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THE ASSESSMENT OF THE PRACTICAL USE OF LIPID PREDICTIVE EQUATIONS IN A DIABETIC POPULATION

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Abstract

Type 2 diabetes is a major risk factor for cardiovascular disease (CVD). Both diseases are strongly linked to diet in terms of prevention and management. One major determinant of CVD risk is blood cholesterol levels. In the 1960s, Ancel Keys created an equation to predict a person's blood cholesterol level based on changes in the amounts of saturated and unsaturated fat in their diet. Over the last 50 years, this equation has been reformulated to include the effects of individual fatty acids, *trans* fat, and total fat. The purpose of this study is to assess the validity of these equations in a population with type 2 diabetes, using the patients who were part of a separate controlled feeding study. The study assessed the patients' habitual diets prior to beginning the study, an Average American Diet (AAD), and a Control diet. Fasting lipid profile values were taken before the start of the study and after the end of two weeks on the AAD. The participants' blood lipid values, after controlled feeding, were compared to the values calculated using the nutrient analysis of the AAD and Control diets. The equations used started with the original Keys Equation and progressed through six subsequent equations to predict total cholesterol, low-density lipoprotein cholesterol (LDL-C), and high-density lipoprotein cholesterol (HDL-C). For all equations, the study results showed poor correlation between the predicted values and the patients' actual values. This lack of correlation may have resulted from inaccurate self-reported calorie and nutrient intake before the controlled feeding period began. However, the performance of the equations was not improved when comparing lipid changes during the two controlled feeding periods. While the predictive equations were not useful in predicting the lipid profile in this population, the principles behind the equations are applicable in the standards of care for individuals with diabetes.

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Literature Review

Overview of CVD

CVD is the number one cause of death for all Americans (Lee and Nieman 2010). Additionally, type 2 diabetes has been one of the top ten leading causes of death in the United States since 1932 (Lee and Nieman 2010). Individuals with diabetes have nearly twice the risk of developing CVD than individuals without diabetes (Silbernagel, Rosinger et al. 2012).

There are many factors that contribute to the development of CVD, and much of the current knowledge in this area comes from the Framingham Heart Study. Based in Framingham, Massachusetts, this prospective, longitudinal study of CVD began in 1948 with the enrollment of 5,209 individuals aged 28 to 62 years (Dawber and Kannel 1958). Children and grandchildren of the original cohort were added in 1971 and 2002, respectively (Splansky, Corey et al. 2007). Extensive medical examinations are conducted every two to four years to evaluate risk factors and incidence of CVD (Yusuf and Anand 2010). The Framingham Study identified a complex interaction between several conditions that led to the onset of the disease. In particular, the investigators identified "risk factors" that most significantly predict CVD: elevated blood pressure; body fat composition; and hypercholesterolemia in men ages 45 to 62 (Dawber 1958; Dawber and Kannel 1958).

It is imperative to understand the etiology of CVD in order to find a solution to the epidemic. In a conference sponsored by the Centers for Disease Control and Prevention and the American Heart Association, inflammation was identified as a key marker for CVD (Sheard, Clark et al. 2004). Factors such as cigarette smoking, hypertension, atherogenic lipoproteins, and hyperglycemia elicit the release of adhesion molecules in endothelial cells and the rapid release of monocyte cells into the arterial space. Cholesterol lipoproteins adhere to the sides of the arteries causing plaque to form and the instigation of a fatty streak in the blood vessel. As the body attempts to clear this damage, collagen in the vessels is

broken down and the atheronecrotic core is exposed to arterial blood (Pearson, Mensah et al. 2003). This exposure induces inflammation which increases the likelihood that plaque will rupture and impede arterial blood flow (Silbernagel, Rosinger et al. 2012). Chronic inflammation can be identified by elevated C - reactive protein (CRP) levels in the blood. Other markers include cell adhesion molecules, cytokines, acute-phase reactants, fibrinogen, and white blood cell count (Pearson, Mensah et al. 2003).

Overview of Type 2 Diabetes

Type 2 diabetes prevalence is growing worldwide. The World Health Organization reported an increase from 30 million to 170 million cases of type 2 diabetes from 1985 to 2000. These numbers are expected to increase by 69% by 2030 (Shaw, Sicree et al. 2010). Also, it is predicted that the number of cases of type 2 diabetes in adults will rise from 6.4% affected to 7.7% affected by 2030 (Shaw, Sicree et al. 2010). Another trend is being seen as instances of type 2 diabetes increase in younger generations, correlating to the obesity epidemic (Lipscombe and Hux 2007). Factors including physical inactivity, a higher percentage of body fat, and insulin resistance have been identified as the major causes for type 2 diabetes and are, thus, the most targeted point for primary interventions (Gress, Nieto et al. 2000).

The risk factor of major concern in patients with type 2 diabetes is hyperglycemia or high concentrations of sugar in the blood (Silbernagel, Rosinger et al. 2012). Elevated glucose levels can have several serious effects on the lining of blood vessels. First, hyperglycemia increases free-radical production, which causes spontaneous cell death (Graves, Liu et al. 2006). Second, it reduces the body's ability to provide nitric oxide, which is responsible for relaxing blood vessels and allowing blood to freely flow to the heart. Type 2 diabetes has been shown to increase glycation of enzymes that produce nitric oxide (Aljada, Ghanim et al. 2002). Such increased glycation leads to the release of hydrogen peroxide by-products, which drives free-radical production and inflammation (Dandona, Aljada et al. 2004).

These changes may explain why individuals with type 2 diabetes are twice as likely to suffer from a heart attack or stroke (Silbernagel, Rosinger et al. 2012).

Many evidence-based dietary guidelines exist for the prevention and treatment of type 2 diabetes. There are multiple benefits to providing dietary interventions for individuals who are at risk for developing type 2 diabetes. Establishing a healthy weight is imperative for reducing the risk of type 2 diabetes and CVD, as obesity is a major gateway to both diseases. It is possible to still enjoy food and eat a healthy diet to manage type 2 diabetes.

Dietary Recommendations from the American Diabetes Association

The American Diabetes Association has very thorough recommendations for preventing and managing type 2 diabetes. These recommendations have been revised to focus on medical nutrition therapy for patients with type 2 diabetes. The overall goal of medical nutrition therapy for individuals at risk for type 2 diabetes is to promote healthy eating and exercise in order to maintain a healthy weight (Sheard, Clark et al. 2004). The diet recommendations are meant to be specific for each individual's lifestyle, as well as for one's nutritional and metabolic needs. Through medical nutrition therapy, the patients and practitioners set realistic goals, and interventions are created to meet them. These goals are evaluated and changed, as necessary, based on a nutritional assessment by a doctor or dietician. Thus, medical nutritional therapy is the optimal way to manage the diet of individuals with type 2 diabetes, because patients' metabolic needs can greatly vary. Setting realistic, specific, and measurable goals allows these patients to meet their needs and manage their type 2 diabetes. The goals include: achieving a healthy body weight; managing blood glucose and lipid levels; and eating a nutritionally adequate diet. Goals are modified as the patient ages and the nutrition profile changes (Lee and Nieman 2010). The United Kingdom Prospective Diabetes Study (UKPDS) indicated using modified,

intensive medical nutrition therapy – in combination with medications to lower blood glucose levels – reduce the risks associated with developing type 2 diabetes by 32% (Blonde 2012).

While it may seem intuitive that a diet lower in carbohydrates would be better for patients with type 2 diabetes, this is not generally recommended by the American Diabetes Association (Association). Carbohydrates are essential for the diet as they provide fuel for the body and brain, along with many vitamins, minerals, and fiber. At the very minimum, individuals must consume at least 130 grams of carbohydrates per day for healthy body functioning (Ryan-Harshman and Aldoori 2006). Epidemiological studies have not reported consistently that a high glycemic index diet leads to type 2 diabetes (Brand-Miller, Hayne et al. 2003). Rather, the consumption of saturated fat and total fat appears to be important, perhaps through their link to obesity (Sheard, Clark et al. 2004). The American Diabetes Association recommends refraining from over consuming calories during meals, eating less complex polysaccharide carbohydrates, limiting saturated fat intake, and maintaining a healthy body weight to prevent type 2 diabetes (Association).

New developments in type 2 diabetes research have changed dietary recommendations over time. For example, the recommended percentage of energy from fat – in particular saturated fat – in the diet has decreased significantly. In the 1920s, fat was recommended to encompass 70% of energy intake (Putnam, Allshouse et al. 2002). Current recommendations state that total fat intake should be individualized depending on diet therapy. However, total fat ingestion should fall between 25-35% of total caloric intake, saturated fat consumption should be less than 7% of energy intake, and up to 10% of energy consumed should come from polyunsaturated fat (Bantle, Wylie-Rosett et al. 2008). Alternatively, carbohydrate recommendations for type 2 diabetics have increased over time, as research has shown that they are not unsafe for type 2 diabetics with proper glycemic control. Carbohydrate recommendations started at 20% of energy intake in the 1920s and have increased to 45-60% of the

diet. Protein recommendations have stayed generally the same, hovering around 20% of energy intake (Lee and Nieman 2010).

It is important to understand that both the type and the amount of carbohydrate in the diet affect blood glucose levels. For example, sucrose does not increase overall blood glucose levels when compared to an isocaloric amount of starch (Franz, Bantle et al. 2004). Starch, which is a form of carbohydrate found as granules of amylose or amylopectin, is the type of carbohydrate found in breads, pastas, and grains. Sugars like sucrose and lactose are polysaccharide forms of carbohydrate that are known to have a much lower glycemic index rating than originally perceived (Pi-Sunyer 2002). Therefore, sucrose does not need to be restricted from a diabetic diet when consumed with an equal amount of starch. Carbohydrates should be simple and easy to digest, such as whole grains, fruits, and vegetables, all of which are very nutrient dense (Franz, Bantle et al. 2004). Soluble fiber is also shown to lower lipid levels (Tuomilehto, Lindström et al. 2001). Fructose does not raise blood glucose levels as much as the other monosaccharides and it does not appear to significantly affect glycemic control. It can, however, raise triglyceride levels (Lee and Nieman 2010).

