was used. ALA has been shown to protect LDL cholesterol from in vivo oxidation (Packer et al., 1995; Kagan et al., 1992; Marangnon et al., 1999; Lodge, 1998). Levels of other functional antioxidants such as, vitamins C and E and glutathione have also been shown to be increased via recycling through supplementation with ALA (Packer et al. 1995, Busse et al. 1992, Hagen et al. 1999). Apart from the antioxidant functions of ALA, effects of ALA on plasma lipid profiles in animals have also been examined yielding inconclusive results. Early studies in the 1970's and 1980's have shown the capacity of ALA to decrease serum total cholesterol in rabbits (Ivanov, 1974) and atherosclerosis in quail (Shih, 1983). In contrast,

more recent research has reported no significant effects of ALA supplementation on cholesterol levels (Ford et al., 2001; Segermann et al., 1991; Marangnon et al., 1999)

Medium chain triglycerides (MCT) have been shown to be more easily absorbed into the intestinal lumen compared to long chain triglycerides (LCT) (Caspary, 1992). MCT also differ from LCT in that they are transported directly to the liver via the portal vein and thus do not pass the adipose tissue prior to hepatic disposal. These characteristics are thought to be responsible for the different rates of fat oxidation for MCT versus LCT. In addition, MCT have been shown to undergo increased oxidation in both animal (Leyton et al., 1987; Johnson et al., 1990) and human studies (Mascioli et al., 1989; Hill et al., 1990; Binnert et al., 1998). These reports of increased oxidative capacity have made MCT appealing as a possible adjunct for the treatment of obesity, however, MCT have also been shown to have deleterious effects on the blood lipid profile causing their use to be less desirable. There is strong evidence in the literature to suggest that MCT increase circulating triglyceride levels (Hill et al., 1990; Swift et al., 1992; Tsai et al., 1999). In addition, MCT have also been shown to increase circulating LDL cholesterol levels (Tsai et al., 1999; Cater et al., 1997). However, some studies have obtained different results demonstrating little effect of MCT on plasma triglyceride (Hainer et al., 1994) and improvements in plasma LDL and total cholesterol (TC) levels (Nicollosi et al., 1998; Geelen et al., 1995; Woollett et al., 1992).

With the existing knowledge of possible negative effects of MCT feeding on blood lipids, the concept of combining MCT with phytosterols and n-3 fatty acids to negate negative effects is provocative. Plant sterols have been shown to decrease both plasma total (Gylling and Miettinen, 1999; Miettinen et al., 1995) and LDL cholesterol

(Pelltier et al., 1995; Jones et al., 1999) without significant alterations in plasma HDL cholesterol and triglyceride concentrations. Phytosterols are known to elicit these actions through inhibition of dietary cholesterol absorption from the intestine (Jones et al., 1995). In addition, supplementation with alpha-linolenic acid, in the form of flaxseed oil has been shown to increase tissue eicosapentanoic (EPA) concentrations in vivo (Mantzioris et al., 1994). EPA is thought to be one of the components responsible for the capacity of fish oils to decrease plasma triglyceride levels (Harris, 1997). Alpha-linolenic acid feeding has been shown to decrease plasma triglyceride levels by 22-24% in humans (Singer, 1992). These results support the rationale for the combined feeding of phytosterols and flaxseed oil in an attempt to temper increases in plasma cholesterol and triglyceride levels caused by MCT feeding.

We tested the null hypothesis that feeding male Golden Syrian hamsters a moderately high cholesterol diet containing an MCT oil mixture (MCTo) composed of; MCT, phytosterols, and n-3 PUFAs alone and in combination with ALA would not elicit beneficial effects on blood lipid concentrations, body weight, and measures of oxidative stress. The main objective of this study was to examine the efficacy of orally administered MCTo and ALA, given independently and in combination, on body weight, lipid profiles, and antioxidant status in the Golden Syrian hamster.

6.3 Animals and Methods

This experimental protocol was approved by the Animal Ethical Review Committee of the Faculty of Agriculture and Environmental Sciences for the School of Dietetics and Human Nutrition at McGill University (Appendix 1).

Diet preparation and animal accommodation: Forty-eight Golden Syrian hamsters weighing 80-100g (Charles River Laboratories, Wilmington, MA) were utilized in this experiment. Hamsters were acclimatized for two weeks while receiving free access to water and were fed standard non-purified laboratory diet (Charles River Laboratories, Wilmington, MA) ad libitum. For the duration of the study hamsters were exposed to a 12 hour light-dark cycle starting at 9:00 am. Following this two-week period, animals were randomized into four groups and switched to semi-purified diets (ICN Pharmaceuticals, Inc.). Diets were prepared weekly and stored at –80°C. Dietary composition is shown in **Table 6.3.1**. All diets were designed to be moderately atherogenic, with a total cholesterol content of 0.25% wt/wt. The total fat content of the diet was 10% fed in the form of a mixture of beef tallow and safflower oil. Once dietary treatment commenced the unmodified atherogenic control diet was fed to one group of hamsters (Group 1). Groups 2-4 were supplied with the same basic diet, with substitutions to the fat content. Group 2 received 75% of the supplied fat as the MCTo with the remaining 25% as the beef tallow/safflower mixture. Meanwhile group 3 received the control fat blend with powdered racemic ALA added at 0.3% wt/wt of diet. Group 4 received MCTo as 75% of dietary fat in addition to 0.3% wt/wt of racemic ALA. Food intake, and food spillage was measured daily and body weight was recorded every three days.

Sample Collection: After thirty days of dietary treatment, hamsters were fasted for a twelve-hour period. Following the fasting period, animals were injected with 0.3g of deuterium oxide, which had been precisely weighed. Three hours post-injection, hamsters

were anaesthetized with carbon dioxide and blood samples were collected by decapitation. Blood was collected in ethylenediamine tetracetic acid (EDTA) tubes and centrifuged at 1500 x g for 15 minutes to obtain red blood cells and plasma. Plasma was immediately separated and aliquoted into micro-centrifuge tubes. Liver, heart, and kidney tissues were harvested, weighed, snap frozen in liquid nitrogen, and stored at –80°C. All samples were coded and maintained in –80°C storage until further analysis.

***Plasma Lipid Measurements*:** Plasma total cholesterol, high-density lipoprotein (HDL) cholesterol and triglyceride levels were measured in duplicate using an Abbott VP Super System Autoanalyser (Abbott, Irving, TX) in conjunction with commercial enzymatic kits (Abbott Laboratories, Montreal, Quebec). Measurement of HDL cholesterol in plasma was carried out after precipitation of apo-B containing lipoproteins with dextran sulfate and magnesium chloride. Results were expressed as concentration of cholesterol in VLDL + IDL + LDL termed "non-HDL", instead of in low density lipoproteins (LDL) because the Friedewald (1972) equation may not be applicable to hamsters. Thus the concentration of lipoprotein (non-HDL) cholesterol was calculated by subtracting HDL cholesterol concentrations from plasma total cholesterol.

Deuterium oxide enrichments: Deuterium analyses were conducted using standard vacuum techniques as previously described by Jones et al. (1988). To determine D_2O enrichment, lengths of 6mm OD Pyrex tubing were attached to a vacuum system containing 0.06 g of zinc. A capillary tube (1µl) filled with plasma was added before immersion in liquid nitrogen. Gases were evacuated and each tube was flame sealed.

Samples were prepared in triplicate. They were then combusted for 1 hour at 520°C to produce hydrogen gas. After reaching room temperature, analyses were carried out using a 903D dual-inlet isotope ratio mass spectrometer (IRMS) (Cheshire, England). Isotope enrichments were determined against a standard curve produced from varying concentrations of deuterium and doubly distilled water, thus enabling the calculation of total body water (TBW). Variation in sample replicates was tolerated within 1%. Calibration of the mass spectrometer was conducted by using Vienna standard mean ocean water (SMOW).

Body composition calculations: Body composition was calculated using total body water (TBW) calculated from deuterium oxide enrichment and final body weight (FBW) on day 30. Total body water was calculated using the enrichment of plasma samples taken at 3 hours after deuterium administration. Based on the assumption that fat free mass (FFM) is 73.2% water, FFM was calculated using the equation (Pace and Rathburn, 1945): FM=TBW/0.732. Fat mass (FM) was then determined using the equation: FM=FBW-FFM.

Analysis of thiobarbituric acid reactive substances (TBARS): Plasma concentrations of TBARS were measured using a modified method of Asakawa & Matsashita, (1980) and Wong et al. (1987). Prior to the TBARS assay, liver and heart tissue, 0.5g and 0.2 g respectively were homogenized in a 1:10 ratio of ice cold KCL. The tissue homogenate was stored on ice and aliquoted into triplicate tubes each containing 250µl.

The reaction of malonyl-dialdehyde (MDA) with thiobarbituric acid (TBA) forms a brightly colored complex, which can be quantified spectrophotometrically with its visible absorbance. Stock solution (1200 μmol/L 0.01 MHCl) was made which contained 1,1,3,3-tetramethoxypropane (TMOP) (Sigma T-9889). TMOP turns into MDA when heated and bound to TBA, forming TBARS. Intensity of the color increases with increasing concentrations of TMOP. These known concentrations were used to make a standard curve, and were used to quantify the TBARS found in the tissue samples.

The TBA reaction was initiated when the sample or standard was added along with butylated hydroxy-toluene (BHT), orthophosphoric acid, and TBA. The mixture was heated for 1 hour in a 100°C water bath, allowing for color change. After color change, butanol:pyridine solution (15:1) was added and centrifuged at 3000 rpm for 15 minutes to obtain an upper butanol phase, which was added to a micro-cuvette and read for absorbance at triple wavelengths of 508, 532, and 556 nm using a Beckman Spectrophotometer (DU 640). A regression curve was calculated from the standards and sample values were obtained.

