THE PENNSYLVANIA STATE UNIVERSITY
SCHREYER HONORS COLLEGE

Department of Nutritional Sciences

Feasibility and Acceptance of a Low-Methionine Diet

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Fall 2011

A thesis
submitted in partial fulfillment
of the requirements
for a baccalaureate degree
in Nutritional Sciences
with honors in Nutritional Sciences

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Abstract

The cancer rates in the United States and other countries of the world continue to rapidly increase and many lives are lost each year to this horrible disease. Advances in surgical procedures, radiation therapy and new chemotherapy as well as other medications have increased survival rates and allowed patients to live abundant lives after cancer. Although these therapies are beneficial and lifesaving, they also produce adverse side effects that can lead to future health problems. Because of these unfavorable side effects, the idea of constructing a diet to aid in the prevention and treatment is of significant interest. Past research has shown that a low-methionine diet may promote overall health, and help prevent and treat cancerous tumors.

The objective of this research is to examine the probability that a healthy adult could maintain and follow a diet low in methionine, as well as supplement the diet with a beverage that provides the necessary nutrients, without experiencing adverse medical effects. Daily monitoring, pre-study and post-study questionnaires were successfully developed to evaluate the ability of participants to tolerate and maintain a low-methionine diet regimen for eight weeks. Overall, this study has shown that the low-methionine diet prescription is feasible and that for the most part, the participants can cope with the required liquid supplement during a limited time. However, in order for the many health advantages of a low-methionine diet to be realized, it is crucial that a drink is developed that is more palatable and can be tolerated over longer periods of time.

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Acknowledgements

I would like to thank Dr. Mary Lou Kiel for all of her help, guidance and encouragement in the past few years that I have had the privilege of working with her on this project. She opened doorways and helped me obtain first-hand experience working in a kitchen setting, entering diet records, helping process blood work etc. I would like to thank Dr. Terryl Hartman for taking me under her wing and accepting me as a thesis student during some very difficult times. I would also like to thank her for the quick responses to emails and all the helpful feedback I needed to produce a finished product. I would like to thank Dr. Rebecca Corwin for being a support system and for making herself available when I had any questions. I would like to thank my father, Dr. Kevin Koudela for putting up with endless complaints on my end and talking me through a step-by-step approach to writing a thesis. I would also like to thank him for helping me edit, and re-edit my thesis. I would like to thank Debbie Corl for helping me format my thesis and for fixing details that I would have missed on my own. I would like to thank my friends and family for supporting me through this process and for loving me and supporting me through the past 22 years of my life; without all of them I would not have made it to where I am today.

Chapter 1

Introduction

1.1 Statement of the Problem:

The cancer rates in the United States and other countries of the world continue to rapidly increase and many lives are lost each year to this horrible disease. Advances in surgical procedures, radiation therapy and new chemotherapy as well as other medications have increased survival rates and allowed patients to live abundant lives after cancer. Although these therapies are beneficial and lifesaving, they also produce adverse side effects that can lead to future health problems. Because of these unfavorable side effects, the idea of constructing a diet to aid in the prevention and treatment is of significant interest. Calorie restriction has been examined and has been shown to control tumor growth; however, it is an unrealistic dietary change that may actually have more adverse physical effects than health benefits (1).

Other holistic approaches have been studied in advanced stage tumors. These include various diet regimes such as the garlic diet, the Brandt Grape Cure diet, and the High Dose and Intravenous Vitamin C diet (2). A Low-methionine diet has consistently shown a positive correlation with malignancy control and is being researched as a possible cancer treatment (3,4,5).

Methionine is an essential amino acid that must be derived from the diet. Cysteine and methionine are the only two sulfur amino acids. One of methionine's most important functions is supplying sulfur to the body in order to aid in normal growth and metabolism. It also contributes methyl groups to other compounds in order to help with a variety of chemical and

metabolic reactions. Additionally, methionine is necessary for removing heavy metals from the body (6).

Methionine belongs to a group of compounds called lipotropics, which help the liver process fat in the body. Methionine is converted in the liver to S-adenosyl methionine (SAM) to be used as the metabolite of the amino acid. Methionine is mainly found in animal products, such as meat, fish and dairy, but can also be found in whole grains and nuts (6).

1.2 Objectives

The objective of this thesis was to examine the possibility that a healthy adult could maintain and follow a diet low in methionine, as well as supplement the diet with a beverage that provides the necessary nutrients, without experiencing adverse medical effects. Data were gathered during the Biomarker Study of Methionine Restriction in Healthy Adults. Previously, this team of investigators demonstrated that methionine restriction dramatically increased the lifespan of rats, in addition to delaying the onset of age-related diseases, including cancer. The long-term goal of the investigators is to determine possible beneficial effects of using methionine restriction to prevent age-related chronic diseases, specifically in high-risk persons. This pilot study looked to evaluate the feasibility of the diet program by assessing compliance and palatability over the course of the study period. The Biomarker Study of Methionine Restriction in Healthy Adults also aimed to evaluate various biomarkers that may affect oxidative stress and inflammation.

Pre and post-study questionnaires were administered that asked participants to evaluate the taste, appearance, consistency and flavor of both the control and methionine-free drinks. Participants were questioned about whether or not they could follow the diet program for: 1) a

three-week period, 2) a six-month period if faced with a chronic illness, and 3) a six-month period if a chronic illness would be prevented. Pre and post-study questionnaires were then compared and analyzed to contrast the subjects' feelings before they began the study and after completing the eight-week diet program.

In addition to the pre and post-study questionnaires, daily monitoring forms were also completed by each of the participants. These were used to determine how well the participant was adhering to the diet, whether or not the participant was able to consume all the drink and how they felt during each day. These data were also evaluated to determine whether or not a diet, such as this one could be manageable. If it is found that a large number of people experienced adverse effects from the drink, or had adverse physical or psychological affects while on the diet, use of the drink and diet program as an alternative treatment method for cancer or other age-related diseases may not be feasible.

1.3 Specific Aims

The following define the specific aims of this thesis:

1. Assess whether or not the methionine-free commercially available beverage powder (Hominex, Abbott Laboratories) can be combined with other ingredients to create a beverage that people can tolerate.
2. Demonstrate that a low-methionine dietary plan can be developed that meets nutrient needs.
3. Develop and pilot-test questionnaires that can be completed daily, as well as pre-study and post-study questionnaires, to adequately evaluate the participants' feelings toward the taste and compliance of the supplement.

1.4 Hypothesis

Healthy individuals following a low-methionine diet will be able to comply with the diet protocol for three weeks without adverse medical or psychological effects. When feeding a low-methionine diet, it is still necessary to provide the other essential amino acids to sustain normal body functions. For this and previous studies, a methionine-free commercial beverage (Hominex, Abbott Laboratories) has been used in combination with foods that are naturally low in methionine (7). However, a person must be able to tolerate and maintain the diet, which will or will not be determined as part of the current research. Questionnaires were developed in this research to indicate if the participants are able to withstand the low-methionine diet and drink for the three-week study period.

Chapter 2

Review of Literature

The aging process is responsible for the decline in proper bodily function, ultimately leading to death. With the average lifespan increasing over time, many older adults have experienced the onset of chronic and debilitating illnesses, including diabetes, cardiovascular disease and various cancers. The Baltimore Longitudinal Study of Aging looked at aging in two different dimensions: aging as it relates to each individual person and the relationship between disease and aging (8). A number of conclusions were drawn in the study that centered on the overall idea that diet and exercise are the keys to a long and healthy lifespan (8). Over the past few decades, researchers have examined calorie-restriction, as well as methionine-restriction as possible dietary options for increasing lifespan and controlling chronic diseases.

2.1 Calorie Restriction

Animal research has suggested that calorie restriction may provide positive health benefits. Anderson et al. showed the effects of calorie restriction on mice. These researchers compared body tissues from three different age groups of mice: adolescent, middle-aged and older adult. Overall, they were able to conclude that the animals that had restricted calories had an overall decrease in adiposity, increased insulin sensitivity and reduced levels of oxidative stress (9). These three physical and biological findings are markers for good health and indicators of positive aging.