Because carbohydrates have the greatest direct effect on blood glucose, many studies have sought to quantify blood glucose response to carbohydrates. The most well recognized formulas, glycemic index and glycemic load, have been created to do this. The glycemic index indicates how much blood glucose levels change in response to specific carbohydrate-containing foods. Foods are assigned a number of “carbohydrate exchanges” in order to quantify the glucose response to the food and are standardized by weight (Brand-Miller, Dickinson et al. 2007). The change is blood glucose is measured over a two-hour time span after food ingestion. The resulting value is compared to a reference food, usually white bread, which has the same amount of carbohydrates (Sheard 2004).

There are limitations to the glycemic index scale. It considers only the specific type of carbohydrate in a serving. Due to individual differences in metabolism, the exact effect of foods in the index will vary. The index also does not consider mixed dishes that contain several sources of carbohydrates. Estimations based on ingredient listings are simply estimations, not exact values.

On the other hand, the glycemic load formula, shown below as the *Glycemic Load Formula* (Salmeron, Ascherio et al. 1997), takes into account the amount of carbohydrate per serving. This formula helps to explain how the amount and type of carbohydrate affects glycemic response.

Glycemic Load Formula

$$\boldsymbol{Glycemic\ Load\ = \frac{(Glycemic\ Index)}{100}\ x\ Net\ Carbohydrates}$$

The Glycemic Index value can be found by consulting the chart.

Net carbohydrates= total carbohydrates (g) – dietary fiber (g).

Glycemic response to carbohydrates and, therefore, glycemic index rating, are determined by several factors in addition to the type of monosaccharide present in the food. The physical form of food (liquid versus solid), type and degree of processing, kind and length of time of preparation, temperature, and variety of food can all greatly vary the amount of glycemic response in the body (Turner-McGrievy, Jenkins et al. 2011).

Type 2 diabetics are encouraged to closely monitor dietary fat intake and corresponding blood lipid levels. Individuals with type 2 diabetes are advised to keep saturated fat between 25-35% of caloric intake and between 30-35% of caloric intake if triglycerides and very-low-density-lipoprotein (VLDL) cholesterol are elevated (Bantle, Wylie-Rosett et al. 2008). Consumption of *trans* fat should be very limited or avoided, if possible (Lichtenstein, Appel et al. 2006). Generally, saturated fats, which include

solid fats like butter and animal fat, should be avoided. Saturated fat has been linked to many health problems, including CVD (Association 2010). Monounsaturated fatty acid (MUFA) and polyunsaturated fatty acid (PUFA) consumption is encouraged, as these types of fat include oleic acid and the omega-6 and omega -3 essential fatty acids. These "heart healthy" polyunsaturated fats are found in liquid sources of oil, such as canola, flax, fish, and algae. Total cholesterol should be below 200 mg/d for all individuals and potentially lower, if the individual has high LDL-C levels (Association 2010). Recommended protein intake for type 2 diabetics generally follows the same guidelines as those without type 2 diabetes. Alcohol consumption does not appear to significantly affect blood glucose levels, but it should not exceed two servings per day in men and one serving daily in women who are diabetic. Alcohol consumption should be limited regardless, to negate its hypoglycemic effects (Franz, Bantle et al. 2004). Sodium intake should be monitored in patients with type 2 diabetes and kept below 1,500 milligrams per day (Lloyd-Jones, Hong et al. 2010), because these individuals can be more vulnerable to sodium. There seems to be no effect, positive or negative, in type 2 diabetic patients taking mineral supplements, unless the patient is deficient (Franz, Bantle et al. 2004).

Dietary Recommendations from the American Dietetic Association

The American Dietetic Association has its own recommendations for eating habits to manage and prevent type 2 diabetes. The American Dietetic Association has issued the following three goals to manage type 2 diabetes:

- To keep blood glucose levels in a normal range;
- To reduce blood pressure and cholesterol to prevent heart disease and stroke; and
- To adopt a diet and lifestyle that are health-promoting and realistic to reduce complications from type 2 diabetes (Dietetics).

Like the recommendations from the American Diabetes Association, the American Dietetic Association states there is no one specific diet for individuals with type 2 diabetes. Instead, the American Dietetic Association produced a collection of healthful eating and preventative tips.

The American Dietetic Association emphasizes portion control (Dietetics). The organization advises eating smaller meals and snacks regularly, as well as and eating the same amount of food at that meal or snack. The recommendations also state that carbohydrate, fat, and protein intake should be balanced in order to control blood sugar levels (2003). The American Dietetic Association suggests seeking the advice of a registered dietician to develop a meal plan that balances healthy food and any medication. The meal plan should consist of the following foods:

- Starches (bread, cereal, starchy vegetables like potatoes, beans, and corn);
- Bright colored vegetables;
- Fruit;
- Meat (including fish and poultry);
- Dairy (cheese, milk, tofu, and yogurt); and
- Healthy fats (unsaturated fats high in omega-3 and omega-6 fatty acids) (Dietetics).

The American Dietetic Association also recommends using the 2007 Food Exchange Lists for Diabetes. The list contains commonly eaten foods and is classified by food group in specific portion sizes. The foods included are in most cases commercially produced staples in the general United States food supply. Preparation style (i.e. raw versus cooked) is included for some foods. Various serving sizes of individual foods are referenced as one standardized “exchange” and are associated with a certain amount of calories, carbohydrates, fat, and protein. The purpose of the list is to allow for carbohydrate counting and to give individuals an easier method to calculate the nutritional content of their meals (Agriculture 2006).

Dietary Recommendations from the Centers for Disease Control and Prevention

The Centers for Disease Control and Prevention (CDC) has similar recommendations to the American Dietetic Association in terms of using diet to prevent and manage type 2 diabetes (Prevention 2007). Additionally, the CDC suggests consulting a dietician to develop a meal plan. The meal plan should consist of three regular meals and one to two snacks in order to regulate blood glucose levels. The CDC offers many specific suggestions of foods to choose and foods to avoid.

Dietary Recommendations by the Centers for Disease Control

Foods to Avoid	Foods to Choose
Fried foods	Whole grain breads, rice, cereal, tortillas, *etc*.
Anything with added sugar (soft drinks, fruit juice, cookies, cakes, candy, sugary breakfast cereals, *etc*.)	Meats and other foods prepared by grilling, baking, broiling, or steaming
Foods prepackaged in cans and jars, snack foods, pickled foods	Dark green vegetables (broccoli, spinach) and orange vegetables (carrots, sweet potatoes)
Fatty cuts of meat, lunch meats (cold cuts)	Beans and peas
Whole or full-fat milk, cheese, and other dairy products	Low-fat milk, cheese, and yogurt
Lard, shortening, stick butter and margarine	Herbs and spices (preferred over salt)
Alcohol, particularly when on certain medications	

*__Note:__ This figure explains the foods the CDC recommends for consumption and avoidance to prevent and manage type 2 diabetes (Prevention 2007).

More generally, the CDC suggests limiting consumption of fat, sugar, and salt, while consuming a wide variety of fruits and vegetables and high-fiber foods (Prevention 2007).

Predicting CVD Risk with Equations

Keys' Equation.

A large body of evidence suggests that diets high in saturated fat intake are more likely to lead to the development of CVD (Hegsted, Mcgrandy et al. 1965; Keys, Anderson et al. 1965; Yu, Derr et al. 1995; Howell, McNamara et al. 1997). Nutritional therapy programs specifically target saturated fat intake, as it leads to a rise in blood cholesterol levels (Hu, Stampfer et al. 1999). Research on the influence of dietary fat on blood cholesterol levels was initiated by Ancel Keys (Keys, Anderson et al. 1965). Keys began by attempting to establish a correlation between the amount of fat and types of fat, mainly the relationship between saturated and polyunsaturated fats in the diet, and risk of cardiovascular events such as heart attack and stroke (Keys, Anderson et al. 1965). To discover this relationship, Keys colleagues conducted an observational study known as the Seven Countries Study in order to identify relationships between the frequency of cardiovascular incidents and physical, lifestyle, and diet characteristics in certain populations (Association 1970). Additionally, Keys et al. conducted several controlled feeding trials at the University of Minnesota with diets consisting of adequate amounts of calories, vitamins, and proteins but varied the fat concentrations. Diet-periods lasted approximately four weeks, with four week standardized "normal" periods between diets. Each participant was studied on a combination of two out of six diets (Keys, Anderson et al. 1957).

Using the data obtained, Keys and his colleagues developed one of the first published blood cholesterol predictive equations, which allows one to predict change in blood cholesterol levels using dietary saturated and unsaturated fat content seen in Equation 1a. Through the course of time and

improved research, Keys et al. modified these equations to better reflect cholesterol levels. This breakthrough can estimate cholesterol levels in order to evaluate CVD risk.

Equation 1a: Original Keys Equation

$$\Delta \boldsymbol{Total\ Cholesterol}\ \left(\frac{\boldsymbol{mg}}{\boldsymbol{1000\ mL}}\right) = \boldsymbol{2.7\Delta S - 1.3\Delta P}$$

S=percentage of calories from saturated fatty acids; P= percentage of calories from unsaturated fatty acids (Keys, Anderson et al. 1957).