Glutathione (GSH) Measures: Prior to analysis, liver and heart tissues were homogenized in a 1:10 dilution of MES buffer, containing 2-(N-norpholino) ethanesulphonic acid, phosphate, and EDTA. Homogenates were centrifuged at 10 000 x g for 15 minutes. Supernatants were deproteinated using meta-phosphoric acid (MPA), and stored at –20°C until complete kit analysis (Cayman Chemical Company, Ann Arbor, MI, 2000).

Levels of GSH and GSSG were measured using Cayman Chemical Kits (Ann Arbor, MI, GSH Assay Kit Cat# 703002) following the same methodology outlined in Poirier et al. (In Press). The kit employs a carefully optimized enzymatic recycling method, using glutathione reductase, for the quantification of GSH. The sulfhydryl group of GSH reacts with 5,5'-dithiobis-2-nitrobenzoic acid (DTNB) and produces a yellow colored 5-thio-2-nitrobenzoic acid (TNB). The mixed disulfide, between GSH and TNB, is further reduced by GSH reductase to recycle GSH and produce more TNB. The rate of TNB production is directly proportional to this recycling reaction, which is in turn directly proportional to the concentration of GSH in the sample. Measurement of the absorbance was done at 405nm (Wallac Victor 2 1420 Mulilabel Counter).

GSH is readily oxidized to the disulphide dimmer GSSG. GSSG is produced during the reduction of hydroperoxides by GSH peroxidase, GSSG may then be reduced to GSH by GSH reductase. Due to the GSH reductase within the Cayman kit, GSSG can be measured by derivatizing GSH with 2-vinylpyridine (VP), followed by a 60-minute incubation at room temperature. Measurement of the absorbance was done at 405nm (Wallac Victor 2 1420 Mulilabel Counter).

Statistical Methods: All data were tested for normality and are expressed as means ± SD. Endpoint data between treatments were analyzed using one-way analysis of variance (ANOVA). Observed treatment differences were evaluated using Tukey's post-hoc comparison. The level of significance for rejection of the null hypothesis was set at $p<0.05$. Version 8.0 of SAS Software (SAS Institute, Cary, NC, US, 1999) was used to perform all statistical analysis.

Table 6.3.1: Composition of experimental diets.

Ingredients (% wt/wt)	**Group 1** Control %	**Group 2** MCT Oil Mix %	**Group 3** Lipoic Acid %	**Group 4** MCT Oil Mix & Lipoic Acid %
Vitamin Free Casein	20.0	20.0	20.0	20.0
Corn Starch	26.0	26.0	26.0	26.0
Sucrose	33.0	33.0	33.0	33.0
Beef Tallow/ Safflower Mixture[1]	10.0	2.5	10.0	2.5
DL-methionine	0.5	0.5	0.5	0.5
Mineral Mixture[2]	4.0	4.0	4.0	4.0
Vitamin Mixture[3]	1.0	1.0	1.0	1.0
Choline Bitartrate	0.2	0.2	0.2	0.2
Butylhydroxytoluene	0.02% of oil	0.02% of oil	0.02% of oil	0.02% of oil
Cholesterol	0.25	0.25	0.25	0.25
Cellulose	5.0	5.0	5.0	5.0
MCT Oil Mixture[4]	**0.0**	**7.5**	**0.0**	**7.5**
Lipoic Acid[5]	**0.0**	**0.0**	**0.3**	**0.3**

[1] Of the 10% or 2.5% dietary fat content, 98% was beef tallow and 2 % was safflower oil
[2] AIN-93 Mineral Mix, ICN Pharmaceuticals, Costa Mesa, CA (cat# 960401)
[3] AIN-93 Vitamin Mix, ICN Pharmaceuticals, Costa Mesa, CA (cat# 960402)
[4] MCT oil mixture: 64.7% medium chain triglycerides, 3.4% phytosterols, 6.8% flaxseed oil, 12.6% olive oil, 6.8% canola oil, 5.8% coconut oil. The oil was blended once prior to study commencement and was stored at 4°C. The oil blend was predetermined based on previous human studies in our laboratory.
[5] α-Lipoic acid was given as a racemic powder. It was blended into the fat component of the diet and then added to the rest of the dry ingredients twice per week during diet preparation periods. Supplied by DNP International, City of Industry, CA

6.4 Results

Forty-eight hamsters completed the 30 day feeding trial. At all times during the study animals appeared to remain in a healthy condition. There were no signs of impaired growth, unusual behavior, or excessive hair loss, which are often signs that animals are suffering from adverse effects related to treatment.

Food intake and body weight: Daily dietary feed intake of hamsters did not differ among groups over the 30 day study period (**Figure 6.4.1**). In addition, body weight over days 0 to 30 did not show any significant differences across groups (**Figure 6.4.2).**

Plasma lipid profile: Plasma lipid values are presented in **Table 6.4.1**. ALA alone fed to hamsters at 0.3 % wt/wt had no effect on plasma TC. However, MCTo feeding at 7.5% wt/wt of diet increased TC both with ($p<0.03$) and without ($p<0.0003$) ALA compared to the control diet.

α-Lipoic acid alone increased HDL-C levels compared to the control ($p<0.04$) and MCTo/ALA ($p<0.0007$) groups. However, ALA treatment was not significantly different from MCTo feeding. Plasma non-HDL cholesterol fraction was increased with MCTo feeding both with ($p<0.0001$) and without ($p<0.006$) ALA, when compared to the control group.

α-Lipoic acid supplementation decreased the non-HDL:HDL ratio compared to MCTo (45%) and MCTo/ALA (68%) ($p<0.0001$). ALA exhibited a similar though non significant trend of non-HDL:HDL cholesterol decrease (22%) ($p<0.14$) when compared

to the HC control diet (**Figure 6.4.3**). Triglyceride levels were not altered by any of the dietary treatments after 30 days.

Body composition: There were no significant differences observed between groups for total body water, lean body mass, fat mass, final body weight (**Table 6.4.2**). A significant positive correlation (r=0.71) was found between hamster body weight and fat mass ($p<0.0001$).

Liver and heart reduced glutathione concentrations (GSH): Treatments effects on liver and heart tissue GSH are shown in **Table 6.4.3** ALA, MCTo, alone and in combination increased ($p<0.0004$) liver tissue GSH compared to the HC control diet. Similar results were obtained in heart tissue where ALA at 0.3% wt/wt diet, MCTo at 7.5% wt/wt diet and the co-treatment, each increased ($p<0.05$) GSH levels when compared to the HC control diet.

Liver and heart oxidized glutathione concentrations (GSSG): Treatment effects on GSSG in liver and heart tissue are shown in **Table 6.4.3**. In liver, dietary treatment had a significant main effect ($p<0.0001$) on GSSG concentrations. ALA supplementation increased GSSG concentrations compared to the MCTo ($p<0.0001$), MCTo/ALA ($p<0.0007$), and the HC control diet ($p<0.0001$). The MCTo/ALA treatment also resulted in increased GSSG concentrations when compared to MCTo alone ($p<0.03$) and the HC control diet ($p<0.0008$).

A significant main effect of dietary treatment was also seen in heart tissue ($p<0.0006$). ALA supplementation of 0.3% wt/wt increased GSSG concentrations compared to MCTo/ALA ($p<0.0055$), MCTo alone ($p<0.0002$), and the HC control diet ($p<0.0005$). No significant differences were observed between MCTo/ALA, MCTo, or HC control diet for heart GSSG concentrations.

Effects of dietary treatment on liver and heart tissue GSH/GSSG ratio: Hamsters fed ALA had significantly lower liver GSH/GSSG ratios as compared to HC control ($p<0.0001$), MCT ($p<0.0002$), and MCTo/ALA ($p<0.0024$) treatments. Although different from the ALA group, there were no remaining significant differences between the other dietary treatments. This effect was not seen in the heart tissues of hamsters **(Figure 6.4.4)**.

Tissue thiobarbituric acid-reactive substances (TBARS): In both liver and heart tissue there were no significant differences in TBARS concentrations between diet treatments **(Table 5.4.3).**