A study conducted on mice evaluated the physiological and clinical implications of calorie restriction over the lifespan of the mice. Similar to the results of Anderson et al., these researchers found that there was an overall decrease in insulin sensitivity and that glucose

tolerance improved. Another valuable finding was that they were able to show a direct link with body composition and percent calorie restriction. A calorie restriction of 20% caused a body weight change of 20% (1). This connection was also found by other researchers that performed an extensive literature review on a number of calorie-restrictive feeding programs (10).The authors concluded, however, that the link between body composition and percent calorie restriction only correlated with a feeding regime that started very early in life, not one that began in later years. They also showed a number of positive correlations between health and calorie restriction that included a reduction in oxidative damage, in addition to a decrease in chronic inflammation and an overall stronger sympathetic nervous system (10).

In a human trial entitled CALERIE (Comprehensive Assessment of Long-term Effects of Reducing Intake of Energy), researchers examined calorie restriction on an overweight population of individuals (12). The subjects were asked to consume between 20-30% less calories than their typical caloric intake. Results were compared to those found in the mice study, including a lower fasting insulin level and a lower core body temperature (11).

Lifespan relating to calorie restriction was also studied and showed favorable results in B6 and B10C3F mice. Although the authors were unsure if this data could translate into human applications, they found that B6 mice increased their longevity by approximately 20% and B10C3F mice increased their overall maximum lifespan by approximately 10% (12). The B10C3F mice generally live longer than B6 mice, therefore, both experienced approximately the same absolute lifespan increase (12). Nevertheless there were also negative findings, including excessive hunger and signs of malnutrition. Mice would ravenously attack food when placed in front of them and showed constant signs of hunger and psychological distress (1). These observations may indicate that an energy-restricted diet may not be feasible. In a country where

food is abundant and eating is often a social event, people may not respond well to a diet that restricts caloric intake.

2.2 Methionine Background

Because diet changes are reasonable and realistic approaches to creating a healthy lifestyle, specific nutrients have been examined and shown to cause positive effects on health. Methionine, in particular, has been studied and data has shown promising results. Methionine is an essential, sulfur amino acid that can be found in a number of food products, such as beef, poultry, nut, grains, fish and eggs. Because it is essential, a person must consume methionine in the diet. Methionine has many functions in the body including breaking down fats, helping to remove heavy metals from the body, increasing the size of muscles, and assisting in maintaining healthy nails, skin and hair. It provides sulfur in the body to promote muscle development and is part of a group called lipotropics, which assist in fat breakdown (13). Methionine restriction is much more tolerable than calorie restriction and tends to provide similar benefits. An example of this was shown in a study conducted on Drosophilia, a common fruit fly, which examined the effects calorie restriction and methionine restriction had on fecundity and lifespan. These findings allowed the researchers to conclude that calorie restriction did not have an effect on lifespan and decreased fecundity, whereas methionine restriction increased lifespan and did not have an adverse affect on fecundity (14).

2.3 Methionine Restriction and Animal Feeding Studies Relating to Lifespan

Over the past few decades, methionine restriction has been studied in animals, to show the overall health effects it may have on lifespan and chronic disease. One of the first informative studies was conducted by Orentreich et al. on Fisher 344 male rats. Rats in the

experimental group were fed 0.17% methionine of the diet and rats in the control group were fed 0.86% methionine. The methionine levels were closely monitored because methionine intake that dropped below 0.17% showed adverse health effects, and methionine levels dropping below 0.12% caused death amongst the rats. Results showed that the methionine restricted animals did not gain weight over the course of the trial, yet actually ate 93% more food per gram of body weight than the control-fed rats. They also had a greater median and maximum lifespan and a greater lean body mass to fat ratio (15).

A study conducted on 80 female mice showed similar results, yet used methionine-restricted levels of 0.12% as their standard methionine amount, which had been shown in the previous study to cause death among rats (16). This could be attributed to a measurement error or a different response between species. Insulin-like growth factor (IGF-1) and thyroxine (T4) were studied closely in this particular study. Mice on the methionine-restricted diet had significantly lower IGF-1 (257±66 vs. 397±125 ng mL-1) and T4 (2.7±0.7 vs. 3.5±1.1 µg mL-1) than the control group ($P<0.01$ and $P<0.01$, respectively). Methionine restriction also produced less oxidative stress, cataracts and necropsy. The authors concluded that the methionine-restricted diet slowed the effects of aging and produced positive overall effects on body functionality. In addition to the above biological findings, the mice on the methionine-restricted feeding regimen weighed approximately 40% less than the control rats while consuming only about 10% less total calories. They did, however, consume more calories per gram of body weight than the control mice, similar to the findings in the previous rat study (16).

Researchers who conducted a male F-344 rat feeding study looked more specifically at the effects of methionine restriction on tissue glutathione (GSH) levels (17). Glutathione is an antioxidant that helps to prevent oxidative damage in cells by free radicals and peroxides.

Because cancer can be caused by oxidative damage, higher glutathione levels have been linked with health promotion and cancer prevention. In this study, rats in the experimental group were given a methionine-restricted diet of 0.17% and the controlled group was given a diet containing 0.86% methionine. In the control group, GSH levels remained stable and no significant changes were reported on cysteine levels. In the methionine-restricted group, however, GSH levels increased 139% after only one week, and continued to increase until week six, where GSH leveled off at a total increase of 190%. This study found that there is a significant difference in GSH levels in different tissues of methionine-restricted rats. The liver, blood, kidney and pancreas, organs that play a role in production and delivery of GSH through interorgan transport system, showed the greatest effect of methionine-restriction. The kidneys showed a lesser decrease in GSH level because it has high levels of y-glutamyltranspeptidase, which breaks down cysteine-containing precursors following the tissue uptake of GSH. Cysteine levels in the methionine-restricted group decreased by 40% after one week. Although specific metabolic mechanisms for the effects of methionine-restricted diet on GSH need to be further investigated, they found that optimal levels of GSH in most extraheptic tissue were present despite methionine restriction (17).

A study conducted in 2006 by Malloy et al. (18) showed similar results during their methionine-restricted feeding study. Oral glucose tolerance tests were performed followed by the testing of cholesterol, triglycerides, and IGF-1 levels. Plasma insulin, glucose, leptin, adiponectin, IGF-1, T4 and T3 levels were measured after 80 weeks during post-mortem examination of the rats. Growth of methionine-restricted rats was significantly lower than that of the control fed group. This trend was consistent with the previous animal feeding studies (15,16,17). Control fed rats gained 80% body weight after six weeks compared to a 31% weight

gain in the methionine-restricted group ($P<0.0001$). Methionine-restricted rats were found to have a 65% reduction in IGF-1 levels and a 72% reduction in visceral fat. A strong positive correlation was found between leptin levels and adipose tissue, which indicated that despite a 21% reduction in food intake, dietary methionine content played a specific role in leptin and visceral fat levels. Methionine-restricted rats also had higher daily energy expenditure (7.02 kcal±0.59) compared to the control group (5.12 kcal±0.33) and the pair-fed group (5.97 kcal±0.45). Because of the restricted methionine diet, plasma methionine levels were reduced (18).

With the preponderance of research reporting positive effects of methionine restriction related to aging, researchers began questioning the effects of an elevated level of methionine. A study was conducted using Fischer rats that investigated the effects of methionine supplementation (19). They found there was a large amount of oxidative damage in the rat liver, yet the rat heart remained unaffected. Their overall conclusion was that excess methionine is actually detrimental, and could potentially lead to oxidative damage throughout body tissues (19). With the connection between methionine and oxidative damage established throughout various animal studies, scientists looked to the idea that a restricted diet may have potential effects on cancer prevention, growth and treatment.

2.4 Methionine Restriction and Animal Feeding Studies relating to Malignant Tumors

Methionine restriction is becoming a more and more promising approach for the prevention and treatment of cancer. A study conducted by Mecham et al. (20) examined the malignancy of cell lines dependent on methionine. A total 23 cell lines were evaluated covering an abundance of common cancers occurring throughout the body. Of the 23, eleven showed complete methionine dependence, including strains of breast, kidney, prostate, bladder and

osteogenic sarcoma cancer. Of the other 12, three showed moderate dependence, which meant they did not grow as well in the methionine-restricted medium (20).