In establishing what is known as the Original Keys Equation, Keys et al. (Keys, Anderson et al. 1965) discovered that the ratio of saturated to unsaturated fatty acids in a diet alone is not enough to accurately predict serum cholesterol levels. Also, Keys found in this preliminary study that the number of double bonds in the polyunsaturated fats did not seem to proportionately affect increases in cholesterol. However, the cholesterol level did respond accordingly with the level of iodine in the fatty acid, which coordinated with the fat's average degree of unsaturation. The results of the trials demonstrated that saturated fat and unsaturated fat had opposing results on blood cholesterol, with saturated fat raising cholesterol levels and unsaturated fat reducing cholesterol levels. Additionally, saturated fat had about twice the effect of unsaturated fat.

With further trials and experimental diets, Keys et al. found several limitations to their original formula (Keys, Anderson et al. 1965). First, the formula assumed all dietary unsaturated fats have the same degree of impact on cholesterol levels. Keys et al. found through controlled dietary studies that monounsaturated fats, such as oleic acid and eruic acid (fatty acids with less than 12 carbon atoms), had little to no effect on blood cholesterol (Keys, Anderson et al. 1965). These fats are less hydrophobic and are metabolized differently, as they do not have to be transported by the lymphatic system. However,

this was found to have little to no effect in terms of calculating total cholesterol, unless these monounsaturated fats made up the majority on an individual's total fat in their diet.

It was noted that dietary cholesterol had a large effect on total blood cholesterol (Keys, Anderson et al. 1965). Because of this difference, Keys et al. added a correction factor to the original formula, where Z is equal to the square root of mg of dietary cholesterol per 1,000 calories as shown in Equation 1b.

Equation 1b: Modified Keys Equation

$$\Delta \boldsymbol{Total\ Cholesterol}\left(\frac{\boldsymbol{mg}}{\boldsymbol{1000\ mL}}\right) = \boldsymbol{2.7\Delta S - 1.3\Delta P + 1.5\Delta Z}$$

S=percentage of calories from saturated fatty acids; P= percentage of calories from unsaturated fatty acids; Z= square root of mg of dietary cholesterol per 1,000 calories (Keys, Anderson et al. 1965).

Also, Keys et al. found that the updated formula fails to be accurate when fats in the diet are mainly from sources such as cocoa butter, beef tallow, or hydrogenated coconut oils (Keys, Anderson et al. 1965). In diets that contain palmitic acid as the dominating fatty acids (coconut oil and butterfat), the value of S was overestimated by as much as 10%. In diets with large amounts of cocoa butter, Keys et al. saw skewed results and high overestimation by his equation. Cocoa butter is made up of approximately 35% stearic acid, which seemed to be the culprit for the skewed outcomes. Keys et al. hypothesized that the stearic acid did not have an effect on total cholesterol levels, or interact with other fatty acids in the diet. From controlled dietary experiments, Keys et al. (Keys, Anderson et al. 1965)found that stearic acid has no effect on blood cholesterol, and therefore changed his equation to reflect this finding, as shown in Equation 1c.

Equation 1c: Modified Keys Equation

$$\Delta \boldsymbol{Total\ Cholesterol}\left(\frac{\boldsymbol{mg}}{\boldsymbol{1000\ mL}}\right) = (\boldsymbol{2.7}\Delta \boldsymbol{S'} - \boldsymbol{1.3}\Delta \boldsymbol{P}) + \boldsymbol{1.5}\Delta \boldsymbol{Z}$$

$S' = S\ (total\ saturated\ fat) - S"\ (amount\ of\ stearic\ acid\ in\ diet)$

Hegsted Equation.

D.M. Hegsted was the next significant contributor to cholesterol predictive equations (Yu, Derr et al. 1995). Using some assumptions from Keys et al., mainly that saturated fatty acids (SFAs) have about twice the effect of unsaturated fatty acids, and some of his own hypothesis that dietary cholesterol has a larger effect than originally assumed, he developed his own predictive equation (Hegsted, Mcgrandy et al. 1965). His controlled diet studies were designed at three specific calorie levels, provided a lower fat diet, and added oils. He strictly regulated the type and amount of fatty acids in all of the fats and oils in the study, as to observe their effects on total cholesterol.

His equation, Equation 2 (Hegsted, Mcgrandy et al. 1965), like the one proposed by Keys et al., showed that a diet with increased levels of saturated fat increased total cholesterol. Likewise, MUFAs had a minimal effect, and PUFAs had a negative, cholesterol-lowering effect. Hegsted et al. confirmed the Keys et al. finding that stearic acid had little to no effect on overall blood cholesterol.

The main difference from previous equations and Equation 2 was the large effect found from dietary cholesterol. He found that less processed oils had a minimal effect on cholesterol when compared to synthetic oils. Additionally, Hegsted et al. found myristic acid had the most influential effect in raising serum cholesterol, unlike the conclusion of Keys et al, which focused on palmitic acid. Hegsted et al. calculated that myristic acid accounted for approximately 67% of the total variance in cholesterol levels. Hegsted et al. saw that if only palmitic acid and myristic acid were used as total SFAs, the effect of fats in cocoa butter could be better predicted (Hegsted, Mcgrandy et al. 1965).

Equation 2: Hegsted Equation

$$\Delta \boldsymbol{Total\ Cholesterol}\ \left(\frac{\boldsymbol{mg}}{\boldsymbol{1000\ mL}}\right) = \boldsymbol{2.32S + 0.32M - 1.46P + 6.51C + 0.83}$$

S=total saturated fatty acids; M= MUFA; P=PUFA; C=dietary cholesterol

Mensink and Katan Equation.

About 30 years later, Mensink and Katan (Mensink and Katan 1992) performed a series of 27 trials to update the accepted equations from Keys et al. and Hegsted et al. Mensink and Katan worked under the premise that the previous equations were no longer adequate predictors of total cholesterol because they did not differentiate between LDL-C and HDL-C in the blood (Mensink and Katan 1992). This differentiation was an important distinction since they have opposite effects in the prevention of heart disease. Secondly, Mensink and Katan stated that all previous studies were mostly concerned with differentiating between the effects of various SFAs in total cholesterol levels. They wanted to focus on distinguishing the independent effects of various unsaturated fatty acids. Their controlled diets only varied in dietary fatty acids and they eliminated very long chain PUFAs, like fish oils, because data was varied and incomplete on the participants.

Mensink and Katan found that their equation, Equation 3, was generally in agreement with the Keys Equation and Hegsted Equation (Mensink and Katan 1992). Like both of the previous equations, Mensink and Katan found that PUFAs had a cholesterol-lowering effect, although not to the extent previously thought. They confirmed that the effect of unsaturated fatty acids on LDL-C paralleled that of total cholesterol. HDL-C changed with diet, rising 2.8 mg/dL per each increase of 10% of energy from PUFAs and 4.7 mg/dL per 10% of energy from SFAs. Unlike the Hegsted et al. study (Hegsted, Mcgrandy et al. 1965), Mensink and Katan found no discernible interaction from dietary cholesterol in this study

(Mensink and Katan 1992). They also found that the SFAs all seemed to raise cholesterol by the same amount, unlike the Keys et al. and Hegsted et al. studies.

Mensink and Katan's study offered an explanation for what the results mean in terms of predicting CVD risk. The preliminary findings of Mensink and Katan (Mensink and Katan 1992) suggested that replacing SFAs in the diet with unsaturated fatty acids will give a more favorable lipid profile. Replacing 10% of energy intake from saturated fat with carbohydrates would lower both LDL-C by 13 mg/dL and HDL-C by 4.7 mg/dL. Epidemiological studies show that every 1 mg/dL increase of LDL-C leads to approximately a 1% decrease in CVD risk. Mensink and Katan stated several drawbacks to this study, including the uncertainty of the effects on HDL-C, the possibility that the effects may change with the subject's body weight, and the extent of other risk factors for CVD, like blood pressure.

Equation 3: Mensink and Katan Equation

$$\Delta \boldsymbol{Total\ Cholesterol}\left(\frac{\boldsymbol{mg}}{\boldsymbol{1000\ mL}}\right) = \boldsymbol{1.2\ x\ (1.8\Delta S' - 0.1\Delta M - 0.5\Delta P)}$$

S'=lauric acid, myristic, and palmitic acids; M= MUFA; P=PUFA

Yu Equation.

Yu et al. (Yu, Derr et al. 1995) sought to more thoroughly study the effect of stearic acid, MUFAs, and other fatty acids on total cholesterol levels. To do so, they conducted a search for controlled feeding studies, completed between 1970 and 1993, which examined the effect of dietary fatty acids on cholesterol levels (Yu, Derr et al. 1995). The findings were narrowed down to 18 studies. New regression equations used to predict total, were created for men and women, seen in Equation 4a. Equations predicting LDL-C, Equation 4b, and HDL-C, Equation 4c, also were generated.

Their results showed that 12:0 (lauric acid), 14:0 (myristic acid), and 16:0 (palmitic acid) SFAs significantly increased total cholesterol, LDL-C, and HDL-C levels in men and women. MUFAs and PUFAs lead to a decrease in total cholesterol and LDL-C, particularly in men. Additionally, MUFAs and PUFAs significantly increased HDL-C, again, particularly in men. 18:0 SFAs have little measurable effect on any variable. MUFAs did decrease total cholesterol slightly but not significantly.

