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Comparison of a Traditional and Modified Daniel Fast on Blood Lipids, Lipid Peroxidation, and Inflammation

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COMPARISON OF A TRADITIONAL AND MODIFIED DANIEL FAST ON BLOOD LIPIDS, LIPID PEROXIDATION, AND INFLAMMATION

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ABSTRACT

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Dietary modification involving the removal of animal products, protein, and processed foods results in rapid and significant improvements in multiple health markers. We compared the impact of two restriction models on biomarkers of inflammation, oxidative stress, and cardiovascular health over a 21-day intervention. One model was a stringent vegan diet; the other was identical but included ~30g/d of additional protein in the form of meat and dairy. Compared to baseline, both models resulted in similar and significant improvements in blood lipids, as well as a reduction in inflammation. Modification of dietary intake may improve risk factors for cardiovascular disease.

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Introduction

For decades scientists have been investigating the effects of dietary intake on certain parameters of human health. Further advancements in technology have allowed for a more intuitive approach to analyzing the effects of nutrient intake on specific health-related biomarkers [1-3]. Fasting is a period of abstinence from some or all food, drink, or both, usually for a pre-determined period of time (reviewed in [4]). Fasting plans that are frequently studied and presented in the scientific literature include caloric restriction (CR) [5], alternate day fasting (ADF) [6], and dietary restriction (DR) [7].

Individuals who partake in CR plans strive to reduce their overall caloric intake on a daily basis (usually by 20-40% of daily energy requirements), while those partaking in ADF diets will alternate days of *ad libitum* feeding with days in which little to no calories are consumed. Dietary restriction is another plan that is characterized by the reduction or abstinence of certain components of the diet, such as amino acids or animal products. The religiously-motivated Daniel Fast, a stringent vegan diet that has been investigated recently and noted to result in multiple health-related benefits [8, 9], falls into this category of DR.

Many people partake in these fasting regimens in order to meet weight loss goals or to improve certain biomarkers of health, while others become involved for spiritual purposes. Prior investigations involving CR, ADF, and DR have been met with favorable results. Specifically, the benefits associated with CR, without malnutrition, include improved cardiovascular health, increased insulin sensitivity and glucose regulation, decreased oxidative damage, favorable changes in blood lipids, and reduced production of inflammatory mediators (reviewed in [10, 11]). It is postulated that these health-

related benefits are responsible for the life-extending effects of CR, which has been demonstrated in rodents [12] and other animals [13]. Similarly, studies that involve ADF in rodents have reproduced some of the same favorable effects as CR with regards to increasing lifespan, lowering cardiovascular disease (CVD) risk, as well as improving variables related to diabetes (reviewed in [14]). A CR and ADF diet produces similar reductions in total cholesterol (TC), low-density lipoprotein cholesterol (LDL-C), and triglycerides (TAG) [15]. The Daniel Fast has also been reported to reduce TC and LDL-C [8]. Lipid disorders have been identified as independent risk factors for CVD [16] and intervention studies have shown that treating these abnormalities decreases the risk for cardiovascular events [17]. Therefore, improving the blood lipid panel appears important for overall health.

Diets that reduce caloric intake generally reduce markers of oxidative stress such as, malondialdehyde (MDA) and hydrogen peroxide (H_2O_2) [18], which may be due to a decrease in the production of reactive oxygen and nitrogen species (RONS) such as superoxide ($O_2^{\bullet -}$). Similarly, ADF reduces markers of oxidative stress, as measured by protein carbonyls, nitrotyrosine, and 8-isoprostanes [15]. The Daniel Fast has also been reported to result in a lowering in oxidative stress biomarkers [9].

In addition to attenuated oxidative stress, CR has been shown to reduce inflammation, evidenced by a reduction in circulating levels of leukocytes and other inflammatory markers such as tumor necrosis factor alpha (TNF-α) and C-reactive protein CRP [19, 20]. While data are conflicting for the effects of ADF on inflammation, the literature indicates no effect of ADF on IL-6 and TNF- α [14] and little to no effect on CRP [15]. However, 8-weeks of ADF in overweight adults with moderate asthma

significantly reduces TNF-α [15]. With regards to the Daniel Fast, reductions in CRP that approached statistical significance have been reported [8]. Reducing systemic inflammation appears important, considering that increased inflammation and oxidative stress may be an underlying mechanism in the pathophysiology of aging and many age-related illnesses/diseases [21-24].