A similar study conducted by Guo et al. (21), used a technique called sponge-gel supported histoculture to grow 21 human tumors surgically removed from cancer patients to test whether or not methionine dependence occurs in fresh patient tumors and in cell lines. Unlike the results found in Mecham et al. (20) study, only five of the 21 human tumors, including breast, prostate, melanoma, colon and ovarian were methionine dependent (21).

Specific types of cancers have been studied to determine the effects that methionine-restriction had on each type's growth and development. Poirson-Bichat et al. (20) looked at human prostate cancer (PC-3) that had been surgically implanted in mice. The authors found that proliferation rates of the cell lines PC-3 and DU-145, both human prostate cancer lines, were reduced to 29% and 78%, respectively. The investigators concluded that there was methionine dependency in the PC-3 line, but not in DU-145. The addition of ethionine, an antagonist amino acid to methionine, increased the effects of a methionine-restricted diet. When added, cell replication decreased by fivefold, in addition to the decrease that occurred when just using methionine-restriction. The tumor growth of the mice fed a methionine containing diet did not differ from the control group; however, the methionine-restricted diet caused 34% decrease in tumor growth on day 20 and 51% on day 32. When combined with ethionine, a 56% decrease in tumor growth by day 20 occurred, although there was little additional effect by day 32 (22).

Not only has research showed that malignant tumors respond to methionine-restriction, studies have linked a low-methionine diet with various chemotherapies. Because many cancer cell lines respond so well to a low-methionine diet prescription, researchers are optimistic that

linking it with current treatments may increase the effects of both treatment options. Research done by Goncalves et al. (21) examined xenografted human tumors in nude mice with high levels of glutathione and/or an over expression of the P-glycoprotein (Pgp) or the multidrug resistance-related protein (MRP). Both Pgp and MRP lead to drug resistance and are major causes of the failure of anticancer chemotherapy. Cisplatin, a popular chemotherapy drug, was administered in conjunction with a methionine-restricted diet. The overall goal was to evaluate the effectiveness of the chemotherapy drug alone (usual care) and in combination with the diet plan. The growth of TC71-MA, a colon cancer cell line, was specifically studied because colon cancer had been shown previously to be sensitive to a methionine-free diet (21). Under normal circumstances, Cisplatin alone rarely inhibits the growth of TC71-MA. When methionine-restriction was coupled with Cisplatin, there was a 56% growth inhibition of the tumor. When combined with ethionine, results showed a 61% growth inhibition and a 3.3 fold increase in survival rates (3).

2.5 Methionine Restriction and Human Feeding Studies relating to Malignant Tumors

Animal studies are an excellent way to gain pertinent information that can often be applied to human subjects; however the results are not always completely translational. To date, there have only been a few stage-one clinical trials that have looked at methionine restriction and cancer growth in humans. The findings are extremely promising and have prompted additional research beyond acceptability and the basic effects of the diet on health outcomes. A phase one clinical trial conducted by Epner (4) was one of the first research studies to examine methionine restriction in adult patients that had advanced solid tumors. Twelve participants were assigned a diet with no methionine for the first two weeks, and then added approximately 2 mg/kg/day into the diet, which equated to 5-10% of a typical methionine intake. The study was used primarily

to assess the safety of the diet or if modifications needed to be made to preclude adversely affecting the health of the patients. Results concluded that serum albumin levels remained stable, which meant that protein synthesis was not blocked. Although there was no conclusive data on whether tumor activity was reduced, a number of the participants did experience some antitumor activity. For example, one patient with hormone independent prostate cancer experienced over a 25% reduction in serum prostate specific antigen (PSA). PSA is a protein produced by cells of the prostate gland. It is used as a biomarker in prostate cancer detection and follow-up. Because only one participant had this type of cancer, it is difficult to make any general conclusions based on a single participant's response (4).

Epner et al. (5) completed another phase one clinical human feeding study, looking again at the safety of the overall diet prescription and examining more closely the plasma methionine levels. The only side effect, which in some cases was beneficial, was a reduction of approximately 0.5% of each participant's baseline body mass index (BMI). As was seen in Epner's previous trial, serum albumin and prealbumin levels remained stable and for some patients even increased, which allowed them to conclude that malnutrition was not a major concern. Overall plasma methionine levels fell an average of 58% within two weeks. With the indicated tumor cell lines that are sensitive to methionine restriction, Epner concluded that this could be promising if given to patients undergoing treatment of the cancers determined to be dependent on methionine (5).

A more specific human study, similar to studies conducted on other mammalian species, looked at the effects of low-methionine in combination with various chemotherapies. The FOLFOX regimen, a 48-hour combined chemotherapy regimen of 5-FU, leucovorin and oxaliplatin, is used very strictly in patients with advanced, metastatic colorectal cancer. Because

of its harsh side effects, patients who are already in debilitated states often have difficultly responding to the program. This clinical trial enrolled 11 participants undergoing FOLFOX to begin taking it in cycles with a methionine-restricted diet. Only four of the eleven participants were able to complete more than four cycles. From the original eleven patients, the authors were able to demonstrate that by day one, there was a 58% decrease in plasma methionine levels and by day three 43%. Of the four remaining patients, three of them partially responded to treatment and one of them had a complete stabilized remission (23).

Research done by Harris et al. (24) evaluated the effects of methionine restriction on incidence of ovarian cancer. This study raised some interesting and very controversial information. The data gathered showed an inverse association between methionine intake and ovarian cancer risk (OR=0.72%, 95% CI=0.60-0.87), meaning women had a decreased risk of ovarian cancer when intakes of methionine were normal or even elevated. The authors' goal was to evaluate nutrients involved in the one-carbon metabolic pathway such as folate, and vitamins B6 and B12, and alcohol and their impact on ovarian cancer. The researchers were careful in choosing a very large participation pool (n=1910) and eliminated any unusual eaters, whose regular diet may have had an effect on these nutrients. Because most of the data were based on food frequency questionnaires during the year prior to starting the study, these data may be misrepresented. Also, given that there were so many different nutrients studied, the inverse relationship between methionine and ovarian cancer may be a chance finding. The authors noted this in their discussion and recommended more data be gathered before drawing specific conclusions (24).

2.6 Methionine Restriction relating to General Health

With all the positive data surrounding methionine and cancer research, other scientists have pointed out the overall health benefits that methionine restriction may have on aging and chronic diseases.

In a number of studies, one of the side effects of the methionine restricted treatment plan was weight loss (5,15,16,18,23). With the obesity crisis our nation is facing, this may not be an adverse outcome, especially in overweight or obese individuals. A sixteen-week human cohort study, conducted by Plaisance et al. (25) evaluated the effects of restricted methionine on metabolic disorders. Twenty-six subjects classified as being overweight or obese were put on a methionine-restricted diet that provided each participant with 2 mg/kg body weight/day. This had been reduced from a controlled methionine intake of 35mg/kg body weight/day. Reductions in methionine were achieved by consuming foods that did not contain methionine, and supplementing the rest of the diet with a methionine-free drink called Hominex-1 (Abbott Laboratories). Methionine restriction reduced plasma methionine by 13.8%+/- 3.8 compared to 1.2 +/- 5.1% increase in the control group. Plasma cysteine was reduced by 10% in both the control and methionine-restricted groups. Both groups lost weight and had a decrease of 10-15% in fasting plasma glucose and an increase of approximately 25% in plasma adiponectin. Fat oxidation was reduced by 12.1% in the methionine-restricted group compared to 8.1% in the control group. Even though minimal weight loss was noted in some of the participants, compliance for the diet was very low, which led the authors to conclude that additional data would need to be gathered to draw definitive conclusions (25).

Haulrik et al. (26) conducted a six-month controlled dietary intervention study that investigated the effects of high and low overall protein and methionine intakes on homocysteine levels in 65 overweight, yet otherwise healthy individuals. Plasma homocysteine levels have been positively associated with obesity. Each subject was assigned to one of three groups: 1) a low protein, low methionine diet (LP: 12% of energy, 1.4 g methionine/day), 2) high protein, high methionine diet (HP: 22% of diet, 2.7 g methionine/day) or 3) a control diet (an intermediate level of protein and methionine). The findings showed that plasma homocysteine levels were not affected by intakes of methionine and protein, but were inversely associated with intakes of folate, vitamin B6 and vitamin B12. Compliance with this diet was low, so the authors concluded that additional, long-term investigations need to be conducted in order to make a conclusive decision on the overall effects methionine has on homocysteine levels (26).