The study observed a unique effect of stearic acid, among the SFAs. Stearic acid showed a neutral effect on total cholesterol, LDL-C, and HDL-C in men, but the data suggested that it may lower HDL-C in women. Due to these results, Yu et al. (Yu, Derr et al. 1995) suggested separating stearic acid from the other SFA, since it appeared to have a separate effect on total cholesterol but more evidence is necessary. Also, the study by Yu et al. suggested that MUFA may lower cholesterol to a greater extent than originally assumed, based on the negative regression coefficients for total cholesterol and LDL- C and their magnitude compared to PUFAs.

Equation 4a: Yu Equation

$$\Delta \boldsymbol{Total\ Cholesterol}\ \left(\frac{\boldsymbol{mg}}{\boldsymbol{dL}}\right) = (\mathbf{02.02}\Delta \mathbf{12{:}0} - \mathbf{16{:}0}) - \mathbf{0.03}\Delta \mathbf{18{:}0} - \mathbf{0.48}\Delta \boldsymbol{MUFA} - \mathbf{0.96}\Delta \boldsymbol{PUFA}$$

12:0 to 16:0 corresponds to 12 to 16 carbon chain length saturated fatty acids and 18:0 corresponds to 18 carbon saturated fatty acids.

Equation 4b: Yu Equation LDL Cholesterol

$$\Delta \boldsymbol{LDL - C}\ \left(\frac{\boldsymbol{mg}}{\boldsymbol{dL}}\right) = (\mathbf{1.46}\Delta \mathbf{12{:}0} - \mathbf{16{:}0}) + \mathbf{0.07}\Delta \mathbf{18{:}0} - \mathbf{0.69}\Delta \boldsymbol{MUFA} - \mathbf{0.96}\Delta \boldsymbol{PUFA}$$

12 to 0- 16:0 corresponds to 12 to 16 carbon chain length saturated fatty acids and 18:0 corresponds to 18 carbon saturated fatty acids.

Equation 4c: Yu Equation HDL Cholesterol

$$\Delta \boldsymbol{HDL - C}\left(\frac{\boldsymbol{mg}}{\boldsymbol{dL}}\right) = (\boldsymbol{0.62\Delta 12{:}0 - 16{:}0}) - \boldsymbol{0.06\Delta 18{:}0 + 0.39\Delta MUFA + 0.96\Delta PUFA}$$

12 to 0- 16:0 corresponds to 12 to 16 carbon chain length saturated fatty acids and 18:0 corresponds to 18 carbon saturated fatty acids.

Howell Equation.

Howell et al. (Howell, McNamara et al. 1997) sought to examine the effect of dietary cholesterol on total cholesterol since previous findings from researchers, such as Keys et al. (Keys, Anderson et al. 1965), Hegsted et al.(Hegsted, Mcgrandy et al. 1965), and Mensink and Katan (Mensink and Katan 1992), had been somewhat inconsistent. The primary objective of her study was to provide a sensitivity analysis to see if study and participant conditions significantly influenced on the study results and predictive model (Howell, McNamara et al. 1997). Her secondary objective was to validate her own predictive model, assessing not only responses in total cholesterol, LDL-C, and HDL-C, but also VLDL-cholesterol and triglycerides. Howell and her colleagues did their study by examining past data on dietary interventions to determine total cholesterol. Her inclusion criteria consisted of the following:

- Studies of adults conducted between 1966 and 1994;
- Studies reporting single group or multiple group repeated measure comparisons;
- Quantitative measurements on total cholesterol, SFAs, PUFAs, or MUFAs; and
- Serum total cholesterol, LDL-C, HDL-C, VLDL cholesterol, and triglycerides.

Howell et al. narrowed down thousands of studies based on the criteria and organized them into databases using the study type to be compared. The final study group contained 224 published studies, 8,143 total participants, 366 independent groups, and 878 blood lipid comparisons.

The results of Howell's experimental analysis showed that total blood cholesterol was most correlated to SFA and PUFA, as previously predicted. LDL-C and HDL-C were most affected by changes in SFA; although, LDL-C had a stronger association with PUFAs than HDL-C did. PUFA was the only variable with a significant effect on VLDL cholesterol and triglycerides. The predictive model established accounts for 74% variation in total cholesterol change. Howell et al. discovered that a 1 mg/dL increase in dietary cholesterol would increase total cholesterol by 0.022 mg/dL. Howell et al.'s study concluded that MUFA and total fat did not significantly add to the precision of this equation in predicting serum cholesterol changes. Changes in SFA and PUFA accounted for 65% of the variation in total LDL-C. The study concluded that a 1% change in total energy from SFA increased LDL-C by 1.8 mg/dL, and a 1% increase in total energy from PUFA decreased LDL-C by 0.5 mg/dL.

From the study, Howell et al. (Howell, McNamara et al. 1997) developed predictive equations for total cholesterol, Equation 5a, and LDL-C, Equation 5b, and HDL-C, Equation 5c.

Equation 5a: Howell Equation

$$\Delta \boldsymbol{Total\ Cholesterol}\ \left(\frac{\boldsymbol{mg}}{\boldsymbol{dL}}\right) = \mathbf{1.918}\Delta\boldsymbol{SFA} - \mathbf{0.9}\Delta\boldsymbol{PUFA} + \mathbf{0.0222}\Delta\ \boldsymbol{dietary\ cholesterol}$$

SFA=saturated fatty acids; PUFA=polyunsaturated fatty acids

Equation 5b: Howell Equation LDL Cholesterol

$$\Delta \boldsymbol{LDL}\ \left(\frac{\boldsymbol{mg}}{\boldsymbol{dL}}\right) = \mathbf{1.808}\Delta\boldsymbol{SFA} - \mathbf{0.495}\Delta\boldsymbol{PUFA}$$

SFA=saturated fatty acids; PUFA=polyunsaturated fatty acids

Equation 5c: Howell Equation HDL Cholesterol

$$\Delta \boldsymbol{HDL}\left(\frac{\boldsymbol{mg}}{\boldsymbol{dL}}\right) = \mathbf{0.287}\Delta \boldsymbol{SFA} + \mathbf{0.192}\Delta \boldsymbol{total\ fat}$$

SFA= saturated fatty acids

Howell et al. predicted dietary change in total fat consumption would cause a variation of up to 41% in HDL-C. A 1% increase in total energy from SFA indicated a 0.3 mg/dL increase in HDL-C, and a 1% increase in total energy from fat resulted in a 0.2 mg/dL increase in total HDL-C. This indicates that very low-fat diets may not be ideal, as HDL-C is necessary to protect against damage from cholesterol.

Other results of Howell et al. showed a correlation between dietary cholesterol and total serum cholesterol. A 2.2 mg/dL decrease in total cholesterol was seen with a 100 mg decrease in dietary cholesterol. Howell et al. found similar results to Mensink and Katan with plasma lipoproteins in that levels of LDL-C were mainly determined by SFA and PUFA; whereas HDL-C was more correlated with SFA and total fat consumption. Also, Howell et al. confirmed what several of the other studies had shown: lauric acid, myristic acid, and palmitic acid were the saturated fats with the most potent effects on cholesterol (Howell, McNamara et al. 1997).

Triglyceride Predictive Equations

Howell et al. additionally worked with triglyceride equations in her study and created an equation, seen in Equation 5d. Triglycerides are an important indicator of lipid tolerance and help to predict the value of VLDL cholesterol. High triglyceride levels are a risk factor for CVD (Bantle, Wylie-Rosett et al. 2008). Total fat is negative in this equation, meaning a decrease in total fat will increase triglycerides, only if PUFA is <9.4% of total energy (Howell, McNamara et al. 1997).

Equation 5d: Howell Equation for Triglycerides

$$\Delta \boldsymbol{Triglycerides}\left(\frac{\boldsymbol{mg}}{\boldsymbol{dL}}\right) = \mathbf{0.0124}\Delta\boldsymbol{cholesterol} - \mathbf{0.859}\Delta\boldsymbol{PUFA} + (-\mathbf{0.746} + \mathbf{0.079} \cdot \Delta\boldsymbol{PUFA}) \cdot \Delta\boldsymbol{total\ fat}$$

Generally, triglycerides can be calculated by first finding VLDL concentration with the Friedewald formula seen in Equation 6 (Warnick, Knopp et al. 1990).

Equation 6: Friedewald Formula

$$VLDL\left(\frac{mg}{dL}\right) = total\ cholesterol\left(\frac{mg}{dL}\right) - LDL\left(\frac{mg}{dL}\right) - HDL\left(\frac{mg}{dL}\right)$$

Triglycerides can be calculated then from the VLDL cholesterol concentration, seen in Equation 7 (Warnick, Knopp et al. 1990).

Equation 7: Predicting Triglycerides

$$Triglycerides\left(\frac{mg}{dL}\right) = VLDL\left(\frac{mg}{dL}\right) * 5$$

**This formula is not accurate when triglycerides >400 mg/dL* (Lee, 2010).

This calculated triglycerides value can be compared to normal triglycerides values. Normal triglycerides in terms of the National Cholesterol Education Program are considered to be <150 mg/dL; borderline high levels are considered to be 150-199 mg/dL; high levels are 200-499 mg/dL; and very high levels are >500 mg/dL (Lee and Nieman 2010). High serum triglycerides are considered to be a risk factor for CVD. Therefore, it is important to be aware of the high serum triglyceride value, as it is an indicator of high VLDL cholesterol and LDL-C and decreased HDL-C levels.