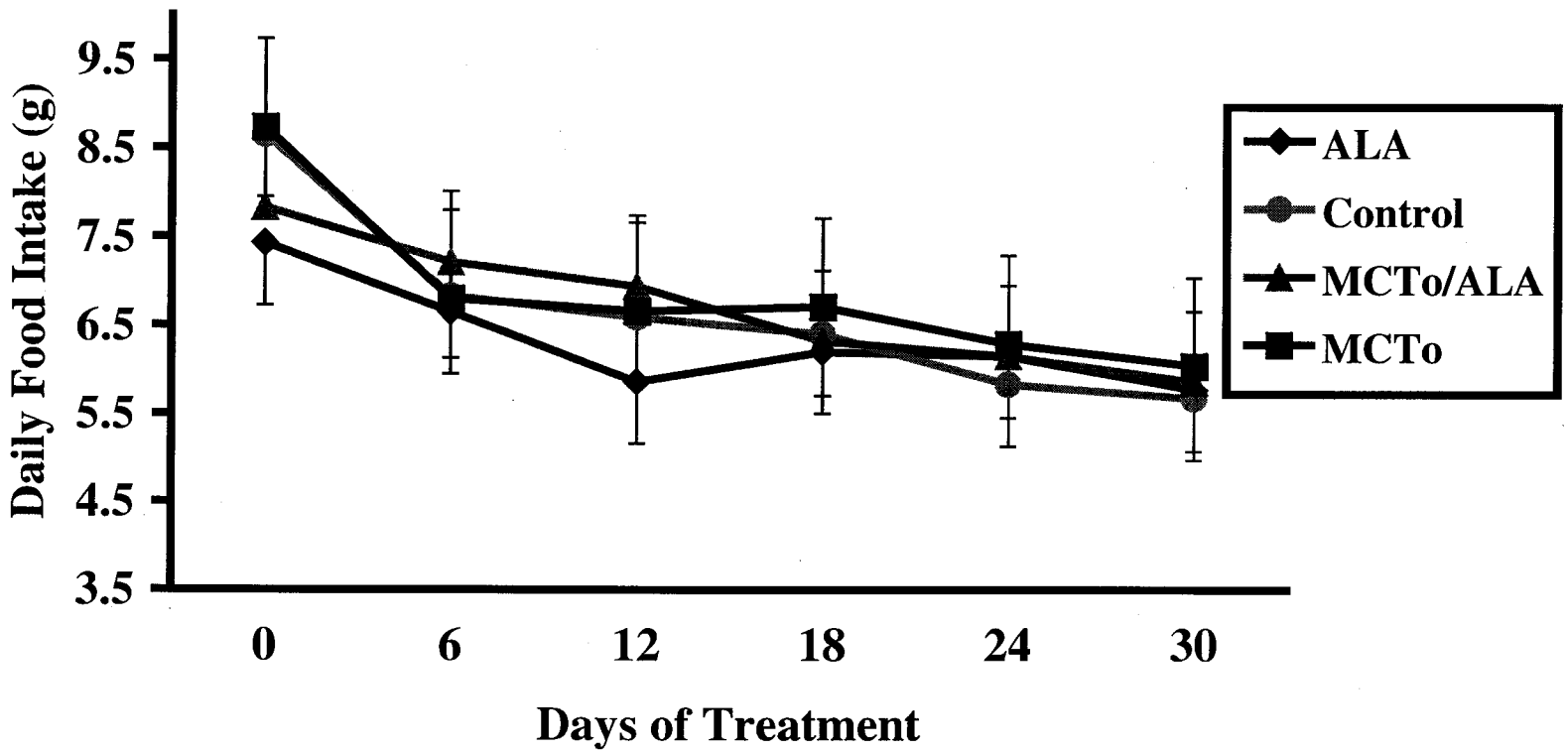


Figure 6.4.1: Effects of dietary treatment on the daily feed intake of hamsters. No significant differences were observed between groups. Data is presented as means ± SD, n=48.
MCTo: medium chain triglyceride oil mixture
ALA: α-lipoic acid

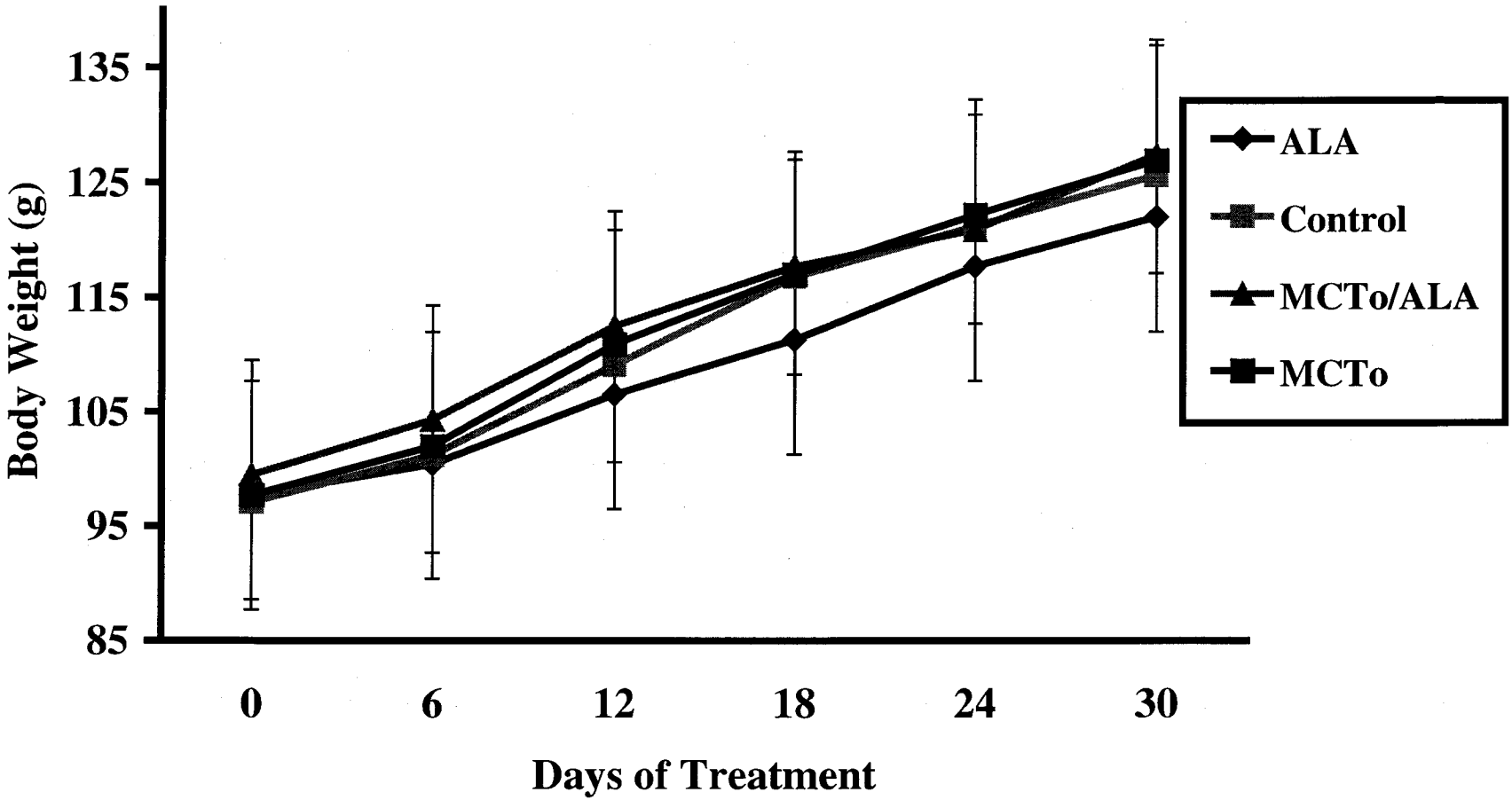


Figure 6.4.2: Effects of dietary treatment on hamster body weight. No significant differences were observed between groups. Data is presented as means ± SD, n=48.
MCTo: medium chain triglyceride oil mixture
ALA: α-lipoic acid

Table 6.4.1: Plasma total-cholesterol (TC), HDL-cholesterol, non-HDL-cholesterol, and triglyceride concentrations[1].

Treatment Group	TC[2]	HDL-C[3]	(non-HDL)-C[4]	TG[5]
Control	6.44 ± 0.94^{c}	4.70 ± 0.69^{bc}	1.74 ± 0.61^{b}	6.15 ± 2.70
ALA[6]	6.79 ± 0.88^{bc}	5.26 ± 0.75^{a}	1.53 ± 0.35^{b}	5.45 ± 1.31
MCTo[7]	7.61 ± 0.65^{a}	5.00 ± 0.64^{ab}	2.61 ± 0.42^{a}	6.65 ± 1.97
MCTo/ALA	7.29 ± 1.10^{ab}	4.30 ± 0.45^{c}	2.99 ± 0.83^{a}	5.02 ± 1.00

[1] Values are expressed as mmol/L ± SD. Values carrying different superscript letters indicate significant differences between diets ($p<0.05$) n=48.
[2] total cholesterol
[3] high-density lipoprotein cholesterol
[4] low, very low, intermediate-density lipoprotein cholesterol
[5] triglycerides
[6] α-lipoic acid
[7] medium chain triglyceride oil mixture

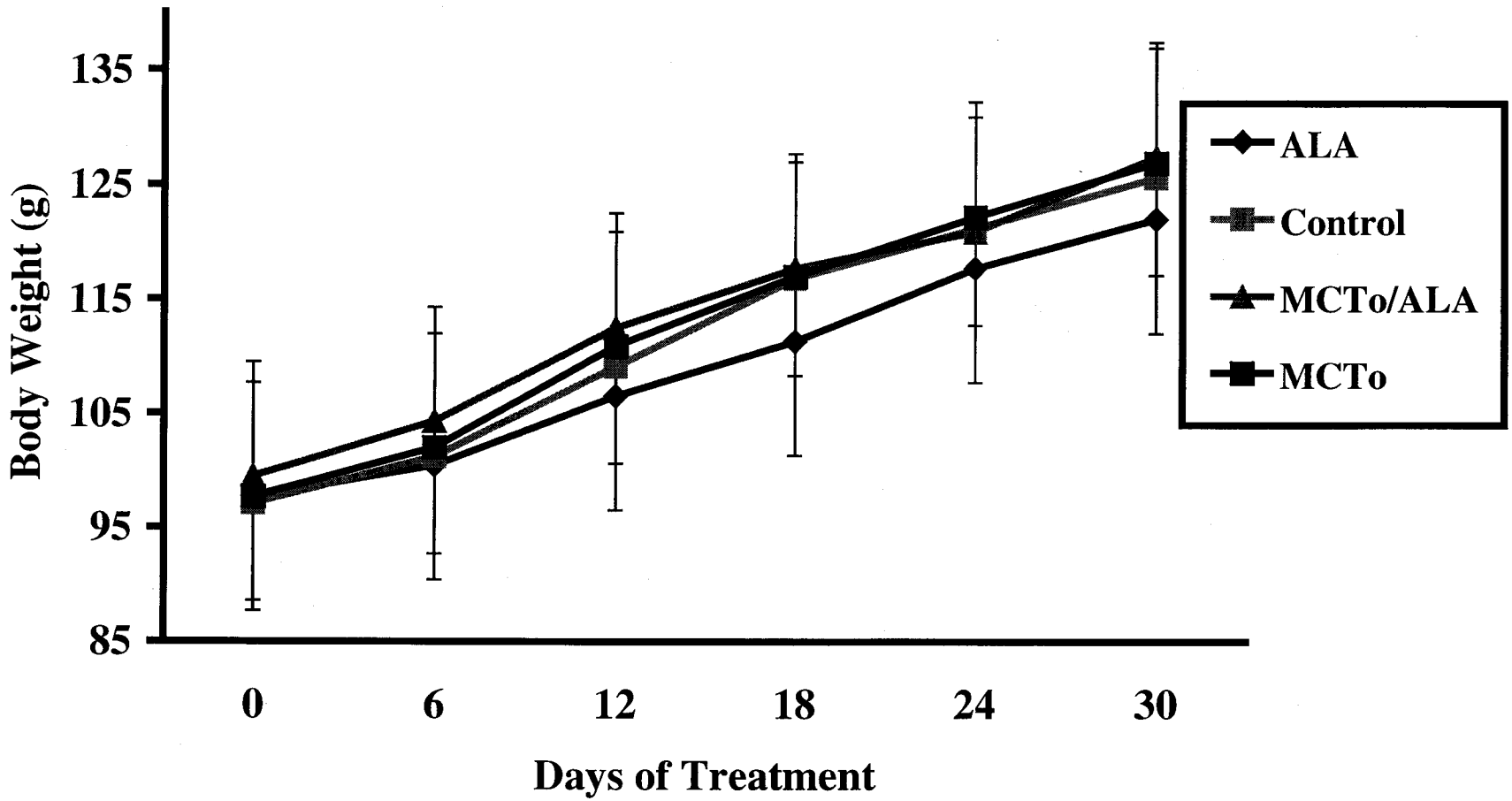


Figure 6.4.2: Effects of dietary treatment on hamster body weight. No significant differences were observed between groups. Data is presented as means ± SD, n=48.
MCTo: medium chain triglyceride oil mixture
ALA: α-lipoic acid