Based on the research found in the animal and human feeding studies, a diet low in methionine shows promising results. More information needs to be gathered; however, if strong data reveals conclusive evidence that low-methionine diets help to treat and prevent cancer, it is crucial that a diet-plan and supplemental drink are developed so that the diet regimen is possible and endurable. This thesis was important for determining whether or not the diet plan is tolerable for participants to sustain, and makes suggestions on how to alter the current program to make it more appealing for individuals. Without a diet program that is tolerable and feasible to follow, results based upon a low-methionine diet do not matter because the program cannot be implemented.

Chapter 3

Methodology

3.1 Subject Characteristics

Subject recruitment for the Biomarker Study of Methionine Restriction in Healthy Adults began during January of 2010 and extended through July of 2010. Flyers and regular newspaper and television advertisements were used to solicit participants for the study in State College, Pennsylvania, and the surrounding areas. A total of twelve subjects were selected to complete an eight-week feeding study. The majority of the subjects were female and ranged in age between 33 and 58. Each participant was required to have a body mass index (BMI) that fell below 35. Table 1 shows a list of all twelve subjects, their gender, age, height, weight and BMI prior to starting the diet plan.

Table 1 – Subject Descriptions

Subject Number	Gender	Age	Weight (lbs)	Height (in)	BMI
1	F	50	198.8	65.5	31.6
2	F	53	159.2	65.6	25.3
3	F	50	156.6	63.6	27.2
4	M	52	162.1	69	23.9
5	F	51	145.8	59.5	29
6	F	48	136.4	59.75	26.9
7	F	33	138.6	67	21.7
8	F	43	164.4	66.25	26.3
9	F	49	139.4	65.75	22.7
10	F	42	120.8	66.3	19.3
11	F	53	162	63.3	28.4
12	F	58	221.8	66.9	34.8

The subjects could not be taking any medications that would interfere with the biomarkers being evaluated as part of the overall study, and had to stop taking any dietary

antioxidant supplements for at least four weeks prior to initiating the study, as these would also interfere with the data being gathered. Initially, prospective subjects were asked to complete a telephone screening that ensured that the subjects meet the above requirements, and evaluated other factors such as health history, family history and lifestyle. Pregnant and lactating women were immediately disqualified from the study because research has not yet shown what effects limiting methionine in the diet may have on a fetus or breast-feeding infants. Because of this, women who participated in the study were also monitored for pregnancy during their regular blood draws. If a woman happened to become pregnant while participating in the study, she would be asked to discontinue the feeding program immediately.

3.2 Study Procedures

Once subjects were proven eligible through the telephone screening, they were asked to come to the General Clinical Research Center (GCRC) in Noll Lab at the Pennsylvania State University to complete additional testing. Pre-study blood tests were performed for each subject to assess their general health status. This blood work was used to further screen for conditions that resulted in the subject's ineligibility to participate in the methionine-restricted feeding study. Once cleared, the subjects were given a physical to determine their height, weight and waist circumference. These measurements were used to verify BMI and served as baseline data for comparison against additional measurements made during the course of the study.

During their initial visit to Noll Lab, subjects were given samples of the low-methionine and control drinks in both the lemon and orange flavors. They were asked whether or not they believed they could consume a significant amount of each drink for the duration of the study. If they indicated that they believed they could tolerate each drink, they were then given a pre-study questionnaire to rate their overall satisfaction with each specific drink and predict their ability to

consume the drinks for an extended period of time. These results were later compared with the results of the post-study questionnaire. During this initial visit, subjects were also provided daily menus, and were asked to screen themselves out if they felt they could not consume all the foods.

Before the participants actually began the methionine restricted feeding study, they were asked to complete three dietary recalls (two weekdays and one weekend) over the telephone with GCRC nutrition staff. Dietary recalls are a retrospective food analysis that collects information from a participant on all foods, beverages and dietary supplements consumed within the previous twenty-four hours. The foods were then entered into NDSR 2008 (University of Minnesota, Minneapolis), MN, a nutrient analysis program to assess usual diet and methionine intake. A resting metabolic rate procedure was completed for each subject to evaluate baseline daily calorie requirements and an appropriate activity factor was added to calculate a calorie intake level that would maintain their current body weight during the study. Daily food menus were custom designed for each participant to provide calorie requirements to the nearest 100 kilocalories, and could be adjusted during the study for weight loss or gain.

3.3 Diet and Drink Supplementation

The feeding study was designed as a randomized, controlled cross-over feeding protocol to be completed over an eight-week period. The subjects were randomized and divided into two groups, group A and group B. Group A was placed on a control diet for the first three weeks, followed by a two-week washout period, where participants were allowed to follow their diet of choice. The washout period was followed by a three-week period, where they were placed on the methionine restricted diet. Group B received the two diets in the opposite order.

Throughout this eight-week period, participants were only allowed to consume food from outside sources during the two-week washout period. For the three weeks that they followed the methionine-restricted diet and the three weeks they followed the control feeding diet, they were asked to only consume foods that the GCRC provided. Breakfast, lunch, dinner and snacks were prepared and two meals were packed out in a cooler for each day the participant was on the diet. Participants were required to eat one meal a day at the GCRC and at that time they were weighed and asked to report any problems with the diet. They were also instructed to report verbally and through written questionnaires any foods they did not consume. This was crucial information to ensure the subject was following the diet accurately.

The control diet and methionine restricted diet foods were the same, so that the subjects did not develop a bias for one diet or the other. All diets were based on a 2500 kilocalorie plan and were factored up or down to meet each participant's energy needs. The foods given provided 20-25% of total protein in the diet. All of the foods contained very low levels of methionine, such as fruits, vegetables and refined grains. An example of a daily menu is provided in Appendix A. The subjects rotated throughout a six-day cycle menu.

In addition to the food provided each day, all of the subjects were given a drink that would meet their protein requirements, as well as provide other nutrients and energy. These drinks differed based on whether the subject was on the control feeding diet or the low-methionine diet. Participants were told they should consume the drink over the duration of the day and make sure they were consuming the entire drink. The size of the drink varied based on caloric need. Drink amounts ranged from 800-1800g/day, depending on calorie level, which could range from 1400-3000 kcal. Subjects were also allowed to add water, diet soda or other non-caloric drinks to the mixture to improve the taste; however, they were informed that if they

did this they still needed to consume the entire drink. The supplemental drink contained 75-80% of total protein. GNC Pro Performance Soy Protein 95 and generic egg white powder were used in the control drink to provide adequate protein and the Recommended Dietary Allowance for methionine. Hominex-1, a medical food available for persons who cannot metabolize methionine, was used as the main ingredient in the experimental drink. Hominex-1 is an FDA approved medical food for treatment of patients with homocystinuria. A picture of the Hominex-1 is shown in Appendix B. With the presence of cysteine, cell proliferation has been shown to progress normally in the absence of methionine (17). Because of the limited amount of solid foods, Benefiber was also added to both drinks to add fiber in the diet to meet recommended levels.

A subject on a typical 2000 calorie diet would go through approximately 1/3 of a can of Hominex-1 a day. According to Abbott Laboratories, as of October, 2011, the price per can is $224.80. This would amount to approximately $500 per week just for the drink alone. Fortunately, we were graciously donated Hominex-1 to accommodate the study. If additional research shows that a low-methionine diet has health benefits and helps to control cancer incidence and progression, then the cost for this product would become an important consideration.

3.4 Data Collection and Questionnaires

To provide further information on the acceptability of the diet, the subjects were given a daily monitoring form in their food pack-out. These included the following queries: 1) Did you eat/drink ALL of the foods/drink provided? 2) Did you eat/drink any foods/drinks other than those provided? 3) How much water did you drink? 4) How many diet sodas did you drink? and 5) Did you take any medication? The subjects were also asked to fill out a survey each day to

monitor their overall satisfaction with their diet. These surveys mirrored the pre and post-study questionnaires the subjects were given, and were designed to draw conclusions on whether or not the diet is feasible. Responses to these questionnaires and surveys were monitored daily by GCRC nutrition staff to ensure that the study was running smoothly and the participant was not experiencing any adverse side effects.