Present Day Equations

Estimating total cholesterol values is something more and more people are becoming interested in, as CVD becomes a greater threat. Current studies have turned to other dietary components, such as *trans* fat, to predict CVD risk. There are presently a wide variety of calculators available on the Internet that will allow users to enter various values from their diet to predict their levels of lipids and lipoproteins. One of the more extensive Web-based calculators comes from the Mensink and Katan equation (Katan 2008). This website (http://www.katancalculator.nl/) allows the user to enter values for saturated (in total or as individual fatty acids), *cis*-monounsaturated, *cis*-polyunsaturated, t*rans*, and very long chain fatty acids in terms of percentage of energy intake, percentage of total fatty acids, and grams of fat. In addition, values may be entered for protein and total cholesterol for up to three separate diets. The calculator outputs a very detailed predicted blood lipoprotein estimate and compares the values for each of the three diets. Estimated values include HDL-C, LDL-C, VLDL cholesterol, total cholesterol, triglycerides, and total cholesterol: HDL ratio, apoprotein A-1, and apoprotein B concentrations. Although this is a very detailed calculator, it includes a disclaimer stating that it should not replace medical advice and will not be the same for every individual.

In addition to total cholesterol, *trans* fats have become a popular topic of nutritional research. *Trans* fats are used in many commercially-produced products, such as partially hydrogenated vegetable oil and margarines, as they are cheaper and have a longer shelf life. Small amounts of *trans* fats occur in meats and dairy products naturally. *Trans* fats are metabolized differently in the body than are natural fatty acids, because of the *trans* bond configuration (Lee and Nieman 2010). The American Heart Association recommends that *trans* fat consumption be restricted to <1% of total energy consumption per day. New equations are being developed to include the effect of *trans* fatty acids on total cholesterol. One such study, (Müller, Kirkhus et al. 2001) developed Equation 9.

Equation 9: Muller Equation

$$\Delta \boldsymbol{total\ cholesterol} = \mathbf{0.01}\Delta(\mathbf{12{:}0}) + \mathbf{0.12}\Delta(\mathbf{14{:}0}) + \mathbf{0.057}\Delta(\mathbf{16{:}0}) + \mathbf{0.039}\Delta(\mathbf{trans\ f}) + \mathbf{0.031}\Delta(\mathbf{trans\ v}) - \mathbf{0.0044}\Delta(\mathbf{18{:}1}) - \mathbf{0.017}\Delta(\mathbf{18{:}2, 18{:}3})$$

12:0 to 16:0 corresponds to 12 to 16 carbon chain length saturated fatty acids, 18:1 corresponds to 18 carbon unsaturated fatty acid with one bond, and 18:2 and 18:3 refers to unsaturated fatty acids with 18 carbon chain length with 2 and 3 double bonds respectively. Trans *F fatty acids are from fish oil and* Trans *V fatty acids are from partially hydrogenated soy bean oil.*

As research on *trans* fatty acids has improved, it has been discovered that *trans* fats are indeed hypercholesterolemic (Lacroix, Charest et al. 2012). *Trans* fat and saturated fat appear to be the principal dietary predictors of LDL-C (Bantle, Wylie-Rosett et al. 2008). More recent studies have shown that *trans* fat intake of <1% does not seem to increase the risk of CVD, however, as research in this field is limited, most studies recommend minimizing *trans* fat intake (Bantle, Wylie-Rosett et al. 2008; Lacroix, Charest et al. 2012).

Overview of Current Study and Hypothesis

Patients with type 2 diabetes are at a twofold increased risk, as opposed to non-diabetic individuals, for developing CVD (Silbernagel, Rosinger et al. 2012). Therefore, it is important to assess changes in dietary aspects that may result in improved blood cholesterol. If information on the fat content of one's diet is obtained, the predictive equations can assess the precision and accuracy of their diet prescription using total cholesterol, LDL-C, HDL-C, and triglycerides. The purpose of this study was to assess the practical use of the lipid prediction equations, listed above, in a population with type 2 diabetes, as participants entered a controlled feeding study. While the equations have been reviewed

by other authors, to the best of the study staff's knowledge, no one has sought to compare them in a population with type 2 diabetes. To examine this population, habitual diet data were collected from 21 adults with type 2 diabetes. As part of this controlled feeding study, a three-day food log was taken before the participants were placed on an AAD and a Control diet. These self-reported diets were analyzed for fat content and their values were used in the predictive equations to predict the participants' blood lipid values. The predicted blood lipid values were compared to the actual lipid levels, which were taken at screening and at the end of each diet period.

The goal of the study was to assess which equation most accurately predicted the actual blood cholesterol values of the participants in the study. We hypothesized that the more recent equations would provide more accurate comparisons of predicted blood lipid levels to actual blood lipid levels. From the research described in the literature review above, it can be seen that each equation made a claim as to why it was an improved version of the previous equations using more recent research and studies. Accordingly, it may be logical to conclude that the latest equations would be the most accurate because they included a wider range of dietary components, beyond a simple focus on degree of unsaturation

Because self-reported diet data may be biased, we used the same equations to predict lipid changes when participants moved from the AAD to a heart healthy Control diet. The study staff hypothesized that the predictive equations would be more accurate in predicting lipid response when the patients were consuming highly controlled, metabolic diets provided in the clinic, when compared to their habitual diets.

Materials and Methods

Participants

This study included 23 healthy, non-smoking participants with type 2 diabetes (baseline demographics are displayed in Table 1). Four of these participants did not complete all three diet periods; two participants did not provide 24-hour recall data; and two individuals did not complete the control portion of the study. Volunteers were recruited from the Pennsylvania State University campus and surrounding community through fliers, email listservs, radio, newspaper, and television advertisements, and information tables at community events.

Participants were diagnosed with type 2 diabetes (defined as fasting glucose > 126 mg/dl and/or two-hour, post-meal glucose above 200 mg/dl), were between 40 and 74 years old, and were free of all other chronic diseases. All type 2 diabetes medications except insulin were allowed, and if medicated, participants were required to be on a stable dose for a minimum of three months prior to study entry. Other allowed medications included statins, selective serotonin reuptake inhibitors (SSRIs), and thyroid replacement therapy. Individuals were excluded from the study if they met any of the following criteria:

- Oral steroids, hormone replacement therapy, blood pressure lowering medications (or unwilling to discontinue medication with permission from a physician), oral contraceptives, or daily aspirin;
- Positive history of CVD, retinopathy, neuropathy, gastroparesis, Crohn's Disease, irritable bowel syndrome, or ulcers;
- Any major surgery within the last six months;
- Allergies to pistachios, other nuts, latex, or adhesive tape;
- Premenopausal status for women;

- Unwillingness to consume all food provided to the exclusion of other meals or snacks during the eight-week diet intervention period; and
- Unwillingness to discontinue nutritional supplements for the duration of the study.

In addition, participants were required to meet the following criteria at screening: HbA1c < 7.4%, body mass index (BMI) 18.5 – 39.0, and blood pressure < 160/100 mm Hg.

Experimental Procedures

Screening.

Participants were screened for eligibility during telephone interviews, which included questions regarding medical history, medication use, dietary habits, and ability to comply with study procedures. Participants meeting the initial criteria completed a clinic visit to confirm eligibility. During the in-person screening visit, height and weight were obtained to calculate BMI. A 12-lead EKG and blood draw were completed to evaluate heart health, HbA1c, lipids, and inflammatory status. If the results of any of these tests were found to be outside study guidelines, the participant was excluded from the study and referred for care. Written informed consent was obtained from all participants and the study was approved by The Pennsylvania State University Institutional Review Board.

Study Design.

This protocol was conducted within a larger clinical trial assessing the effects of pistachios on cardiovascular risk factors in type 2 diabetes. In the two weeks prior to starting the study, participants were required to complete a three-day food log to review their habitual eating habits. The participants then completed a controlled feeding study with three diet periods in which all meals and snacks were provided. All diets were nutritionally adequate and energy-balanced for weight maintenance. Allowed beverages included: water (as much as desired), calorie-free caffeinated or decaffeinated beverages such as coffee, tea, or diet soda (not exceeding five servings per day), and up to two alcoholic

beverages per week (one drink consisting of 12 ounces of beer, five ounces of wine, or 1.5 ounces of hard liquor with a non-caloric beverage). The diet periods consisted of a two-week run-in diet, the AAD, followed by random assignment to either a low-fat Control diet or a moderate fat experimental diet consisting of pistachios. After four weeks on either the Control or Pistachio diet, participants completed a four-week washout period, and then crossed over to the other diet. Compliance was assessed daily with a self-completed questionnaire. Weight was measured daily by study staff, and calorie adjustments were made to keep weight stable throughout the study.

Study Diets.

Only data from the AAD diet and Control diets were used in this analysis, because a previous study by this staff showed that predictive equations were not effective in estimating the LDL-C response to a pistachio-rich diet (Gebauer, West et al. 2008). The detailed nutritional information for each of these diets is displayed in Table 2. The diets were created by Melissa Hendricks, a Metabolic Diet Center dietitian, and Mary Lou Kiel, a Penn State dietitian. The AAD diet was designed to resemble the typical American diet, containing full fat cheeses and more dairy products, oil, and butter. It was used as a "run-in diet" in order to start the participants off at a similar point before intervention. The Control diet was similar to the Pistachio diet, with the removal of the pistachios, with the major differences in fat and carbohydrates in order to keep calorie content consistent. As pistachios were added, total fat content increased, particularly MUFA and PUFA amounts, and total carbohydrate and sodium content decreased. Protein content remained fairly similar between the two diets but was slightly higher in the Control diet. Carbohydrates, fat, protein, SFAs, MUFAs, and PUFAs were kept at a constant percentage throughout the various calorie levels. Cholesterol levels were held constant across all calorie levels with the addition of egg yolks, where necessary. Both diets contained fruits, vegetables, lean meats, and whole grains. The study diets were matched for sodium and

potassium. Sodium, potassium, and fiber were increased proportionally as the calorie level increased; therefore, the sodium-to- potassium ratio remained constant across all calorie levels.

Nutritional Analysis.