Table 6.4.2: Hamster body composition measures: total body water (TBW), lean body mass (LBM), and fat mass (FM)[1].

Treatment Group	TBW	LBM	FM
Control	74.3 ± 6.6	101.8 ± 9.1	15.8 ± 6.9
ALA	77.8 ± 6.4	106.6 ± 8.7	16.6 ± 9.2
MCTo	78.2 ± 7.6	107.1 ± 10.4	22.6 ± 14.7
MCTo/ALA	79.3 ± 4.2	108.6 ± 5.8	19.4 ± 13.7

[1] Values are expressed as grams ± SD. There were no significant differences between treatments n=48.

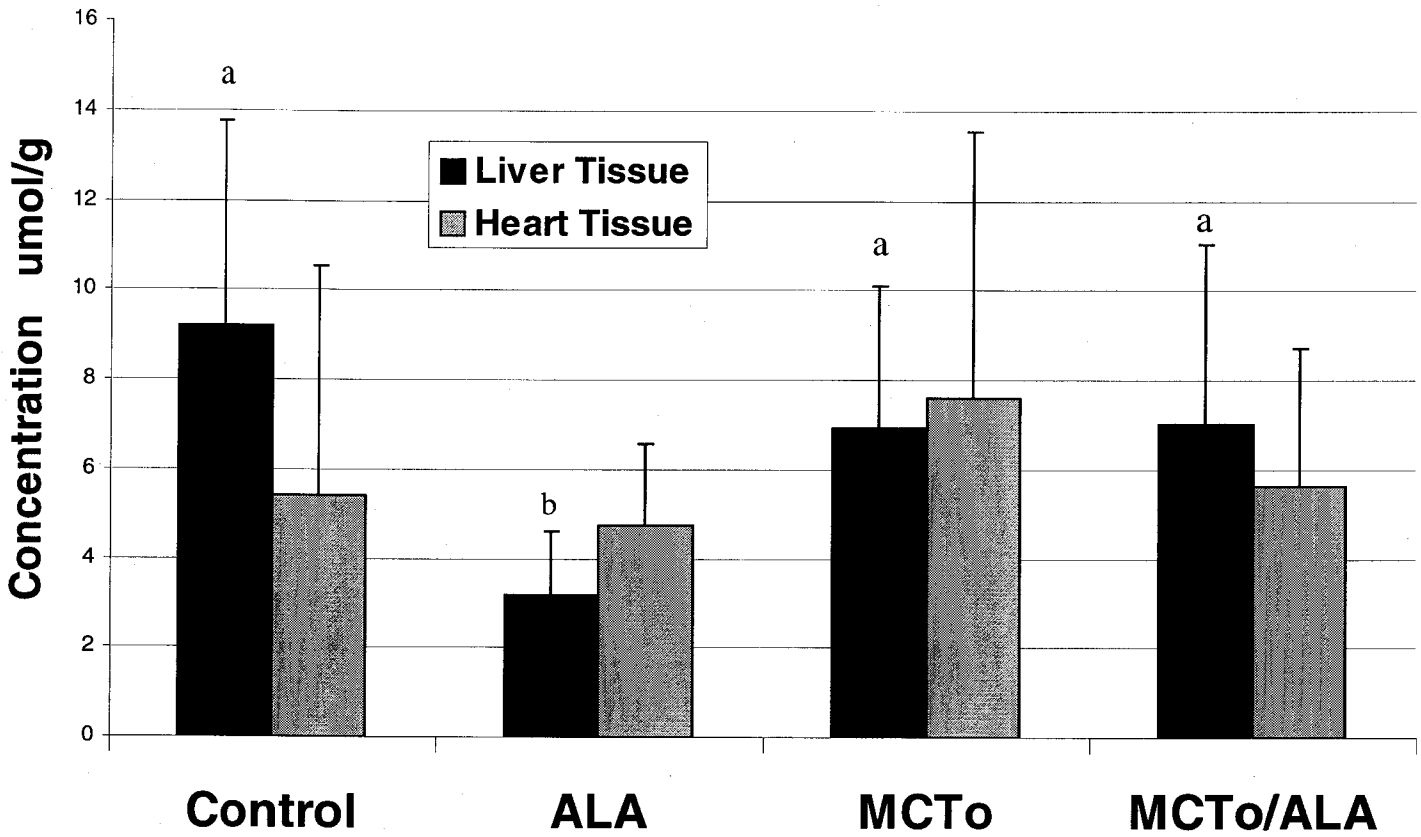
Table 6.4.3: Liver and heart tissue reduced glutathione (GSH), oxidized glutathione (GSSG) and thiobarbituric acid reactive substances (TBARS) concentrations[1].

	GSH		GSSG		TBARS	
Treatment Group	LIVER	HEART	LIVER	HEART	LIVER	HEART
Control	3.05 ± 0.92^{b}	0.46 ± 0.26^{b}	0.37 ± 0.12^{c}	0.11 ± 0.034^{b}	87.44 ± 28.54	80.76 ± 28.20
ALA[2]	3.75 ± 1.29^{a}	0.61 ± 0.21^{a}	1.29 ± 0.37^{a}	0.14 ± 0.057^{a}	76.28 ± 16.43	83.09 ± 21.81
MCTo[3]	3.92 ± 1.01^{a}	0.57 ± 0.23^{a}	0.58 ± 0.39^{bc}	0.10 ± 0.037^{b}	83.26 ± 28.37	78.35 ± 17.86
MCTo/ALA	4.23 ± 1.02^{a}	0.60 ± 0.26^{a}	0.73 ± 0.35^{b}	0.11 ± 0.036^{b}	83.19 ± 26.06	77.86 ± 13.08

[1] Values are expressed as mean µmol/g tissue concentrations ± SD. Values carrying different superscript letters indicate significant differences between diets ($p<0.05$) n=48.

[2] α-lipoic acid

[3] medium chain triglyceride oil mixture



GSH/GSSG RATIO

Figure 6.4.4: Effects of dietary treatment on the reduced glutathione (GSH) to oxidized glutathione (GSSG) ratio. Significant differences between groups are shown by letter subscripts ($p<0.05$) n=48.
MCTo: medium chain triglycerides
ALA: α-lipoic acid

6.5 Discussion

Our results demonstrate that in the hamster model, MCT oil mixture (MCTo) treatment was atherogenic, despite the addition of ALA. In addition, supplementation of ALA did not appear to offer improved oxidative status in our animals.

Reports of the effects of MCT feeding on circulating lipid levels in animal (Nicollosi et al., 1998; Woollett et al., 1992) and human studies (Cater et al. 1997; Hill et al., 1990; Swift et al., 1992; Hainer et al., 1994; Tsai et al., 1999) have been well documented. Recent studies in our laboratory have shown that in humans, MCT oil in combination with phytosterols and flaxseed oil, has the capacity to negate the negative effects of plain MCT feeding (Bourque et al., 2002 (Submitted)). Unexpectedly, this was not observed using the hamster model. We report that MCTo feeding increased circulating plasma total and non-HDL cholesterol fractions, which is a risk factor for the development of CVD. This result is not consistent to previous reports in hamsters (Nicollosi, 1998) and rats (Geelen, 1995), where MCT feeding was shown to decrease plasma LDL and total cholesterol levels. More specifically, our findings are in contrast to those of Woollett et al. (1992) who found that dietary treatments composed of C8:0 and C10:0 plus 0.12% pure cholesterol resulted in no detrimental effects on plasma LDL-cholesterol in hamsters. Contradictory findings may be attributed to the fact that our animals were fed MCTo in combination with 0.25% wt/wt pure cholesterol. Therefore, in our laboratory the same MCTo tested in both humans and animals has elicited different results leading us to focus our attention to the effects of ALA treatment.

α-Lipoic acid influenced the lipid profile through a significant increase in circulating HDL-cholesterol levels, which resulted in a concomitant decrease in the non-

HDL:HDL ratio. This shift in HDL provides evidence that ALA on its own may offer improvement to the CVD risk profile through a beneficial alteration in blood lipid components. Several authors have commented on the cardiovascular benefits of increasing circulating HDL-cholesterol levels (Williams, 1996; Wood, 1987; Manninin et al., 1988). Specifically, Williams (1996) reported that an increase of 1 mg/dL in HDL cholesterol translates into a 4.7% decrease in CVD mortality and a 29% decrease in the risk of developing heart disease in humans. Furthermore, there is growing evidence suggesting that HDL protects against the progression of atherosclerosis. The benefits of HDL-cholesterol are not only attributed to its capacity to scavenge cholesterol from tissues to be sent back to the liver but also by protecting blood vessel endothelium from injury (Holvoet et al., 1998). HDL has the capacity to inhibit smooth muscle cell proliferation (Holvoet et al., 1998), by offering protection against LDL oxidation through the removal of hydroperoxides (Holvoet et al., 1998; Singh et al., 1996), and by countering the formation of oxygen free radicals (Singh et al., 1996).