Data from pre-study and post-study questionnaires were also used to draw conclusions about the methionine-restricted diet and liquid supplement. Pre-study questionnaires were filled out during each participant's initial visit. A total of six questions (three for experimental and three for control) were on the pre-study questionnaires. The survey asked the participants to rate 1) How well they believed they could tolerate consuming a quart of each beverage every day for three weeks, 2) the overall consistency and appearance of each drink, and 3) the overall flavor of each drink. The same six questions were repeated on the post-study questionnaire, in addition to general questions about the overall diet regimen. The six questions from the pre-study questionnaire and the post-study questionnaire were compared to show any changes that had occurred after each subject experienced the diet and liquid supplementation.

3.5 Analysis

During the eight-week period, blood work was conducted three times: the beginning and end points of each diet, as well as the mid-point. This thesis does not focus on the results of the blood work profile. Instead it examines results from the questionnaire responses, by calculating averages for each of the question responses at baseline and follow-up time points and changes in subject responses over time, as well as analyzing other feedback given by the participants.

Chapter 4

Results

4.1 Summary of Results

The results of the daily monitoring forms and pre and post-diet questionnaires discussed in Section 3.4 were used to test the initial hypothesis that healthy individuals following a low-methionine diet will be able to comply with the diet protocol for three weeks without adverse medical or psychological effects. To ensure an accurate assessment, each person was evaluated individually based on their daily monitoring surveys, as well as their pre-study questionnaire and post-study questionnaire response data. In addition, these data were then compared to data compiled for the entire participant pool, and averages as well as standard deviations were calculated. Both individual and pooled data were evaluated to determine feasibility of the diet, compliance with the diet prescription and acceptance of the low-methionine drink.

4.2 Individual Monitoring Form Data

During the course of the diet program, participants were asked to fill out a daily monitoring form including a series of questions related to their satisfaction with the diet plan, hunger between meals, fullness and satisfaction during meals, ease of following the diet plan, and ease of drinking the supplemental drink. They were also asked to provide the investigators with any additional comments or concerns. Answers were based on a seven-point Likert scale, with one always correlating to an unfavorable value and seven always correlating to a favorable value. Daily scores were averaged and standard deviations were calculated to provide information on how well the subjects felt on a day-to-day basis. Table 2 shows the results from one representative participant's daily monitoring form while on the control feeding diet. The

columns are labeled by question and the numbers in the columns correspond to the participant's responses to each specific question. Each row correlates to the day that the participant completed the survey.

Table 2 – Daily Monitoring Survey from a Representative Subject on the Control Diet

Subject Name	Subject ID#	Date Reporting About	Estimated oz H20	Diet Soda oz	1.Satisfaction with diet plan	2.Hunger between meals	3.Satiety value	4.Ease of following diet plan	5.Ease of drinking formula	Comments/Medications
BF		8/25/10	48	12	7	5	5	7	5	Synthroid 100ug daily
		8/26/10	24	0	5	5	5	6	5	
		8/27/10	48	0	6	6	6	6	5	
		8/28/10	48	0	6	7	7	7	5	
		8/29/10	16	8	6	6	6	6	5	
		8/30/10	16	0	6	6	6	6	5	
		8/31/10	16	0	6	3	3	6	6	Two bouts of muscle cramps
		9/1/10	16	12	6	5	4	6	6	Muscle cramp in foot
		9/2/10	16	0	6	6	6	6	6	Muscle cramp in leg
		9/3/10	16	0	6	6	6	6	6	
Drink A	1	9/4/10	24	0	6	6	6	6	6	
		9/5/10	16	0	6	4	4	6	6	
		9/6/10	24	0	6	6	6	6	6	
		9/7/10	16	0	6	6	6	6	6	
		9/8/10	24	0	6	6	6	6	6	
		9/9/10	16	0	6	6	6	6	6	Muscle cramp in leg and foot
		9/10/10	20	0	6	4	5	6	6	Leg muscle cramp
		9/11/10	16	0	6	6	6	6	6	Leg cramp
		9/12/10	24	0	6	6	6	6	6	
		9/13/10								Missing daily monitoring form
		9/14/10	24	0	6	6	6	6	6	Achy muscles all over
Averages - Control					6.0	5.6	5.6	6.1	5.7	
Standard Deviation					0.3	0.9	0.9	0.3	0.5	

Table 3 presents the same data for a participant on the experimental diet. The format of Table 3 is identical to Table 2. On average, very favorable responses were given with little change (STD) for all questions except for overall drink satisfaction. Almost all the participants

filled out questionnaires for every day they were on the diet. There were a few participants who missed a few days during the course of the two diet cycles. There were also two participants who did not finish the experimental diet period due to the inability to tolerate the drink, so therefore, they do not have the same number of dates (rows) as the other participants do. These two tables are from a representative subject, who completed all days for both diet

Table 3 - Daily Monitoring Survey from a Representative Subject on the Experimental Diet

Subject Name	Subject ID#	Date Reporting About	Estimated oz H20	Diet Soda oz	1.Satisfaction with diet plan	2.Hunger between meals	3.Satiety value	4.Ease of following diet plan	5.Ease of drinking formula	Comments/Medications
		9/29/10	24	0	6	7	7	5	2	
		9/30/10	24	0	6	7	7	5	2	
		10/1/10	24	0	6	6	7	5	2	Cramping in legs
		10/2/10	16	12	6	6	6	5	2	Muscle cramps
		10/3/10	24	12	6	4	4	6	2	Slight nausea
		10/4/10	32	0	6	6	6	6	3	
		10/5/10	32	0	6	5	5	6	3	
		10/6/10	24	0	5	5	5	6	3	
		10/7/10	48	12	5	5	5	6	3	
		10/8/10	24	0	6	5	5	6	3	Muscle aches in legs
Drink B	1	10/9/10	32	0	6	4	4	6	3	Muscle aches in legs
		10/10/10	32	0	6	5	5	6	3	Muscles aches in legs
		10/11/10	24	0	5	5	5	6	3	Muscles aches
		10/12/10	32	12	6	5	5	6	3	
		10/13/10	32	12	6	5	5	6	3	Likes lemon flavor better
		10/14/10	32	0	6	5	5	6	3	
		10/15/10	32	0	6	5	5	6	3	Didn't eat mandarin oranges
		10/16/10	24	0	6	5	5	6	3	Leg achy, twitching, sore
		10/17/10	24	12	5	5	4	6	3	Achy legs
		10/18/10	32	0	6	4	4	6	3	
Averages - Hominex					5.8	5.2	5.2	5.8	2.75	
Standard Deviation					0.41	0.83	0.95	0.41	0.44	

4.3 Averages and Standard Deviations of Daily Monitoring Forms

From the tables created for each individual, averages for each question over the course of each of the diet periods, control and experimental, were calculated, along with standard deviations. Table 4 shows the averages for each question during the control-feeding period for all twelve participants and table 5 displays this information during the experimental period. Participants eight and ten discontinued the study; however, it was during each of their second (experimental) diet periods and had no effect on the control questionnaires.

Table 5 displays the averages for each question during the experimental-feeding period for all twelve subjects. As indicated above and in the table, participants eight and ten both discontinued the study during the experimental-diet period due to the inability to continue consuming the liquid supplement. Because of this, their averages and standard deviations are computed only using the scores input for the amount of time they were able to comply with the diet (#8 completed 12 days of the experimental diet and #10 completed 5 days of the experimental diet).

Table 6 is a summary of all the averages for both the control-feeding study and the experimental-feeding study. The columns in this table correspond to the questions on the daily monitoring form. The first row represents the overall averages of all participants during the experimental diet period and second row represents the overall averages of all the participants during the control diet period. The table is used to show an overall comparison of the experimental and control diets for each question.