The diets used in the study were initially designed in *Food Processor*, and then transferred into *Nutritional Data System (NDS) 2007*. The diets were designed for a range of calorie requirements, between 1,800 and 3,900 kcals. Diets were designed with a 6 day menu cycle. Each of these menus was entered into the *NDS* software, and menus for each calorie level were independently entered. The amount of food was entered in grams. Brand name foods were used when specified (for example, cereals). Nutrient analysis included all macro- and micronutrients, fatty acid profile, amino acids, electrolytes, and added sugars (see Table 2).

Blood Draw Procedures.

Fasting blood draws were completed on two separate days at the end of each diet period for analysis of total cholesterol, LDL-C (direct and calculated methods), HDL-C, and triglycerides. Prior to the blood draw, participants were questioned regarding food and beverage intake within 12 hours, alcohol consumption within 48 hours, and vigorous exercise within two hours. If the participant did not meet these study guidelines to qualify as a fasting blood draw, the visit was rescheduled. All fasting blood draws were completed by a registered nurse at the GCRC.

Data Analysis

Predictive Equations.

The predictive equations included in this study are fully discussed above in the *Literature Review*. The equations included the Keys Equations (Equations 1a, 1b, and 1c), the Hegsted Equation (Equation 2), the Mensink and Katan Equation (Equation 3), the Yu Equations (Equations 4a, 4b, and 4c), and the Howell Equations (Equations 5a, 5b, 5c, and 5d). Seven of these equations predict total

cholesterol (mg/dL), two predict LDL-C (mg/dL), two predict HDL-C (mg/dL), and one predicts triglycerides (mg/dL). Each lipid-predicting equation was applied to the data from the participants' personal diets and the actual nutrient profile of study diets. The predicted values were then compared to the actual blood lipid values, which were reported at the end of each diet period.

Statistical Approach.

Our first analysis compared the nutrient profile of the participants' personal diets with the AAD, and the lipid changes from screening to the end of the run-in diet. To do this, we calculated the difference in types of fat and dietary cholesterol (as indicated by the various equations) between the diets and entered this data into each of the predictive equations to determine the predicted change in lipids. Then, the study staff calculated the actual change in total cholesterol, LDL-C, HDL-C, and triglycerides between the screening visit and the blood draw at the end of the AAD. Finally, we used the correlation procedure in SAS® (v9.2, Cary, North Carolina) to assess the association between the predicted and actual lipid changes. This analysis included 19 out of 21 participants, as two participants did not provide sufficient food logs. Statistically significant effects ($p \leq 0.05$) were evaluated and reported.

A similar analysis was performed to compare the lipid effects of the AAD and Control diets. Macronutrient profiles for the two diets were entered, and predicted change in the serum cholesterol profile was calculated for each equation. This analysis included only the 12 participants who were randomized to the Control diet immediately following the AAD diet. Results are presented in descriptive tables, as further statistical analysis was unwarranted given the degree of discrepancy between habitual diet reporting and demonstrated calorie needs during the controlled feeding study.

Results

Participants' Habitual Diet Compared to AAD Diet

The participants' personal diets varied considerably from the AAD study diet (**Table 3**). Total calories and carbohydrates were lower and total fat was higher on the personal diets compared to the AAD diet; dietary cholesterol was approximately the same between the two diets, but higher in the personal diets. We observed decreases in all lipid parameters between the screening visit and the end of the AAD diet period (**Table 4**). Three of the lipid equations predicted increases in total cholesterol, and four predicted decreases. One of the lipid equations predicted an increase in LDL cholesterol, and one predicted a decrease. Both HDL equations predicted an increase in HDL cholesterol, and the sole triglyceride equation predicted a decrease in triglycerides. The lipid changes predicted by the Yu, 1995 Equation 4C HDL equation and the Howell, 1997 Equation D triglyceride equation were significantly correlated with the observed changes (**Table 4**); however, neither remained statistically significant after the Bonferroni adjustment for multiple comparisons was applied ($p<0.004$ for 12 comparisons).

AAD Diet Compared to Control Diet

As designed, the AAD and control diets were similar in total calories, but differed according to relative content of total fat, particularly saturated fat, and dietary cholesterol (**Table 3**). Following the control diet, total, LDL, and HDL cholesterol were reduced and triglycerides were increased relative to the AAD diet (**Table 5**). The prediction equations followed a similar pattern, with the total, LDL, and HDL lipid equations predicting reductions and the triglyceride equation predicting an increase. However, none of the predicted changes were significantly correlated with the observed changes in lipids from the end of the AAD diet to the end of the control diet (**Table 6**).

Discussion

This study attempted to apply equations used for predicting change in lipids and lipoproteins in relatively healthy adults with type 2 diabetes. We found that these equations were not helpful in predicting the change in the serum lipid profile in this sample. No previous studies of which we are aware have used these equations in a diabetic sample. It may be that dyslipidemia in diabetes is less influenced by dietary factors. However, several previous studies of adults with diabetes (Kendall 2010; Jenkins 2011; Jenkins, Jones et al. 2011; Kendall 2011) have shown improvements in lipids and lipoproteins when nuts are consumed. A more likely explanation relates to errors in evaluating their habitual diet and medications that may have blunted dietary effects. In analyses comparing the AAD to the person's habitual diet, we see evidence of substantial under-reporting. The self-reported calorie intake is significantly lower than the calories provided by the study diets. These supposedly "higher calorie" diets did not result in significant weight gain during the controlled feeding portion of the study.

We note that under-reporting was not an issue when we compared the predicted vs. observed change in lipids between the two controlled diets. The equations tested were ineffective for predicting lipid change between the two controlled periods. We considered several explanations for this pattern of results. The most likely explanation is the range of medications that the participants were prescribed throughout the study. The standard of care for diabetes (Association 2010) requires that newly diagnosed diabetics be prescribed a statin drug and some kind of insulin sensitizing agent (Metformin for example). Twenty of our participants were taking diabetes medications, including Metformin, and thirteen were taking a statin drug. Thus, it is possible that these medications, or the underlying disease process of diabetes, had a greater influence on their lipid profile than their dietary intake. To evaluate if these drugs has a profound effect on the results, we repeated the correlation

analysis with participants who were not on statins. However, the predictive equations again failed to produce any correlations to actual changes in the participants total, LDL, and HDL cholesterol, as well as triglycerides. In the design and implementation of this study, we first attempted to recruit patients who were not taking statin drugs. However, these drugs are highly recommended for people at risk of heart disease, including diabetics. Thus, for results of the experiment to be generalizable to the larger population of adults with diabetes, accepting volunteers on statin therapy was the more pragmatic and biologically-relevant option. Our future work will examine whether change in lipids will be achieved with a moderate fat diet which includes pistachios, and we note that others have reported lipid lowering with nuts – even in volunteers taking statins (Jenkins, Jones et al. 2011).

From a historical perspective, the development of lipid-predictive equations over the last 60 years has served as a useful tool in generating hypotheses about which dietary changes would make the greatest difference in serum cholesterol values. Data from large observational studies, such as the Seven Countries Study, have been very important for generating hypotheses about what the optimal diet may consist. However, as the equations were refined over time, it became apparent that serum lipids and lipoproteins were influenced by a variety of causes, not just the fatty acid profile of the diet. At the present time, controlled feeding studies are considered the state of the art methodology for truly evaluating change in CVD risk factors with dietary modification.

Study Limitations

For future epidemiologic studies, it is critical that under-reporting be addressed in order for accurate conclusions to be drawn from datasets collected in large, nationally representative samples. The majority of studies are in agreement that the tendency to underreport energy intake increases with an individual's BMI and age (Macdiarmid 1998; Warwick 2006) and can be as high as 70% in

certain subpopulations (Macdiarmid 1998). This is particularly important as underreporting energy intake is associated with an underreported nutrient profile (Livingstone 2003). There have been several suggestions made as to how to avoid underreporting when assessing a subject's diet. Memory lapses, one of the main contributors to misreporting, can be minimized by using a multi-pass dietary interview and using plastic food models or color pictures to aid in estimating portion sizes (Poslusna 2009). Method of recall also plays an important role in avoiding misreporting. Many researchers agree that 24-hour recalls are superior to food frequency questionnaires, as food frequency questionnaires may not contain every food the participant consumes (Lutomski, van den Broeck et al. 2011). Also, it is important to address potential psychological effects of having an investigator take a diet recall. The investigator must be trained to control aspects such as body language, tone of voice, and phrasing to avoid creating bias from a subject (Livingstone 2003; Poslusna 2009). Underreporting may have played a critical role in this study. In comparing the participants' habitual diets with the study diets, an energy deficiency should have occurred. However, no participant lost weight during the study.

A control population was not tested and compared to the diabetic population in the study. Therefore, it is difficult to identify all of the factors, other than underreporting, in the diabetic populations that made the equations predict the values incorrectly. Diabetics may not be expected to fit into the population assess previous by the equations because the equations were previous assessed on healthy individuals (Keys, Anderson et al. 1957; Hegsted, Mcgrandy et al. 1965; Mensink and Katan 1992; Yu, Derr et al. 1995; Howell, McNamara et al. 1997). The majority of the type 2 diabetic participants in this study were overweight or obese, which may cause altered fat metabolism (Lee and Nieman 2010). The total cholesterol mean suggests that these individuals also possessed high cholesterol at screening (Silbernagel, Rosinger et al. 2012). This could contribute to the inaccuracy of

the equations, as this was not seen in the participants tested in previous lipid predictive equation studies. Future population studies in this area should examine the specific group in comparison to a control group.