Despite the encouraging increase in HDL levels, it is important to keep in mind that clinically although we recognize that the beneficial value of an increase in HDL-cholesterol may exist, the predictability of a treatment agent may be dependent on a number of other factors (Furberg, 2001). Certainly, this may be the case for our present findings within the hamster model. Clearly, the predictability of ALA-mediated effects on human lipid metabolism require further exploration and confounders such as dietary habits, lifestyle, individual cholesterol metabolism, genetics, and environment could all play a key role in the magnitude of treatment effects and should be addressed in future research with humans.

One of the major concerns when feeding MCT is a potential increase in plasma triglyceride concentrations (Swift et al., 1992; Hill et al., 1990; Cater et al., 1997, Tsai et al., 1999). This was not observed in our study. This may be attributed to alpha-linolenic acid contained in flaxseed oil being converted to the long chain n-3 eicosapentanoic acid (EPA) and tempering any increase in triglycerides elicited from MCT feeding. One study in particular found that alpha-linolenic acid can suppress the triglyceride increasing capacities of MCT through an increase in EPA production (Singer, 1992), however, the author supplemented 60 ml of flaxseed oil per day in humans. It has also been reported that MCT do not alter plasma triglyceride levels (Hainer et al., 1994; Wardlaw et al. 1995; Asakura et al., 2000). This discrepancy regarding the effects of MCT on triglyceride levels makes it difficult to ascertain if the flaxseed oil is in fact down-regulating any potential rise in triglyceride levels. However, in a review by Harris (1997), the conclusion was drawn that hamster plasma triglyceride levels may not respond to n-3 PUFAs in the same manner as humans. If this is the case, then our study supports the findings that MCTo feeding does not affect circulating triglyceride levels in hamsters as reported in human studies.

α-Lipoic acid, MCTo, and MCTo/ALA all exhibited increased GSH levels compared to the HC control diet in both liver and heart tissues. GSH is one of the body's most important endogenous antioxidants responsible for free radical scavenging in all cell types (Busse et al., 1992; Arivazhagan et al., 2000). Thus all three dietary treatments containing bioactive components offered increased antioxidant protection to hepatic and cardiac tissues when compared to the hyper-cholesterolemic control diet. However, neither diet proved to be more effective than the other.

Similar results of oxidized glutathione (GSSG) concentrations in liver and heart tissues were observed, with both tissues unexpectedly having increased GSSG levels after supplementation with ALA. After absorption into the cells of tissues ALA is reduced to its dithiol form, dihydrolipoic acid (DHLA). DHLA is a strong reducing agent that is capable of converting GSSG to GSH (Haramake & Handekman, 1997). However, despite this action we observed increased GSSG levels in both tissues. Packer et al. (1997) comment that this action by dehyrolipoamide dehydrogenase to reduce ALA to DHLA shows a marked preference for the R-enantomer of ALA. Thus, in the current study where a racemic mixture was supplemented the overall cellular levels of the highly active DHLA may not have reached a beneficial threshold.

In relation to the observed findings surrounding tissue glutathione status, there are a number of pertinent issues, which lend themselves to further discussion. Firstly, Upchurch et al. 1997 reported that elevated concentrations of homocysteine have the capacity to decrease the expression of cellular glutathione peroxidase in vitro. Interestingly, previous results from our laboratory (Bourque et al., 2002 (Submitted)) have shown that consumption of the same MCTo containing phytosterols and n-3 fatty acids cause an increase in plasma homocysteine concentrations in overweight women. Although these assays were not preformed in the present study, glutathione peroxidase activity may be altered thus making interpretation of the results increasingly difficult. Furthermore, it is known that disulphides can be reduced by other thiols (Biewenga et al., 1997b). The same authors in 1996 reported that in principle, GSH is able to reduce the disulphide DHLA, however, this reaction is said to proceed slowly and has never been observed by this research group. In light of such facts, it is feasible in theory that GSH

was recycling ALA in our hamster model causing an abundance of GSSG formation, however, proof of this mechanism remains to be seen in vivo.

In a recent study by Jones et al. (2002), examined the uptake and antioxidant actions of ALA in endothelial cell cultures. Results indicated that at concentrations of ALA greater than 0.5mM in cell culture, there is a concomitant fall in cellular GSH, NADPH, and NADH. The authors comment that the reducing capacity of the cellular system is taxed at high ALA concentrations, such that GSH is oxidized in response to increased oxidative stress within the cells. Unfortunately, cellular concentrations of ALA were not measured in the current study, and therefore it is not possible to know if our animals experienced ALA concentrations that reached this threshold, however, we did see a significant increase in oxidized glutathione in both liver and heart tissues. Thus, the importance of measuring ALA concentrations in both plasma and tissues should not be overlooked in future studies examining oxidative status in animal models.

The lack of change observed in hamster body weight and body composition do not support the advantages proposed of MCT utilization as an adjunct to weight management. In addition, our results do not support findings in rats where MCT feeding led to a decrease in fat tissue deposition and overall weight loss (Baba et al., 1982; Geleibter et al., 1983; Chanez et al., 1991; Hwang et al., 1993). However, it is noted that the aforementioned studies fed between 30-50% of total kcal in the form of dietary fat. The present study utilized 10% of energy as fat, which is double that of the outlined requirements for hamsters. In contrast, our findings that MCTo feeding had no effect on overall body weight does support previous work in rats published by Hill et al. (1993).

Moreover, it was shown that feeding MCTo and ALA exhibited no adverse effects on the normal growth and development of hamsters.

A possible explanation as to why no changes in body weight were observed could be related to the immature age of the hamsters used in this trial. Young animals likely have immature digestion, absorption, and therefore transport of lipids (Bach et al., 1996). Since our animals began the experimental period at 5 weeks of age, it is possible that the animals were too young to manifest an effect from the dietary treatments. Bach et al. (1996) also state that the proportion of C8:0 and C10:0 in the MCT oil tested has the potential to alter the oils functioning. Octanoate has been described to exhibit increased oxidation rates, a lower energy supply, and a decreased ability to form complex lipids. Therefore, it is possible that an unfavorable ratio of C8:0 to C10:0 fatty acids may have led to our varying results of MCTo feeding in the hamster.

Studies by Gleiter et al. (1996) and Hermann et al. (1996) have examined the influence of dietary components and the bioavailability of ALA in humans. The overall bioavailability of ALA has been reported to range from 20-38% depending on the isomer [(R)-lipoic acid or (S)-lipoic acid] and the formulation tested (Hermann et al., 1996). Our study utilized a powdered synthetic racemic mixture of ALA. With regards to absorption, Hermann et al. (1996) found that ALA is absorbed more slowly as an oral tablet compared to the rapid absorption of a prepared oral solution. The present study, outlines the effects elicited from a powdered compound which may have greater potential for action if provided to the animals in the form of an oral solution, there by, improving the overall absorption into the biological system. Hermann et al., 1996 also discuss the structural similarity between ALA and MCT. In fact, it has been reported that de novo

synthesis of ALA originates from octanoic acid (C8:0) and cysteine within the mitochondria (Biewenga et al., 1997b; Morikawa et al., 2001). Hermann et al. (1996) go on to report that the hepatic uptake of ALA may be carrier-mediated and selectively inhibited by medium chain fatty acids. Hence, in our study where ALA and MCTo were fed in combination, there exists the potential for competitive absorption into the liver which may have affected the results of our combination treatment group (MCTo/ALA). In addition, Gleiter et al. (1996) found that in humans the absorption of racemic ALA decreased significantly when given with a meal. Thus, this group of researchers suggests that in order to achieve maximal absorption and hence a therapeutic effect, ALA is best ingested on an empty stomach. In contrast, we incorporated the ALA into the lipid fraction of the synthetic diet, therefore the dose received was always in the presence of food. Therefore, it seems reasonable to propose that possible interactions with other dietary components may have reduced the overall absorption of ALA although conclusive evidence of this phenomenon was not measured.

In conclusion, MCT administered in combination with phytosterols, flaxseed oil, and ALA does not offer increased benefits to the risk factor profile of CVD when tested in the hamster model. This study does, however, provide significant additions to the scientific knowledge of ALA supplementation. ALA was not shown to offer any measured benefits on hamster oxidant status, however, ALA was shown to significantly increase circulating HDL-cholesterol levels in hamsters which lends evidence to a protective role of ALA in the development of cardiovascular disease.

7. GENERAL CONCLUSION

This study examined the efficacy of a MCT oil mixture (MCTo) containing cholesterol lowering phytosterols and triglyceride modulating n-3 fatty acids and α-lipoic acid (ALA) on plasma lipid levels and oxidative status in the hamster. The novelty of the present study was the combination of such a wide range of potentially bioactive components into a single feeding treatment. The MCTo tested in our study was prepared by Forbes Medi-Tech Inc. (Vancouver, BC) and has been used in two other human feeding studies conducted by our research lab. This study was different in that the antioxidant ALA was incorporated to determine whether any synergistic effects could be elucidated. However, as the results indicate the feeding of MCTo to hamsters in this study was clearly atherogenic and ineffective in eliciting changes in weight or body composition. Since the MCTo was designed for use in humans, the phytosterol content was established to be 22 mg/kg of body weight in order to provide a daily dose of 1.81 g and a mean concentration of 2.1% wt/wt of oil in order to provide cholesterol-lowering benefits. However, when you examine this dose in a small hamster fed the same oil as 7.5% wt/wt of diet, the phytosterol intake becomes approximately 12 mg/ day. This value is significantly less than phytosterol levels fed in previous hamster trials in our lab where hamsters were receiving upwards of 79 mg/day in cholesterol-modulation trials (Ntanios et al., 1998). This difference may be the reason that the MCTo remained atherogenic despite the addition of phytosterols. The major finding of the present study is the favorable shift in the HDL component of blood lipids which translated into a significant decrease in the non-HDL:HDL ratio when hamsters were supplemented with ALA. This shift has a positive influence on the overall CVD risk profile.