Table 4 – Participants' Control Diet Daily Monitoring from Responses[1]

	1. Satisfaction with Diet Plan	2. Hunger between meals	3. Satiety Value	4. Ease of following diet plan	5. Ease of drinking formula
Subject 1 - BF					
Averages	6.00±0.32	5.55±0.94	5.55±0.94	6.10±0.31	5.70±0.47
Subject 2 - TB					
Averages	5.75±0.55	2.85±0.75	3.40±0.82	6.05±0.51	6.15±0.67
Subject 3 - MY					
Averages	3.84±0.76	3.95±0.78	3.74±0.65	3.37±0.90	3.89±1.24
Subject 4 - NY					
Averages	4.67±0.69	5.06±0.64	4.78±0.81	4.44±0.62	5.50±0.62
Subject 5 - PD					
Averages	5.95±0.22	5.45±0.69	5.60±0.68	5.95±0.22	4.90±0.72
Subject 6 -DS					
Averages	3.68±0.82	2.58±0.84	2.95±1.27	6.84±0.37	6.89±0.32
Subject 7 - LK					
Averages	5.47±0.51	4.630.83	4.84±0.50	5.32±0.48	5.42±0.51
Subject 8 - TM					
Averages	5.10±0.31	4.60±0.60	4.75±0.44	5.00±0.00	4.65±0.49
Subject 9 - DP					
Averages	5.80±0.41	5.85±0.37	5.85±0.37	5.75±0.44	4.95±0.22
Subject 10 - TM					
Averages	5.80±0.41	5.53±1.41	6.00±0.93	5.27±1.03	5.60±0.51
Subject 11 - CR					
Averages	5.63±0.50	4.32±0.67	5.00±0.47	6.16±0.60	6.00±0.94
Subject 12 - EB					
Averages	5.63	5.89	5.63	2.58	2.58
Standard Deviation	0.50	0.32	0.96	0.69	0.61
Total Averages	5.28±0.80	4.69±1.11	4.84±1.00	5.24±1.24	5.19±1.13

[1] An answer of 1 is unfavorable and an answer of 7 is favorable

Table 5 – Participants' Experimental Diet Daily Monitoring Form Responses

	1. Satisfaction with Diet Plan	2. Hunger between meals	3. Satiety Value	4. Ease of following diet plan	5. Ease of drinking formula
Subject 1 - BF					
Averages	5.8±0.41	5.2±0.83	5.2±0.95	5.8±0.41	2.75±0.44
Subject 2 - TB					
Averages	6.15±0.37	5.80±0.52	5.70±0.57	5.85±0.59	1.95±0.60
Subject 3 - MY					
Averages	4.56±0.53	3.56±0.88	4.00±0.00	4.89±0.60	4.11±0.60
Subject 4 - NY					
Averages	5.22±0.83	5.33±1.12	5.33±1.22	5.11±0.78	4.44±0.53
Subject 5 - PD					
Averages	6.30±0.47	6.15±0.49	6.55±0.51	6.25±0.55	4.50±0.69
Subject 6 - DS					
Averages	3.25±0.64	2.10±1.12	2.15±0.99	7.00±0.00	2.35±0.49
Subject 7 - LK					
Averages	5.10±0.72	4.85±0.67	4.80±0.41	4.50±0.83	1.95±0.22
Subject 8 - TM					
Averages	4.08±0.29	4.17±0.39	4.17±0.39	4.00±0.00	1.33±0.49
Subject 9 - DP					
Averages	5.30±0.66	4.10±1.29	4.70±1.26	5.10±0.85	4.85±0.67
Subject 10 - TM					
Averages	6.00±0.00	6.25±0.50	6.25±0.50	4.25±0.96	1.75±0.50
Subject 11 - CR					
Averages	5.78±0.43	4.39±0.78	5.44±0.51	6.94±0.24	7.00±0.00
Subject 12 - EB					
Averages	4.90±1.65	6.05±0.39	6.00±0.32	2.55±0.51	2.05±0.51
Total Averages	**5.20±0.91**	**4.83±1.24**	**5.02±1.20**	**5.19±1.28**	**3.25±1.71**

As mentioned previously participants eight and ten quit before completing the entire experimental diet period; therefore, the summary averages could have been affected, and the values for the experimental diet period, specifically for the liquid supplement, could have been influenced.

Table 6 –Overall Averages of Diet Questionnaires

Diet Period	1. Satisfaction with Diet Plan	2. Hunger between meals	3. Satiety Value	4. Ease of following diet plan	5. Ease of drinking formula
Experimental Diet	5.20±0.91	4.83±1.24	5.02±1.20	5.19±1.28	3.25±1.71
Control Diet	5.28±0.80	4.69±1.11	4.84±1.00	5.24±1.24	5.19±1.13

4.4 Pre-Questionnaire and Post-Questionnaire Data

A pre-study questionnaire was conducted before each participant began the program. They were asked a series of six questions, three pertaining to the control drink and three pertaining to the experimental drink. These results are presented in Table 7. The columns are labeled and correspond with each of the six questions asked on the pre-study questionnaire. Drink X corresponds to the control diet and Drink Y was used during the experimental diet. The rows represent the responses given by every participant for each of the questions. The last row provides the overall averages of each question for all the participants.

Table 7 – Pre-Study Questionnaire Results

Subject Number	DX1: Rate how well you feel you could drink a quart of the beverage for 3 weeks	DX2:Rate the consistency and appearance of the drink	DX3: Rate the overall flavor of the drink	DY1: Rate how well you feel you could drink a quart of the beverage for 3 weeks	DY2:Rate the consistency and appearance of the drink	DY3: Rate the overall flavor of the drink
1	7	6	6	7	7	7
2	6	7	5	5	5	5
3	7	3	3	7	3	5
4	6	5	5	7	6	6
5	7	6	7	7	6	6
6	6	6	6	7	6	7
7	6	5	5	6	6	6
8	3	3	3	5	5	6
9	7	5	5	7	5	5
10	6	5	4	6	4	5
11	7	6	6	7	4	4
12	7	6	7	5	3	5
Averages	6.25	5.25	5.17	6.33	5.00	5.58

These same questions were asked again on the post-study questionnaire and compared with the answers from the pre-study questionnaire to see if the answers were comparable to each other, or if the subjects had changed their opinions based on their experience with the diet. Three additional questions were added to the post-diet questionnaire for each liquid supplement to survey which flavor they enjoyed the best (orange or lemon), whether or not the subjects believed they could complete the program for six months to treat a chronic illness and whether or not the subjects believed they could complete the program for six months to prevent a chronic illness. Table 8 shows post-diet questionnaire results for the control drink (X). The columns are labeled with the specific questions and each row represents all twelve of the subjects and their corresponding responses. Table 9 shows the results for the experimental drink (Y). Like Table

8, the columns are labeled with the specific questions and each row represents all twelve of the subjects and their corresponding responses.

Table 8 – Post-study Questionnaire Results for the Control Drink and Diet

Subject Number	DX1: Rate how well you feel you could drink a quart of the beverage for 3 weeks	DX2:Rate the consistency and appearance of the drink	DX3: Rate the overall flavor of the drink	DX4: Rate how well you feel you could follow the diet for 6 months to treat an illness	DX5: Rate how well you feel you could follow the diet for 6 months to prevent an illness
1	6	4	6	5	4
2	7	7	6	7	5
3	7	2	3	5	3
4	5	4	5	4	4
5	6	4	4	7	6
6	5	3	4	5	2
7	6	6	6	4	3
8	Withdrew				
9	7	6	7	7	7
10	Withdrew				
11	5	5	6	6	5
12	6	4	5	6	6
Averages	6	4.5	5.2	5.6	4.5

Table 10 is a comparison of the pre and post-study questionnaires to gain an understanding of whether or not the participant's opinions changed after being on the diet. The columns are labeled with each question. The first row represents the averages from the pre-diet questionnaire and the second row represents the averages from the post-diet questionnaire.