Study Implications

The implications for this study's results for dietetic practice are complex. On the one hand, the equations were not useful for predicting lipids in adults with diabetes. On the other hand, the equations have been judged to be effective in well-designed studies of other populations. At the present time, the standard of care in dietary advice for diabetes is influenced most by controlled feeding studies in which specific nutrients and foods are compared under controlled conditions. While the equations may not have been useful in predicting the change in cholesterol in the subjects, the principles behind their development are applicable to a diabetic population. Type 2 diabetics are encouraged to limit SFAs and increase PUFAs as the majority their fat content, in order to maintain a healthy weight and avoid obesity (Sheard, Clark et al. 2004; Lee and Nieman 2010). As these fats can be highly caloric, the American Diabetes Association recommends keeping levels of SFA <7% of total energy intake and PUFA levels <10% (Bantle, Wylie-Rosett et al. 2008). It is emphasized that diabetics should focus on maintaining healthy and controlled levels of carbohydrates in order to keep their blood glucose at acceptable and safe levels (Association 2010).

Summary of Study Conclusions

In conclusion, the equations described in this paper were incredibly important in early efforts to identify dietary risk factors for dyslipidemia and CVD risk, beginning in the 1950's. Use of the equations facilitated important discoveries about optimal nutrition, and many of these conclusions have been validated in controlled feeding studies. From the Keys et al. study alone, we learned that dietary SFA greatly affects changes in cholesterol (causing cholesterol to increase) more than any other

type of fat, particularly PUFAs (causing cholesterol to decrease). In more recent times, dietary guidelines have been driven by results of controlled feeding studies and by observational studies with advanced methods for detecting and ameliorating underreporting. While this study was unsuccessful in predicting changes in total cholesterol in a diabetic population, it is important to reflect upon the implications of the research. Although the specific formulas did not accurately predict the lipid values, the ideas behind them (increasing MUFAs and PUFAs and decreasing SFA) are very applicable to most populations and are in agreement with recommendations from the American Diabetes Association. This study was successful in identifying cases of underreporting in subjects with a BMI in the overweight and obese category and did hypothesize correctly that the predictive equations were more accurate when the AAD diet was compared to the Control diet.

Table 1. Lipid predictive equations.

Source	Equation	Key
Keys, 1956	1a: ΔTotal Cholesterol (mg/(1000 mL))=2.7ΔS-1.3ΔP	ΔS = percentage of calories from saturated fatty acids ΔP = percentage of calories from unsaturated fatty acids
	1b: ΔTotal Cholesterol (mg/(1000 mL))=2.7ΔS-1.3ΔP+1.5ΔZ	ΔZ= square root of mg of dietary cholesterol per 1,000 calories
	1c: ΔTotal Cholesterol (mg/(1000 mL))=(2.7ΔS'-1.3ΔP)+1.5ΔZ	ΔS' = S (total saturated fat)-S" (amount of stearic acid in diet)
Hegsted, 1965	2: ΔTotal Cholesterol (mg/(1000 mL))=2.32S+0.32M-1.46P+6.51C+0.83	S=total saturated fatty acids M= MUFA P=PUFA C=dietary cholesterol
Mensink and Katan, 1992	3: ΔTotal Cholesterol (mg/(1000 mL))=2.32S+0.32M-1.46P+6.51C+0.83	ΔS'=lauric acid, myristic, and palmitic acids ΔMUFA = Monounsaturated fatty acids ΔPUFA = Polyunsaturated fatty acids
Yu, 1995	4a: ΔTotal Cholesterol (mg/dL)=(02.02Δ12:0-16:0)-0.03Δ18:0-0.48ΔMUFA-0.96ΔPUFA	12:0 to 16:0 = 12 to 16 carbon chain length saturated fatty acids 18:0 = 18 carbon saturated fatty acids.
	4b: ΔLDL-C (mg/dL)=(1.46Δ12:0-16:0)+0.07Δ18:0-0.69ΔMUFA-0.96ΔPUFA 4c: ΔHDL-C (mg/dL)=(0.62Δ12:0-16:0)-0.06Δ18:0+0.39ΔMUFA+0.96ΔPUFA	
Howell, 1997	5a: ΔTotal Cholesterol (mg/dL)=1.918ΔSFA-0.9ΔPUFA+0.0222Δ dietary cholesterol 5b: ΔLDL (mg/dL)=1.808ΔSFA-0.495ΔPUFA 5c: ΔHDL (mg/dL)=0.287ΔSFA+0.192Δtotal fat 5d: ΔTriglycerides (mg/dL)=0.0124 Δdietary cholesterol-0.859ΔPUFA+(-0.746+0.079·ΔPUFA)·Δtotal fat	ΔSFA=saturated fatty acids

Table 2. Characteristics of the study participants.

	Screening *n*=21		AAD Endpoint *n*=23		Control Endpoint *n*=12	
	Mean ± SE at Screening	Range	Mean ± SE at AAD Endpoint	Range	Mean ± SE at Control Endpoint	Range
Age *years*	56	40-74	---	---	---	---
Weight *kg*	97 ± 5	58 – 1467	97 ± 5	59-148	96 ± 6	64-139
Body Mass Index kg/m^2	34 ± 1	23-44	33 ± 1	23-44	33 ± 2	24-41
Total cholesterol *mg/dL*	177 ± 9	113-254	153 ± 8	103-234	148 ± 11	107-224
LDL-C *mg/dL*	98 ± 8	39-168	80 ± 7	40-148	77 ± 12	39-17
LDL-C Direct *mg/dL*	-	-	90 ± 7	42-153	84 ± 10	43-151
HDL-C *mg/dL*	46 ± 3	29-83	42 ± 3	256-78	38 ± 5	25-72
Triglycerides *mg/dL*	165 ± 20	57-378	154 ± 18	40-336	161 ± 29	38-303

Table 3. Nutrient profiles and composition of self-reported Personal Diet, Average American Diet and the Control Diet.

	Personal Diet *n* = 21	AAD *n* = 23	Control Diet *n* = 12
Mean calorie intake (kcal)	1951.7	2613.6	2704.0
Carbohydrates	43.9	49.9	49.9
Total fat	38.1	36.3	27.5
Protein	18.0	15.9	15.9
Saturated fat	13.0	11.7	6.8
Monounsaturated fatty acids	13.4	13.2	11.3
Polyunsaturated fatty acids	8.4	8.6	7.1
Cholesterol (mg/dL)	321.0	295.0	214.8
Saturated fatty acids 12:0	1.1	0.9	0.4
Saturated fatty acids 14:0	2.6	3.2	1.4
Saturated fatty acids 16:0	15.5	18.0	12.3
Saturated fatty acids 18:0	7.2	7.8	4.5

***Note:** Data is presented as percent of total calories, unless otherwise specified.

Table 4. Average observed vs. predicted change in lipid values from the study entry (screening) to the end of the Average American Diet (AAD) period (n=21).

	Observed Value at Screening	Observed Change at End of the AAD	Keys, 1956			Hegsted, 1965	Mensink and Katan, 1992	Yu, 1995	Howell, 1997
			Equation 1A	Equation 1B	Equation 1C				
Total cholesterol	174.5	-21.7	-3.7	-6.3	9.4	-4.1	7.0	6.5	-3.2
LDL-C	95.7	-15.8	-	-	-	-	-	4.8	-2.4
HDL-C	45.1	-3.3	-	-	-	-	-	2.1	3.6
Triglycerides	168.5	-12.4	-	-	-	-	-	-	-12.4

***Note:** All values represented as mg/dL.

Table 5. Average observed vs. predicted change in lipid values from the end of the Average American Diet (AAD) to the end of the Control Diet Period (n=12).

	Observed Value at End of AAD	Observed Change at End of the Control Diet	Keys, 1956			Hegsted, 1965	Mensink and Katan, 1992	Yu, 1995	Howell, 1997
			Equation 1A	Equation 1B	Equation 1C				
Total cholesterol	152.9	-3.5	-11.4	-14.0	-29.6	-14.3	-17.2	-14.7	-9.9
LDL-C (Calculated)	79.8	-2.0	-	-	-	-	-	-9.9	-8.2
LDL-C (Direct)	88.9	-5.6	-	-	-	-	-	-9.9	-8.2
HDL-C	41.8	-3.1	-	-	-	-	-	-7.2	-6.2
Triglycerides	156.1	7.9	-	-	-	-	-	-	21.9

***Note:** All values represented as mg/dL.

Table 6. Correlation between predicted and actual lipid changes.