Similar to the above mentioned plans, a specific form of DR involving methionine restriction has been shown to be inversely correlated with maximum lifespan potential in rats [25]. While there is still much to be understood with regards to methionine restriction and longevity in humans, vegetarian diets tend to be low in methionine content and studies have consistently demonstrated improvements in blood lipids and decreased age-associated oxidative stress (reviewed in [26]). Additionally, investigations have noted significantly lower CRP in vegetarians when compared to age-matched non-vegetarians [27, 28].

Considered a variant of veganism, the Daniel Fast has demonstrated favorable outcomes on blood lipids [8]. Specifically, a 21-day Daniel Fast has indicated significant reductions in TC and LDL-C, but also resulted in a decrease in high-density lipoprotein cholesterol (HDL-C). Additional work involving a 21-day Daniel Fast has noted reductions in the TC/HDL-C ratio and LDL-C/HDL-C ratio, with similar effects on the other blood lipids (unpublished data). While vegetarian diets appear to have little effect on oxidative stress markers in younger women (age: 20-40 years) [29], a 21-day Daniel Fast in men and women (age 35 ± 1 years) has demonstrated decreases in both MDA and H_2O_2 [9]. With regards to antioxidant status, Trolox Equivalent Antioxidant Capacity (TEAC) has been reported to significantly increase following a 21-day Daniel Fast, with

no changes in the oxygen radical absorbance capacity (ORAC) [9]. The noted downside of this form of DR is that HDL-C is reduced [8]. Maintaining HDL-C would be advantageous due to the associated atheroprotective benefits [30].

Although reductions in HDL-C is common during low-fat vegetarian diets, without being associated with poor cardiovascular health (reviewed in [31]), efforts to maintain HDL-C during a DR period should be considered. One possible plan is to consider the inclusion of small amounts of animal protein to the diet during a traditional Daniel Fast plan. While studies indicate that meat consumption is directly related to an increased risk for disease [32], the quality of the meat (i.e. lean meat) is more important when considering the effects on blood lipids and disease [33-35]. Diets that include moderate consumption of lean beef and chicken [36, 37], as well as lean fish [43], are associated with modest increases in HDL-C. Alternatively, removing meat from the diet tends to lower HDL-C while having a greater effect on reducing TC and LDL-C [38]. It appears as though the protein from meat is not what causes an increase in oxidative stress and inflammation [39]. Rather, the type of fat appears to be more important when considering oxidative stress and inflammation, with exacerbated responses from increasing amounts of saturated fatty acids (SFA) [40].

Using a case study approach, we have recently noted that minimal consumption of low-fat meat (poultry and fish) and dairy (skim milk) allows for a similar, albeit not as striking, reductions in TC and LDL-C as compared to the traditional Daniel Fast, with little change in HDL-C. Moreover, the TC/HDL-C ratio was better with the modified plan compared to the traditional plan. Hence, further study with this modified plan needs to be considered, with direct comparison being made to the traditional Daniel Fast plan in

regards to blood lipids, as well as biomarkers of oxidative stress and inflammation. This was the purpose of the present investigation.

Methods

Subjects

Twenty-nine healthy men and women between the ages of 18 and 75 years were recruited to participate. Subjects were not current smokers, and did not have a history of controlled cardiovascular or metabolic problems (e.g., high blood pressure, diabetes). Subjects were not required to engage in any strenuous tasks during the course of their participation. They were instructed to simply alter their dietary intake in favor of more healthy food choices.

Health history, medication and dietary supplement usage, and physical activity questionnaires were completed by all subjects and reviewed in detail by the study coordinator (with consultation with the PI as needed) to determine eligibility. Prior to participation, each subject was informed of all procedures, potential risks, and benefits associated with the study through both verbal and written form in accordance with the procedures approved by the University Institutional Review Board for Human Subjects Research. All subjects signed an informed consent form prior to being admitted as a subject.