Table 9 – Post-study Questionnaire Results for the Experimental Drink and Diet

Subject Number	DY1: Rate how well you feel you could drink a quart of the beverage for 3 weeks	DY2:Rate the consistency and appearance of the drink	DY3: Rate the overall flavor of the drink	DY4: Rate how well you feel you could follow the diet for 6 months to treat an illness	DY5: Rate how well you feel you could follow the diet for 6 months to prevent an illness
1	3	4	3	3	3
2	1	5	2	4	1
3	7	2	6	6	5
4	4	3	4	5	5
5	3	3	3	6	5
6	2	3	2	3	1
7	2	3	2	3	2
8	Withdrew				
9	4	4	2	5	5
10	Withdrew				
11	5	5	3	6	6
12	2	1	2	6	6
Averages	3.30	3.30	2.90	4.70	3.90

Table 10 – Comparison of the Averages from the Pre and Post-Study Questionnaires

Diet Period	DX1: Rate how well you feel you could drink a quart of the beverage for 3 weeks	DX2:Rate the consistency and appearance of the drink	DX3: Rate the overall flavor of the drink	DY1: Rate how well you feel you could drink a quart of the beverage for 3 weeks	DY2:Rate the consistency and appearance of the drink	DY3: Rate the overall flavor of the drink
Pre-diet Questionnaire	6.25±1.14	5.25±1.22	5.17±1.34	6.33±0.89	5.00±1.28	5.58±0.90
Post-diet Questionnaire	6.00±0.82	4.50±1.51	5.20±1.23	3.30±1.77	3.30±1.25	2.90±1.29

4.5 Weight Changes during the Study

Body weight of each participant was measured on a daily basis beginning with the first day and continuing through the last day of each diet period. Table 11 shows the percent body weight changes during the control diet period. Each subject's starting weight, ending weight, weight change and percent body weight lost is calculated and shown in the table. Table 12 shows the percent body weight changes during the experimental diet period. Like Table 11, each subject's beginning weight, ending weight, weight change and percent body weight lost is calculated and presented.

Table 11 – Body Weight Changes for Participants during the Control Diet

Subject #	Beginning Weight (lbs)	End Weight (lbs)	Weight Change (lbs)	% Body Weight Loss
1	197	192	5	0.03
2	157	153.6	3.4	0.02
3	155	152.8	2.2	0.01
4	160.6	156.4	4.2	0.03
5	142.4	135.2	7.2	0.05
6	138.4	130.8	7.6	0.05
7	137.4	135	2.4	0.02
8	168.4	163.2	5.2	0.03
9	137	135.4	1.6	0.01
10	121.2	118.2	3	0.02
11	163.8	159.8	4	0.02
12	221.2	213.2	8	0.04
Average % Body Weight Loss on Control Diet				0.03

Table 12 - Body Weight Changes for Participants during the Experimental Diet

Subject #	Beginning Weight (lbs)	End Weight (lbs)	Weight Change (lbs)	% Body Weight Loss
1	194.2	190.4	3.8	0.02
2	155.2	153.4	1.8	0.01
3	159.4	155.6	3.8	0.02
4	161.8	159.6	2.2	0.01
5	144.6	140.4	4.2	0.03
6	135.2	128.5	6.7	0.05
7	134.2	130.8	3.4	0.03
8	164.8	Withdrew		
9	138.8	133.6	5.2	0.04
10	117.8	Withdrew		
11	168	163	5	0.03
12	225.4	215.2	10.2	0.05
Average % Body Weight Loss on Experimental Diet				0.03

Chapter 5

Discussion of Results

5.1 Summary of Results

Daily monitoring, pre-study and post-study questionnaires were successfully developed as part of this thesis to adequately evaluate the ability of participants to tolerate and maintain a low-methionine diet regimen for eight weeks. The results showed that on average, the majority of the participants did not have any severe adverse opinions about the diet and in general, most people believed they would be able to maintain and comply with the diet. For all the questions on the daily monitoring survey pertaining to the diet, average answers were all high except for participant 12 (Table 4 and Table 5). A few people indicated hunger between meals, but these numbers generally leveled out over time as people got used to consuming a smaller food volume. Generally, people who experienced the most difficulty were those whose normal diets contained more meat. Because both the control and experimental diet did not contain any meat products and resembled a vegan diet, these people were thrown off from their typical feeding routine. Mostly everyone felt satisfied with the overall diet plan and their satiety levels remained stable.

Of the twelve participants, only two participants were unable to complete the experimental-diet period. Both of these subjects experienced nausea and gastrointestinal problems while drinking the liquid supplement. They also both indicated hunger throughout the day while on the diet, as well as mild headaches. According to their personal daily monitoring forms, they both struggled with finishing the drink daily, which became a serious problem. Other findings show that a number of the subjects who completed the study were also dissatisfied with the low-methionine drink. The average rating for all the patients was

3.25±1.71, compared to 5.19±1.13 for the control drink (see Table 6). This number is not too unreasonable, but it may become an issue for future subjects who may be facing other complications. As stated by Epner et al (5), subjects enduring advanced stage malignant tumors were very willing to maintain a diet low in methionine (5). These subjects were not given a liquid supplement; however, it could be hypothesized that they would also be willing to comply with the diet prescription investigated in this thesis. It is a concern, however, that "healthy" patients on the diet faced difficulties consuming the drink when research shows that typically patients on chemotherapy have altered tastes. How their tastes are affected could further hinder their ability to maintain a low-methionine diet.

When analyzing the pre-study questionnaire (see Table 7), it is apparent that most people felt that they could consume both the control and methionine-free drink for a three-week period with limited complications. It is important to point out that the participants were only given approximately three ounces of each of the two drinks to taste test before the trial feeding period began. This is a very limited amount compared to what they were required to consume during the diet (approximately 32-64 ounces, based on calorie intake), which could have contributed to elevated positive responses for this questionnaire. The averages for both the control and experimental drinks were all five or above, indicating that the participants comfortably consumed the drinks. In fact, two of the three questions were rated higher for the methionine-free drink than the control drink.

When the pre-study questionnaires were compared to the post-study questionnaire data (Table 8 through 10), it was very apparent that after the diet period, opinions about the methionine-free drink had changed drastically. Averages for the control drink barely changed from the pre-study questionnaire to the post-study questionnaire, although numbers plummeted

for the experimental drink by approximately three points per question. This is critically important because the beverage provides most of the protein and calories in the diet. If the patient is unable to consume the drink, they would not be able to receive the necessary nutrients to maintain a healthy nutrient balance. A diet low in methionine not supplemented by a drink is nutritionally inadequate because it also is missing other essential amino acids and would ultimately promote protein breakdown.

Additional questions on the post-study questionnaire were included to determine whether or not the participant felt they could continue on the diet for six months to treat a chronic disease and for six months to prevent a chronic disease. These results were slightly better, showing an average response of 4.7 for treatment of a chronic disease and 3.9 for prevention of a chronic disease. Some additional comments added by participants were that they would have a "difficult time consuming the drink daily," and "the drink would be difficult to manage," although they would still be able to complete the diet program if in fact the diet was shown to prevent and treat a debilitating disease. This is important to note because the prescribed liquid supplement needs to be consumable for the diet to be successful.

When looking at body weight changes on the experimental diet and the control diet (Table 11 and 12), both diet periods saw an average 3% decrease in total percent body weight. Weight loss occurred during both diet periods, making it impossible to conclude that the low-methionine diet was more likely to contribute to weight loss. An extended trial with more participants would need to be conducted to gather conclusive evidence about body weight.

5.2 Results Compared to Past Findings

Years of research have concluded that low-methionine diets limit the rate of certain types of cancer cell growth in specific mammalian species. Healthy cells are not methionine-dependent. Because of this, researchers have hypothesized that a diet low in methionine could ultimately be used to help treat and prevent certain types of cancers (20,21,22). Low-methionine diets have been paired with chemotherapies and other treatments (3,21), but have not yet been tested on healthy individuals. This overall pilot study (summarized in Section 1.2) tested the effects that a low-methionine diet had on healthy participants in hopes of finding blood work correlations to conclude that it would be safe and manageable. If these results prove to be positive, further studies could be conducted to show if the low methionine diet could produce positive results in patients experiencing malignant tumors.

In previous studies, research has shown a correlation with weight loss and methionine-restriction (5,15,16,18,23). A study conducted by Plaisance et al. (25) did research on the effects of methionine-restriction on obese adults. Fat oxidation was reduced, and weight loss was noted; however, compliance was not high so the authors were unable to conclusively say that methionine-restriction directly influences weight loss. From the findings in this study, it is also difficult to make any correlations between methionine-restriction and weight loss because weight loss was experienced in the control group as well. Participants experienced a 3% average total body weight loss on both diet periods. This may be attributed to the controlled feeding regimen in both diet periods that restricted calories to the specific amounts needed, which limited outside snacking and did not include processed foods or desserts.

To date, no acceptance data about a methionine-free drink has been gathered among any participants. The goal of this thesis was to draw conclusions on compliance with all components

of the diet and whether or not the subjects had any adverse effects or feelings towards the diet protocol.