In conclusion, the present study established that our MCT oil mixture, developed for human consumption, was not an appropriate dietary treatment to test any synergistic actions with ALA, as the actions of the MCTo previously shown to exist in human trials did not exert themselves in the hamster model. In addition, ALA in a racemic mixture fed at 0.3% wt/wt of diet did not appear to offer any improvement in liver and heart tissue antioxidant status as measured through GSH, GSSG, and TBARS concentrations.

Future research should include a number of feeding trials in conjunction with ALA supplementation in order to clarify which isomer of ALA is most effective be it *R*, *S* or a racemic blend. Once the most effective form of ALA is identified the supplementation methods should be studied in order to discover the best dosing method, such as tablets, powder, oral solutions, and/or intravenous injections. Further, a dose response trial is necessary to work toward identifying an active and efficacious threshold for supplementation. Once these questions are answered then the concomitant addition of ALA to other bioactive components can be attempted. Moreover, when examining the potential effects of an antioxidant the importance of designing an experiment that encompasses a full array of assays covering absorption, tissue and plasma concentrations, enzyme functioning, and antioxidant status must be attempted in order to draw valuable results and a complete picture of the substances mechanism of action.

BIBLIOGRAPHY

Abbey M., P. Clifton, M. Kestin, B. Belling, P. Nestel. 1990. Effect of fish oil on lipoproteins, lecithin :cholesterol acyltransferase, and lipid transfer protein activity in humans. Atherosclerosis. 10:85-94.

Angelucci L., E. Mascitelli-Coriandoli. 1958. Anticholesterol activity of α-lipoic acid. Nature. 181: 911-912.

Arivazhagan P., P. Juliet, C. Panneerselvam. 2000. Effect of DL-α-lipoic acid on the status of lipid peroxidation and antioxidants in aged rats. Pharmacological Research. 41:299-303.

Asakura L., A.M.P. Lottenburg, M.Q.T.S Neves et al. 2000. Dietary medium chain triacylglycerol prevents the postprandial rise in plasma triacylglycerols but induces hypercholesterolemia in primary hypertriglyceridemic subjects. American Journal of Clinical Nutrition. 71:701-705.

Asherio A., E.B. Rimm, M.J. Stampfer, E.L. Giovannucci, W.C. Willett. 1995. Dietary intake of marine n-3 fatty acids, fish intake, and the risk of coronary disease among men. The New England Journal of Medicine. 332:977-982.

Asakawa T., S. Matsushita. 1980. Thiobarbituric acid test for detecting lipid peroxides. Lipids. 14:401-406.

Baba N., E. Bracco, S. Hashim. 1982. Enhanced thermogenesis and diminished deposition of fat in response to overfeeding with diet containing medium-chain triglyceride. American Journal of Clinical Nutrition. 35:678-682.

Bach A.C., Y. Ingenbleek, A. Frey. 1996. The usefulness of dietary medium-chain triglycerides in body weight control: fact or fancy? Journal of Lipid Research. 37:708-726.

Bell S.J., D. Bradley, R.A. Forse, B.R. Bistrian.1997. The new dietary fats in health and disease. Journal of the American Dietetic Association. 97:280-286.

Biewenga G. P., G.R.M.M. Haenen, A. Bast. 1997a. The pharmacology of the antioxidant lipoic acid. General Pharmacology. 29:315-331.

Biewenga G.P., G.R.M.M. Haenen, A. Bast. Ed. J. Fuchs, L. Packer, G. Zimmer. 1997b. An Overview of Lipoate Chemistry. In: Lipoic Acid in Health and Disease. Marcel Dekker, New York. pp.1-32.

Binnert C., C. Pachiaudi, M. Beylot, D. Hans, J. Vandermander, P. Chantre, J.P. Riou, M. Laville. 1998. Influence of human obesity on the metabolic fate of dietary long- and medium-chain triacylglycerols. American Journal of Clinical Nutrition. 67:595-601.

Bourque C., MP. St-Onge, A.A. Papamandjaris, J.S. Cohn, P.J. Jones. 2002. Consumption of a functional oil composed of medium chain triglycerols, phytosterols, and n-3 fatty acids improves the overall cardiovascular risk profile of overweight women. Metabolism. Submitted.

Busby R.W., J.P.M. Schelvis, D.S. Yu, G.T. Babcock, M.A. Marletta. 1999. Lipoic acid biosynthesis: LipA is an iron-sulphur protein. Journal of the American Chemical Society. 121:4706-4707.

Busse E., G. Zimmer, B. Schopohl, B. Kornhuber. 1992. Influence of alpha-lipoic acid on intracellular glutathione in vitro and in vivo. Arzneimittel-Forschung. 42:829-831.

Bustamante J., J.K. Lodge, L. Marcocci, H.J. Tritschler, L. Packer, B.H. Rihn. 1998. α-Lipoic acid in liver metabolism and disease. Free Radical Biology & Medicine. 24:1023-1039.

Calabrese C., S. Myer, S. Munson, P. Turet, T.C. Birdsall. 1999. A cross-over study of the effect of a single oral feeding of medium chain triglyceride oil vs. canola oil on post-ingestion plasma triglyceride levels in healthy men. Alternative Medicine Reviews. 4:23-28.

Caspary W.F. 1992. Physiology and pathophysiology of intestinal absorption. American Journal of Clinical Nutrition. 55S:299S-308S.

Cater N.B., H.J. Heller, M.A. Denke. 1997. Comparison of the effects of medium-chain triacylglycerols, palm oil, and high oleic acid sunflower oil on plasma triacylglycerol fatty acids and lipoprotein concentrations in humans. American Journal of Clinical Nutrition. 65:41-45.

Chanez M., B. Bois-Joyeux, M. Arnaud, J. Peret. 1991. Metabolic effects in rats of a diet with a moderate level of medium-chain triglycerides. Journal of Nutrition. 121:595-594.

Christian W.G., C.H. Hennekens. Ed. J-C. Tardif, M.G. Bourassa. 2000. Antioxidant vitamins and cardiovascular disease: Evidence from observational epidemiologic studies and randomized trials. In: Developments in Cardiovascular Medicine: Antioxidants and Cardiovascular Disease. v. 233. Kluwer Academic Press, Netherlands. pp. 135-145.

Clement J. 1980. Intestinal absorption of triglycerides. Reproduction Nutrition and Development. 20:1285-1307.

Conner W.E. 2000. Importance of n-3 fatty acids in health and disease. American Journal of Clinical Nutrition. 171:171S-175S

Constatinescu A., U. Pick, G. Handelman, N. Haramaki, D. Han, M.Podda, H.J. Tritchler, L. Packer. 1995. Reduction and transport of lipoic acid by human erythrocytes. Biochemistry and Pharmacology. 50:253-261.

Crozier G.L. 1988. Medium chain triglyceride feeding over the long term: The metabolic fate of [^{14}C] Octanoate and [^{14}C] Oleate in isolated rat hepatocytes. Journal of Nutrition. 118:297-304.

Estrada E., H. S. Ewart, T. Tsakiridis, A. Volchuk, T. Ramlal, H. Tritschler, A. Klip.1996. Stimulation of glucose uptake by the natural coenzyme α-lipoic acid/thioctic acid: Participation of elements of the insulin signalling pathway. Diabetes. 45:1798-1805.

Evans J.L., I.D. Goldfine. 2000. α-Lipoic acid: A multifunctional antioxidant that improves insulin sensitivity in patients with Type II diabetes. Diabetes Technology & Therapeutics. 2:401-413.

Furberg C.D. 2001. The usefulness of information on HDL-cholesterol: potential pitfalls of conventional assumptions. Current Controlled Trials in Cardiovascular Medicine. 2:107-108.

Ford I., M.A. Cotter, N.E. Cameron, M. Greaves. 2001. The effects of treatment with α-lipoic acid or evening primrose oil on vascular haemostatic and lipid risk factors, blood flow, and peripheral nerve conduction in the streptozotocin-diabetic rat. Metabolism. 50:868-875.

Forgione M., J. Loscalzo. Ed. J-C. Tardif, M.G. Bourassa. 2000. The antioxidant hypothesis In: Developments in Cardiovascular Medicine: Antioxidants and Cardiovascular Disease. v.233. Kluwer Academic Press, Netherlands. pp. 47-57.

Friedewald W.T., Levy R.I., Fredrickson D.S. 1972. Estimation of the concentration of low density lipoprotein cholesterol in plasma, without use of the preparative ultracentrifuge. Clinical Chemistry 18:499-502.

Geelen M.J.H., W.J. Schoots, C. Bijleveld, A.C. Beynen. 1995. Dietary medium chain fatty acids raise and (n-3) polyunsaturated fatty acids lower hepatic triacylglycerol synthesis in rats. Journal of Nutrition. 125:2449-2456.

Geliebter A., N. Torbay, E. Bracco, S. Hashim, T. Van Itallie. 1983. Overfeeding with medium-chain triglyceride diet results in diminished deposition of fat. American Journal of Clinical Nutrition. 37:1-4.

Gleiter C.H., B.S. Schug, R. Hermann, M. Elze, H.H. Blume, U. Gundert-Remy. 1996. Influence of food intake on the bioavailability of thioctic acid enantiomers. European Journal of Clinical Pharmacology. 50:513-514.

Gylling, H., and Miettinen, T.A. 1999. Cholesterol reduction by different plant stanol mixtures with variable fat intake. Metabolism 48:575-580.

Hainer V., M. Kunesova, V. Stich, A. Zak, J. Parizkova. 1994. The role of oils containing triacylglycerols and medium-chain fatty acids in the dietary treatment of obesity. The effect on resting energy expenditure and serum lipids. (Abstract in English). Casopis Lekaru Ceskych. 133:373-375.