		Personal vs. AAD (n=21)		AAD vs. Control (n=12)	
		Correlation coefficient	**p-value**	**Correlation coefficient**	**p-value**
Total cholesterol	Keys, 1956 Equation 1A	0.18	ns	-0.14	ns
	Keys, 1956 Equation 1B	0.09	ns	-0.16	ns
	Keys, 1956 Equation 1C	0.07	ns	0.22	ns
	Hegsted, 1965	-0.04	ns	-0.14	ns
	Mensink and Katan, 1992	0.09	ns	0.20	ns
	Yu, 1995 Equation 4A	0.07	ns	0.20	ns
	Howell, 1997 Equation 5A	0.04	ns	-0.15	ns
LDL cholesterol (calculated)	Yu, 1995 Equation 4B	0.21	ns	0.16	ns
	Howell, 1997 Equation 5B	0.27	ns	-0.36	ns
LDL cholesterol (direct)	Yu, 1995 Equation 4B	-	-	0.37	ns
	Howell, 1997 Equation 5B	-	-	-0.57	ns
HDL cholesterol	Yu, 1995 Equation 4C	0.43	0.049	0.16	ns
	Howell, 1997 Equation 5C	0.26	ns	0.11	ns
Triglycerides	Howell, 1997 Equation 5D	0.45	0.039	0.09	ns

***Note:** LDL measured by calculation only at screening visit.

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Personal Academic Vita

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EDUCATION

The Pennsylvania State University, University Park, PA
B.S., Nutritional Science, Applied Science Option with Honors in Biobehavioral Health, Expected May 2012
Dean's List – Spring 2009, Fall-Spring 2010, Fall-Spring 2011
Schreyer Honors College Scholar

PROFESSIONAL CERTIFICATES

ServSafe Food Handler Certification (May 2010, expires May 2015)
American Red Cross CPR & AED Certification (September 2011, expires September 2013)

RESEARCH EXPERIENCES

Vascular Health Interventions Lab, The Pennsylvania State University, Undergraduate Research Assistant, 2009-present

- Involved with the implementation, data collection, and data entry of a controlled feeding study, "Pistachio Diabetes", which examines the effects of a diet rich in pistachios on cardiovascular reactivity to stress in participants with Type 2 diabetes.
- Utilized techniques of blood pressure and measurement of pulse wave amplitude to conduct "EndoPat" exams to measure blood flow to finger tips and therefore, arterial elasticity.
- Attending the Experimental Biology Conference in San Diego, CA this April to present "The Assessment of the Practical Use of Lipid Predictive Equations in a Diabetic Population."
- Participated in monthly/bi-montly journal discussions with other undergraduate and graduate students in the lab to discuss present research relative to the lab research.
- Mentor/P.I.: Sheila G. West, Ph.D., Associate Professor of Biobehavioral Health and Laboratory Director

CLINICAL EXPERIENCES

Mount Nittany Medical Center Volunteer – Nutrition Department, August 2010-May 2011

- Conducted patient interviews with a diet aid, then eventually on my own, to identify patient's food preferences, allergies, and nutrition concerns.
- Entered data from the patient visits into a medical notes program for the dietitians to evaluate.

- Performed phone interviews with recently discharged patients on the quality of the food and experience with food service staff at the hospital.

NUTRITION EDUCATION EXPERIENCES

Teaching Assistant – NUTR 100: Contemporary Nutrition Concerns, August 2011-present

- Served as a Teaching Assistant for a 1.5 credit introductory nutrition course for non-nutrition majors, meeting once a week for 1.5 hours.
- Responsibilities included attending all lectures, taking notes, assisting the instructor during class, entering homework and class assignment grades, and completing project grading.
- Given the opportunity to teach a lecture during Spring 2012. Using the premade PowerPoint slides on weight management and eating disorders, designed a lecture with additional pieces on fad diets. The lecture was given to the 200 person class and lasted approximately 1 hour. Additionally, conducted a class activity on weight management.
- Required to lead at least one review session for exams during the semester and assist in completing study materials beforehand.
- Received 1 course credit.

Penn State Learning Center Tutor- NUTR 251 Group Leader, August 2010-May 2011

- Worked at the Penn State Learning center holding tutoring group sessions for an introductory nutrition course.
- Required preparing a study guide for what the class had covered in a week and tutoring 1-4 students in a study group setting, through 1-2 sessions per week for approximately 1 hour, as well as some individual tutoring for students who's schedules did not accommodate the session time.
- The position required tutors to take a course on tutoring for this position, which focused on group and individual study practices, how to address questions, and how to plan a study session.
- Received payment for tutoring sessions and planning hours.

Teaching Assistant- NUTR 251: Introductory Principles of Nutrition, Fall 2010

- Served as a Teaching Assistant for a 3 credit introductory nutrition course for nutrition and many other pre-health majors, meeting twice a week for 1.5 hours.
- Responsibilities included attending all lectures, assisting the instructor during class, entering homework and class assignment grades, and completing project grading for approximately 60 students.
- Required to lead at least one review session for exams during the semester and assist in completing study materials beforehand.
- Received 1 course credit.

FOOD SERVICE & NUTRITION EXPERIENCES

Sodexo Future Leaders Program Ambassador – Student Ambassador to Penn State, August 2011-present

- Served as a Sodexo Ambassador to promote the Future Leader's Internship to other students at Penn State.

- Selected from a group of 40 interns from the Sodexo Future Leaders Summer Program as per recommendations from my managers.
- Responsibilities include working with Sodexo recruiters to plan and execute events on campus at Penn State, utilizing social media to promote the program through twitter, Facebook, and Sodexo blogging.
- Collaborated with the other Sodexo Ambassador at Penn State to plan and execute a food drive in December.
- Required to submit a monthly report detailing activities, as well as attending a monthly conference call to collaborate with the other Ambassadors.

Sodexo Future Leaders Program Intern – Food Service Management Intern at the University of South Carolina, June-August 2011

- Worked as a Food Service Management Intern in the Sodexo Future Leaders Summer Program, completing a 10 week rotational program, serving in different departments of the University of South Carolina's dining services, including human resources, marketing, finance, quality assurance, operations, catering, and four of their dining locations..
- Personal accomplishments during the internship included designing several of the menus and creating a nutritionally adequate value meal based on calories, fat, and sodium content, as well as planning and helping to execute the campus's first job fair to the public.
- The program also required interns to complete five web presentations with other interns around the country to learn about topics including communication and leadership, as well as complete a group presentation to present to the other interns.

OTHER EXPERIENCES

Penn State Sailing Club – General Member, January 2011-present

- Participated as an active member of the Penn State sailing club in order to acquire more knowledge regarding the sport of sailing.

Omega Phi Alpha – Active member, Historian/Alumnae Liaison, Morale Chair, and Scholarship Chair, January 2010-present

- Earned the achievement as a lifetime member of Omega Phi Alpha, a national service sorority, through a pledging process.
- Completed over 75 hours of community service each semester as a member, with activities included helping at local animal shelters, cleaning the Penn State arboretum, collecting and sending supplies to soldiers, and raising over $70,000.00 for the Penn State Dance Marathon "THON," the largest student run philanthropy in the world.
- Elected as morale chair to keep the group morale high and celebrate birthdays in Fall 2010.
- Served as Historian/Alumnae Liaison for the 2011 Executive Board to keep the sorority's records up to date, plan the annual alumni weekend, create an annual newsletter with information on current sisters and alumnae, and work with other members of the executive board to make decisions for the sorority as a whole.
- Served as Scholarship chair in Spring 2012 to choose another member to receive a scholarship from the Omega Phi Alpha National Organization.

Schreyer Honors College "SHOtime" – Mentor and Team Leader, August 2009-September 2011

- Participated in the Schreyer Honors College Orientation Program, "SHOtime," for three years, which takes place three days earlier than other students arrive to campus for incoming freshman students.
- Mentored incoming freshman students in my area of study during year one to acclimate them to the Honors College and life at Penn State.
- Served as a team leader for the Finale Events committee during year two to plan a finale presentation by the Dean of the Honors College and provide support to the other twelve team leaders.
- Served as a team leader for the Logistics committee to plan all meals during a three day period, including menu planning for themed dinners, creating a meal wave system for crowd control, planning a carnival with booths and food for 500 people, transporting all signage and materials to various events around campus, and supporting the other members of the team and leader mentor.
- Both team leader positions required a bi-monthly meeting with all team leaders and the lead mentor to plan the orientation event starting in February and continuing into the summer.
- Assistant in the development of a "Make-Up Orientation Program" in 2009 for students who could not come to campus early. This required developing a schedule of events and activities, contacting all speakers, recruiting student volunteers, and running the event.

Schreyer Honors College Student Council – General Member and Recruitment Committee, September 2008-April 2010

- Participated in the Schreyer Honors College Student Council as a tour guide to give tours to visiting parents and students to the Schreyer Honors College, serve on question and answer panels, and speak to visitors at open houses for the Honors College.

Eberly College of Science Student Council – General Member and Secretary, August 2008-December 2009

- Active member of the Eberly College of Science Student Council at Penn State, requiring attendance to 4 of 6 meetings a semester.
- Served as secretary for the Fall 2009 semester, with responsibilities including taking meeting attendance and sending all communication to the club members.

SCHOLARSHIPS

Penn State Schreyer Honors College Academic Scholarship ($10,000 scholarship awarded to the approximate 300 incoming Schreyer Honors College scholars)

HONORS/AWARDS

Outstanding Service Award, Omega Phi Alpha

DIDACTIC PROGRAM IN DIETETICS COURSES COMPLETED

ENGL 030- Honors Freshman Composition
PSYCH 100H- Honors Introduction to Psychology
MICRB 201- Introduction to Microbiology
CHEM 210H- Honors Organic Chemistry I
BIOL 141- Physiology
NUTR 251H- Honors Introductory Principles of Nutrition
NUTR 120- Food Preparation
HRIM 329- Introduction to Food Production and Service
HRIM 330- Food Production and Service Management
STAT 200- Elementary Statistics
NUTR 456- Community Nutrition
NUTR 358- Assessment of Nutritional Status
ECON 002- Microeconomics Analysis
MICRB 107- Elementary Microbiology Lab
BMB 211- Elementary Biochemistry
NUTR 445- Nutrient Metabolism I
CAS 100H- Honors Effective Speech
NUTR 360- Disseminating Nutritional Information
NUTR 370- Professions in Dietetics
NUTR 451- Nutrition throughout the Life Cycle
NUTR 400- Introduction to Nutrition Counseling
NUTR 446- Nutrient Metabolism II
ENGL 202C- Technical Writing
NUTR 490W- Nutrition Seminar
NUTR 452- Nutritional Aspects of Disease
NUTR 453H- Honors Diet and Disease
NUTR 386- Managing Food and Nutrition Services

*Upon graduation in May 2012, will be eligible to complete a Dietetic Internship from completion of all require DPD courses.