5.3 Implications of Results

A diet treatment for cessation of cancer growth and development is novel and an extremely encouraging possibility. For years, scientists have been researching prevention plans, as well as treatment options for metastatic tumors. If a simple diet restriction could help to prevent or even treat tumors, death rates could drop. This study examined whether or not a diet low in methionine is feasible and realistic, as well as healthy and beneficial. Even if the diet proves to be clinically successful and safe, it is crucial that the patients are able to tolerate and maintain the diet. From the questionnaires and daily monitoring forms, it is clear that the food plan for the diet can be followed and tolerated. Other than mild complaints of hunger between meals, adherence was relatively easy and the diet was well accepted. The low-methionine beverage, on the other hand, needs some modifications to improve the taste. Even though only 17% of the participants (2 out of 12) quit the study while on the experimental diet, the drink was disliked by many, if not all, of the participants. There were a number of complaints, including taste dissatisfaction and nausea caused by consumption of the liquid supplement. Although most subjects concluded that they could potentially drink the beverage as an alternative treatment for a chronic illness, almost all were dissatisfied with it and claimed they could not consistently consume Hominex-1 for prevention of minor health problems.

5.4 Limitations

The current pilot study conducted by Dr. John Richie, Penn State Milton S. Hershey Medical Center, included a limited number of participants. To this point, no extensive human

studies have been performed, therefore conclusive data is not yet available on whether or not a low-methionine diet will produce positive effects on aging, cancer prevention or cancer growth. In the Epner et al. (5) stage 1 clinical trial, a low-methionine diet did not affect serum albumin levels or protein synthesis. The investigators concluded preliminarily that the diet was safe and would not have any severe adverse effects (4,5). Another clinical trial using human participants also showed that the prescribed diet did not have any strong negative effects on the patient's body. In fact, when combined with a regime entitled FOLFOX, some patients experienced a remission of their malignant tumors (23). If the results of the blood work from this study are promising, it will provide evidence that a low-methionine diet would be healthy and sustainable for body cells. A more comprehensive clinical investigation will then need to be conducted to confirm the benefits of a low-methionine diet on aging and cancer prevention and cancer growth. At this point, however, the subjects' poor tolerance of the liquid supplement observed in my research may become an issue.

From the daily monitoring forms, we can conclude that the majority of the subjects complied with the diet. About half of the subjects complained of being hungry throughout the day, but seemed to adjust as the diet period progressed. These results were found on both the control and experimental diet periods. The averages of all the questions surveying their opinions about the food diet remained positive during both diet periods. The main issue for almost all participants was the low-methionine drink. Patients dealt with nausea and diarrhea, as well as cramps and indigestion while on the low-methionine drink. Some of these problems escalated in a few of the subjects, and as stated above, two of the twelve quit the diet all together. In addition, we received comments such as "I could never drink this drink for longer than a few weeks," and "I would never comply with this diet even under devastating circumstances." These

and other similar comments raise concerns about the overall compliance of long-term consumption of the beverage. This also prompts other questions about potentially feeding this diet to cancer patients, who already may have a suppressed appetite. It was hypothesized by Epner (4) that the majority of terminal cancer patients are very compliant with treatment options, but it will be crucial that the drink, which would likely provide most of their calories, is tolerable by the patients.

For this study, Hominex-1 was donated by Abbott Laboratories. Hominex is a methionine-free powder supplement that is mixed as a drink and given to patients who must follow a diet low in methionine due to inborn errors of metabolism. It supplies all the other necessary amino acids to maintain protein synthesis that the food provided would be lacking. In the initial phases of this feeding trial, we had the opportunity to taste test this drink mix, as well as a methionine-free powder supplement called X-Met by Nutricia North America. Because of extenuating circumstances, only Hominex was donated to complete the study. Through a number of taste-tests, however, we determined that the X-Met tasted more appealing and could potentially be tolerated better. This finding is promising if the blood test results from this pilot study are positive. If the drink continues to be the only negative factor, a different liquid supplement could be implemented in the experimental diet regimen.

5.5 Future Research

This pilot study is an important first step for the research that will need to be completed to obtain conclusive results about the risks and benefits of a low-methionine diet. With only a few human pilot studies to date, it will be crucial that long-term evaluations take place over a large population of people with a wide variety of demographic characteristics. Animal studies have proven to be very promising, and results from initial human studies have also indicated

potential benefits from methionine-restricted diets. These studies have so far concluded that there is no direct damage done to healthy cells in the body and that protein synthesis is maintained. If results continue to show these positive findings in relation to cancer growth and development, this could provide an alternative cancer treatment or supplement conventional treatment protocol.

For the purpose of this thesis, it was important that the liquid supplement was manageable and tolerable, so that the patients were able to comply with the diet and consume the drink. It will be a major step forward if a low-methionine diet creates positive results, but without a diet prescription that can be managed, the many benefits of a low-methionine diet will not be realizable. Therefore, the primary recommendation for future research is to partner with methionine-free powder supplement vendors to improve taste, and reduce the propensity for patients to experience nausea, diarrhea, cramping and indigestion caused by their supplement.

5.6 Conclusion

Overall, this pilot study has shown that the low-methionine diet prescription is feasible and that for the most part, the participants can cope with the required drink during a limited time period of three weeks. As stated above, the methionine-free drink is essential to maintain a healthy balance of nutrients and must be consumed daily. It will be crucial in future research that a drink is developed that can be better tolerated and accepted. If this can occur, a low-methionine diet may be revolutionary in helping prevent and treat cancerous tumors among high risk populations.

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Appendix A – One Day Sample Menu for Both the Control and Experimental Feeding

Low Methionine Sample Menu

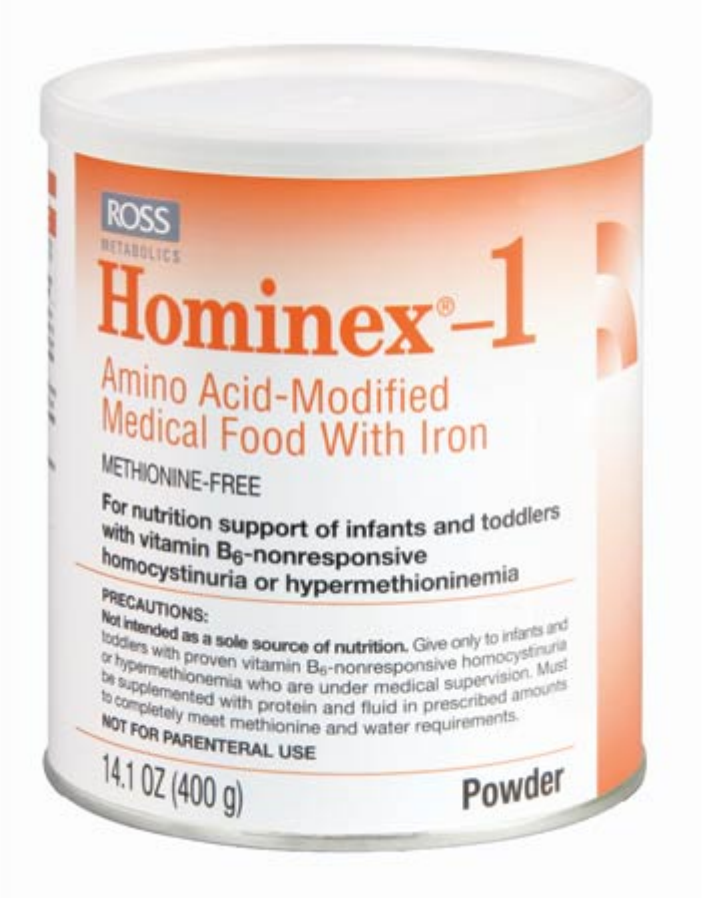
Breakfast
New formula Centrum Vitamin
Oatmeal
Coffee Creamer
Strawberries

Lunch
Celery
Cucumbers
Peppers
Ranch Dip
Tomato Soup
Saltines
Banana Chips

Dinner
Hash brown potato casserole
Ketchup
Romaine Lettuce
Onion
Green Pepper
Italian Dressing

Snack
Macadamia Nuts
Dried Figs
2 pieces hard candy
2 pieces sugar-free chewing gum

Appendix B: Hominex-1 Can



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