Han D., G. Handelman, L. Marcocci, C. Sen, S. Roy, H. Kobuchi, H.J. Tritschler, L. Flohe, L. Packer. 1997. Lipoic acid increases *de novo* synthesis of cellular glutathione by improving cystine utilization. Biofactors. 6:321-338.

Hagen T.M., J. Liu, J. Lykkesfeldt, C.M. Wehr, R.T. Ingersoll, V. Vinarsky, J.C. Bartholomew, B.N. Ames. 2002. Feeding acetyl-$_L$-carnitine and lipoic acid to old rats significantly improves metabolic function while decreasing oxidative stress. Proceedings of the National Academy of Sciences of the United States of America. 99:1870-1875.

Hagen T.M., R.T. Ingersoll, J. Lykkesfeldt, J. Liu, C.M. Wehr, V. Vinarsky, J.C. Bartholomew, B.N. Ames. 1999. (R)-α-lipoic acid-supplemented old rats have improved mitochondrial function, decreased oxidative damage, and increased metabolic rate. FASEB Journal. 13:411-418.

Haramake N., G.J. Handelman. Ed. J. Fuchs, L. Packer, G. Zimmer. 1997. Tissue specific pathways of alpha-lipoate reduction in mammalian systems. In: Lipoic Acid in Health and Disease. Marcel Dekker, New York. pp. 145-162.

Harris W.S. 1997. n-3 fatty acids and serum lipoproteins: animal studies. American Journal of Clinical Nutrition. 65S:1611S-1615S.

Harrison E.H., D.B. McCormick.1974. The metabolism of dl-[1, 6-14C] lipoic acid in the rat. Archives of Biochemistry and Biophysiology. 160:514-522.

Health Canada. 1997. Heart Disease and Stroke in Canada. Health and Protection Branch-Lab Center for Disease Control. Ottawa (ON).

Hermann R., G.Niebch, H.O. Borbe, H. Fieger-Büschges, P. Ruus, H. Nowak, H. Riethmüller, M. Peukert, H. Blume.1996. Enantioselective pharmacokinetics and bioavailability of different racemic α-lipoic acid formulations in healthy volunteers. European Journal of Pharmaceutical Sciences. 4:167-174.

Hill J.O., J.C. Peters, L.L. Swift, D. Yang, T. Sharp, N. Abumrad, H.L. Greene. 1990. Changes in blood lipids during six days of overfeeding with medium or long chain triglycerides. Journal of Lipid Research. 31:407-416.

Hill J.O., J.C. Peters, D. Lin, F. Yakubu, H. Greene, L. Swift. 1993. Lipid accumulation and body fat distribution is influenced by type of dietary fat fed to rats. International Journal of Obesity. 17:223-236.

Hoffman M.A., H.J. Tritschler, A. Bierhaus, R. Zeigler, P. Wahl. P. Nawroth. Ed. J. Fuchs, L. Packer, G. Zimmer. 2000. Lipoate effects on atherogenesis. In: Lipoic Acid in Health and Disease. Marcel Dekker, New York. pp. 321-336.

Holvoet P. and D. Collen. 1998. Oxidation of low density lipoproteins in the pathogenesis of atherosclerosis. Atherosclerosis. 137S:S33-S38.

Hwang S.G., H. Yano, R. Kawashima. 1993. Influence of dietary medium-chain and long-chain triglycerides on fat deposition and lipogenic enzyme activities in rats. Journal of the American College of Nutrition. 12:643-650.

Ivanov V.N. 1974. Effect of lipoic acid on tissue respiration in rabbits with experimental atherosclerosis. Cor et Vasa. 16:141-150.

Jacob S., E.J. Henriksen, H.J. Tritschler, H.J. Augustin, G.J. Dietze.1996. Improvement if insulin-stimulated glucose-disposal in type 2 diabetes after repeated parenteral administration of thioctic acid. Experimental and Clinical Endocrinology and Diabetes. 104: 284-288.

Johnson R.C., S.K. Young, R. Cotter, L. Lin, W.B. Rowe. 1990. Medium chain triglyceride lipid emulsion: metabolism and tissue distribution. American Journal of Clinical Nutrition. 52:502-508.

Jones W., X. Li, Z. Qu, L. Perriott, R.R. Whiteshell, J.M. May. 2002. Uptake, recycling, and antioxidant actions of α-lipoic acid in endothelial cells. Free Radical Biology and Medicine. 33:83-93.

Jones P.J.H., F.Y. Ntanios, M. Raeini-Sarjaz, C. Vanstone. 1999. Cholesterol-lowering efficacy of a sitostanol-containing phytosterol mixture with a prudent diet in hyperlipidemic men. American Journal of Clinical Nutrition. 69:1144-1150.

Jones P.J., MacDougall D.E., Ntanios F., Vanstone C.A. 1997. Dietary phytosterols as cholesterol lowering agents in humans. Canadian Journal of Physiology and Pharmacology. 75:217-227.

Khanna S., M. Atalay, D.E. Laaksonen, M. Gul, S. Roy, C.K. Sen.1999. Alpha-lipoic acid supplementation: Tissue glutathione homeostasis at rest and after exercise. Journal of Applied Physiology. 86:1191-1196.

Kagan W., B. Kuklinski, C. Ruhlmann, C. Plotz. 1992. Recycling of vitamin E in human low density lipoproteins. Journal of Lipid Research. 33:385-97.

Kishi Y., J.D. Schmelzer, J.K. Yao, P.J. Zollman, K.K. Nickander, H.J. Tritschler, P.A. 1999. Low. α-Lipoic acid: Effect on glucose uptake, sorbitol pathway, and energy metabolism in experimental diabetic neuropathy. Diabetes. 48:2045-2051.

Kokkinos P.F., B. Fernhall. 1999. Physical activity and high density lipoprotein cholesterol levels. What is the relationship? Sports Medicine. 28:307-314.

Konrad T., P. Vicini, K. Kusterer, A. Hoflich, A. Assadkhani, H.J. Bohles, A. Sewell, H.J. Tritschler, C. Cobelli, K.H. Usadel. 1999. α-Lipoic acid treatment decreases serum lactate and pyruvate concentrations and improves glucose effectiveness in lean and obese patients with Type II diabetes. Diabetes Care. 22:280-287.

Kritchevesky D. 1958. Anticholesterol activity of α-lipoic acid. Nature. 182:396.

Kubow S., N. Goyette, S. Kermasha, J. Stewart-Phillip, K.G. Koski. 1996. Vitamin E inhibits fish oil induced hyperlipidemia and tissue lipid peroxidation in hamsters. Lipids. 31:839-847.

Law M. 2000. Plant sterols and stanol margarines and health. British Medical Journal. 320:861-864.

Leaf A., H.A. Halleq. 1992. The role of nutrition in the functioning of the cardiovascular system. Nutrition Reviews. 50:402-406.

Leyton J., P.J. Drury, M.A. Crawford. 1987. Differential oxidation of saturated and unsaturated fatty acids in vivo in the rat. British Journal of Nutrition. 57:383-393.

Ling W.H., P.J.H. Jones. 1995. Dietary phytosterols: A review of metabolism and side effects. Life Sciences. 57:195-206.

Lodge J.K., M.G. Traber, L. Packer. 1998. Thiol chelation of Cu2+ by dihydrolipoic acid prevents human low density lipoprotein peroxidation. Free Radical Biology & Medicine. 25: 287-297.

Lykkesfeldt J., T.M. Hagen, V. Vinarsky, B.N. Ames. 1998. Age-associated decline in ascorbic acid concentration, recycling, and biosynthesis in rat hepatocytes- reversal with (R)-α-lipoic acid supplementation. FASEB Journal. 12:1183-1189.

Manninen V., M.O. Elo, M.H. Frick. 1988. Lipid alterations and decline in the incidence of coronary heart disease in the Helsinki Heart Study. Journal of the American Medical Association. 260:641-651.

Mantzioris E., M.J. James, R.A. Gibson, L.G. Cleland. 1994. Dietary substitution with an α-linolenic acid-rich vegetable oil increases eicosapentaenoic acid concentrations in tissues. American Journal of Clinical Nutrition. 59:1304-1309.

McDonald G.B., D.R. Saunders, M. Weidman, L. Fisher. 1980. Portal venous transport of long-chain fatty acids absorbed from rat intestine. American Journal of Physiology. 239:G141-G150.

Marangon K., S. Devaraj, O. Tirosh, L. Packer, I. Jialal. 1999. Comparison of the effect of α-lipoic acid and α-tocopherol supplementation on measures of oxidative stress. Free Radical Biology & Medicine. 27:1114-1121.

Marquet A., B. Tse Sum Bui, D. Florentin. 2001. Biosynthesis of biotin and lipoic acid. Vitamins and Hormones. 61:51-94.

Mascioli E.A., S. Lopes, S. Randall, K.A. Porter, G. Kater, Y. Hirschberg, V.K. Babayan, B.R. Bistrian, G.L. Blackburn. 1989. Serum fatty acid profiles after intravenous medium-chain triglyceride administration. Lipids. 24:793-798.

Meijer G.W., A. van Tol, T.J.C. van Berkel, J.A. Weststrate. 2001. Effect of dietary elaidic versus vaccenic acid on blood and liver lipids in the hamster. Atherosclerosis. 157:31-40.

Midaoui A., J. de Champlain. 2002. Prevention of hypertension, insulin resistance, and oxidative stress by α-lipoic acid. Hypertension. 39:303-307.

Miettinen T.A., P. Puska, H. Gylling, H. Vanhanan, E. Vartiainen. 1995. Reduction of serum cholesterol with sitostanol-ester margarine in a mildly hypercholesterolemic population. The New England Journal of Medicine. 333:1308-1312.

Moini H., O. Tirosh, Y.C. Park, K.-J. Cho, L. Packer. 2002. R-α-lipoic acid action on cell redox status, the insulin receptor, and glucose uptake in 3T3-L1 adipocytes. Archives of Biochemistry and Biophysics. 397:384-391.

Morikawa T. R. Yasuno, H. Wada. 2001. Do animal cells synthesize lipoic acid? Identification of a mouse cDNA encoding a lipoic acid synthase located in mitochondria. Federation of European Biochemical Societies. 498:16-21.

Nicollosi R.J., T.A. Wilson, E.J. Rogers, and D. Kritchevsky.1998. Effects of specific fatty acids (8:0, 14:0, *cis*-18:1, *trans*-18:1) on plasma lipoproteins, early atherogenic potential, and LDL oxidative properties in the hamster. Journal of Lipid Research. 39:1972-1980.

Obrosova I., X. Cao, D.A. Greene, M.J. Stevens. 1998. Diabetes-induced changes in lens antioxidant status, glucose utilization and energy metabolism: Effect of DL-α-lipoic acid. Diabetologia. 41:1442-1450.

Pace N., E.N. Rathburn. 1945. Studies on body composition III: The body water and chemically combined nitrogen content in relation to fat content. Journal of Biological Chemistry. 158:685-691.

Packer L., E.H. Witt, H. Tritschler. 1995. Alpha-lipoic acid as a biological antioxidant. Free Radical Biology & Medicine. 19:227-250.

Packer L., H.J. Tritschler. 1996. Alpha-lipoic acid: The metabolic antioxidant. Free Radical Biology & Medicine. 20:625-626.

Packer L. S. Roy, C.K. Sen. 1997. α-Lipoic acid: A metabolic antioxidant and potential redox modulator of transcription. Advances in Pharmacology. 38:79-101.

Packer L., Kraemer K., Rimbach G. 2001. Molecular aspects of lipoic acid in the prevention of diabetes complications. Nutrition. 17:888-895.

Papas A.M. (editor). 1998. Antioxidant status, diet, nutrition, and health. CRC Press, Boca Raton, FL. pp. 650.

Pegorier J.P., P.H. Duee, C. Herbin, P.Y. Laulan, C. Balde, J. Girard. 1988. Fatty acid metabolism in hepatocytes isolated from rats adapted to high-fat diets containing long- or medium-chain triacylglycerols. Biochemistry Journal. 249:801-806.

Pelletier X., S. Belbraouet, D. Mirabel, F. Mordret, J.L. Perrin, X. Pages, G. Derby. 1995. A diet moderately enriched in phytosterols lowers plasma cholesterol concentrations in normocholesterolemic humans. Annals of Nutrition and Metabolism. 39:291-295.

Poirier J., K. Cockell, N. Hidiroglou, R. Madere, K. Trick, S. Kubow. 2002. The effects of vitamin E and selenium intake on oxidative stress and plasma lipids in hamsters fed fish oil. Lipids. In Press.

Quinn M.T., Parthasarathy S., Fong L.G., Steinberg D. 1987. Oxidatively modified low density lipoproteins: a potential role in recruitment and retention of monocyte/macrophages during atherogenesis. Proceedings of the National Academy of Science, USA. 84:2995-2998.

Rahnau K.J., H.P. Meissnert, J.R. Finn, M. Reljanovic, M. Lobisch, K. Schütte, D. Nehrdich, H.J. Tritschler, H. Mehnert, D. Ziegler. 1999. Effects of 3-week oral treatment with antioxidant thioctic acid (α-lipoic acid) in symptomatic diabetic polyneuropathy. Diabetic Medicine. 16:1040-1043.

Reed L. 1998. From lipoic acid to multi-enzyme complexes. Protein Science. 7:220-224.

Rimm E.B., M.J. Stampfer, A. Ascherio, E. Giovannucci, G.A. Colditz, W.C. Willet. 1993. Vitamin E consumption and the risk of coronary heart disease in men. New England Journal of Medicine. 328:1450-1456.

Roberts S.S. 2001. The diabetes advisor: Alpha-lipoic acid shows promise for treating diabetic nerve damage. Diabetes Forecast. July:19-22.

Schmidt A.M., O. Hori, J. Brett. 1994. Cellular receptor for advanced glycation end products: Implications for induction of oxidative stress and cellular dysfunction in the pathogenesis of vascular lesions. Atherosclerosis and Thrombosis. 14:1521-1528.

Schupke H., R. Hempel, G. Peter, R. Hermann, K. Wessel, J. Engel, T. Kronbach. 2001. New metabolic pathways of α-lipoic acid. Drug Metabolism and Disposition. 29: 855-862.

Segermann J., A. Hotze, H. Ulrich, G.S. Rao.1991. Effect of α-lipoic acid on the peripheral conversion of thyroxine to triiodothyronine and on serum lipid-, protein- and glucose levels. Arzneim.-Forsch./Drug Research. 41:1294-1298.

Shapiro K., W.C. Gong. 2002. Natural products used for diabetes. Journal of the American Pharmaceutical Association. 42:217-226.

Shih J.C. 1983. Atherosclerosis in Japanese quail and the effect of lipoic acid. Federation Proceedings. 42:2494-2497.

Simon J.A. 1995. Serum fatty acids and the risk of stroke. Stroke. 26:778-782.

Singer P., M. Wirth, I. Berger, B. Heinrich, W. Gödicke, S. Voigt, C. Taube, W. Jaross, S. Gehrisch. 1992. Long chain ω3 fatty acids are the most effective polyunsaturated fatty acids for dietary prevention and treatment of cardiovascular risk factors. World Reviews in Nutrition and Dietetics. 69:74-112.

Singh K., R. Chander, N.K. Kapoor. 1997. High density lipoprotein subclasses inhibit low density lipoprotein oxidation. Indian Journal of Biochemistry and Biophysics. 34:313-318.

Skaper S.D., M. Fabris, V. Ferrari, M.D. Carbonare, A. Leon. 1997. Quercetin protects cutaneous tissue-associated cell types including sensory neurons from oxidative stress induced by glutathione depletion: cooperative effects of ascorbic acid. Free Radical Biology and Medicine. 22:669-678.

Stampfer M.J., C.H. Hennekens, J.E. Manson, G.A. Colditz, B. Rosner, W.C. Willet. 1993. Vitamin E consumption and the risk of coronary disease in women. New England Journal of Medicine. 328:1444-1449.

Steinberg D. 1997. Low density lipoprotein oxidation and its pathobiological significance. Journal of Biological Chemistry 272:20963-6.

Swift L.L., Hill J.O., J.C. Peters, H.L. Greene. 1992. Plasma lipids and lipoproteins during 6- d of maintenance feeding with long-chain, medium chain, and mixed chain triglycerides. American Journal of Clinical Nutrition. 56:881-886.

Tardif J-C., M.G. Bourassa (editors) .2000. Developments in Cardiovascular Medicine: Antioxidants and Cardiovascular Disease. Kluwer Academic Press, Netherlands. v 233. pp. 258.

Teichert J., J. Kern, H.-J. Tritschler, H. Ulrich, R. Preib.1998. Investigations on the pharmacokinetics of α-lipoic acid in healthy volunteers. International Journal of Clinical Pharmacology and Therapeutics. 36:625-628.

Tsai Y.H., S. Park, J. Kovacic, J.T. Snook. 1999. Mechanisms mediating lipoprotein responses to diets with medium-chain triglyceride and lauric acid. Lipids. 34:895-905.

Upchurch G.R., G.N. Welch, A.J. Fabian, J.E. Freedman, J.L. Johnson, J.F. Keany, J. Loscalzo. 1997. Homocyst(e)ine decreases bioavailable nitric oxide by a mechanism involving glutathione peroxidase. The Journal of Biological Chemistry. 272:17012-17017.

Vasdev S., C.A. Ford, S. Parai, L. Longerich, V. Gadag. 2000. Dietary α-lipoic acid supplementation lowers blood pressure in spontaneously hypertensive rats. Journal of Hypertension. 18:567-573.

Vallot A., A. Bernard, H. Carlier. 1985. Influence of the diet on the portal and lymph transport of decanoic acid in rats. Simultaneous study of its mucosal metabolism. Comparative Biochemistry and Physiology. 82:693-699.

Wada H., D.Shintani, J. Ohlrogge. 1997. Why do mitochondria synthesize fatty acids? Evidence for involvement in lipoic acid production. Proceedings of the National Academy of Sciences. 94:1591-1596.

Wardlaw G.M., J.T. Snook, S. Park. 1995. Reflective effects of serum lipids and apolipoproteins of a caprenin-rich diet compared with diets rich in palm oil/palm kernal oil or butter. American Journal of Clinical Nutrition. 61:535-542.

Westhuysen J. 1997. The oxidation hypothesis of atherosclerosis: an update. Annals of Clinical Laboratory Science. 27:1-10.

Williams P.T. 1996. High-density lipoprotein cholesterol and other risk factors for coronary heart disease in female runners. The New England Journal of Medicine. 334:1298-1303.

Wood P.D. 1987. A round table: The health benefits of exercise (part 1 of 2). The Physician and Sportsmedicine, Minneapolis. 15(10):115-132.

Wong S.H.Y., J.A. Knight, S.M. Hopfer, O. Zaharia, C.N. Jr. Leach, F.W. Sunderman. 1987. Lipidperoxides in plasma as measured by liquid-chromatographic separation of malondialdehyde-thiobarbituric acid adduct. Clinical Chemistry. 33: 214-220.

Woollett L.A., D.K. Spady, J.M. Dietschy.1992. Regulatory effects of the saturated fatty acids 6:0 through 18:0 on hepatic low density lipoprotein receptor activity in the hamster. Journal of Clinical Investigation. 80:1133-1141.

Ziegler D., M. Reljanovic, H. Mehnert, F.A. Gries. 1999. α-Lipoic acid in the treatment of diabetic polyneuropathy in Germany: Current evidence from clinical trials. Experimental and Clinical Endocrinology, Diabetes. 107:421-430.

APPENDIX 1