Study Timeline

Week 0	Initial screening: Paperwork and diet instructions/logs; plan assignment
Week 1	Complete 7 day diet record and prepare for Fast
Week 2-4	Day 1: Fasting blood sample, measurements; Start Fast (for 3 weeks)
	Week 4: Complete 7 day diet record
	End Week 4: Fasting blood sample, measurements, post-fast questionnaire

Daniel Fast and Modified Daniel Fast

Subjects were assigned to either the traditional Daniel Fast (vegan diet) or the Daniel Fast inclusive of one serving per day of *lean meat (3 ounces of chicken, fish, beef, pork, or turkey) and skim milk (8 ounces).* Following all baseline assessments and the initial week of dietary recording, subjects underwent their Daniel Fast assignment for 3 weeks. While numerous websites are available with information and recipes related to this fast, subjects were provided an outline of the foods that are routinely allowed, as well as commonly consumed foods that are not allowed. For simplicity sake, subjects were informed that the diet essentially consists of fruits and vegetables, nuts, seeds, legumes, vegetable, oil, and grains. In addition, no additives, preservatives, flavoring, caffeine, or alcohol were allowed. Subjects were allowed caffeinated coffee and tea, as well as water, to drink. This applied to subjects in both plans (traditional and modified). Those subjects assigned to the traditional Daniel Fast plan were not allowed any animal products, while subjects assigned to the modified Daniel Fast plan were required to consume one serving per day of lean meat (3 ounces) and one serving per day of skim milk (8 ounces). Subjects were exposed to food models to have a better understanding of portion sizes.

Lab Visits

During the initial visit to the lab, subjects completed the informed consent form, health, and physical activity questionnaires. They were also informed of their assigned condition (traditional or modified Daniel Fast plan), and received study instructions and a study schedule. During the other lab visits (end of weeks 1 and 4), all assessments were performed. Heart rate (via palpation) and blood pressure (via cuff and stethoscope) was

recorded following a 10 minute period of quiet rest. A blood sample was then taken from subjects, as described below. Subjects' height, weight, and circumference measures were recorded. Subjects then had a scan performed to determine their body composition (via dual energy x-ray absorptiometry [DEXA]), as described below.

Blood Collection and Biochemistry

Venous blood samples (20mL; 4 teaspoons) were taken from subjects via needle and Vacutainer® by a trained phlebotomist. Blood samples were collected in a rested and fasted state at the end of week 1 (before beginning the fast) and week 4. Following collection, blood collected in tubes with no additives was allowed to clot at room temperature for 30 minutes and then separated to serum by centrifugation at 1500g for 15 minutes at 4°C. Samples were sent to Laboratory Corporation of America (LabCorp) for a lipid panel and CRP evaluation. Blood collected in tubes containing ethylenediaminetetraacetic acid (EDTA) was immediately separated to plasma by centrifugation at 1500g for 15 minutes at 4°C. Plasma was stored and analyzed for MDA. The lipid panel was determined using enzymatic procedures (Roche/Hitachi Modular). C-reactive protein was determined using a high-sensitivity, particle-enhanced turbidimetric immunoassay (Roche Integra 800). Malondialdehyde was analyzed in plasma following the procedures of Jentzsch et al [41] using reagents purchased from Northwest Life Science Specialties (Vancouver, WA). Specifically, 75µL of plasma was added to microcentrifuge reaction tubes and 75µL of 1M phosphoric acid and 75µL of 2-thiobarbituric acid reagent was added to each reaction tube and mixed thoroughly. Samples and reagents were incubated for 60 minutes at 60°C. Following incubation, tubes were removed, and the reaction mixture was transferred to a microplate. The

absorbance was read using a spectrophotometer at both 535 and 572nm to correct for baseline absorption. Malondialdehyde equivalents were calculated using the difference in absorption at the two wavelengths. Quantification was performed with a calibration curve using tetramethoxypropane in a stabilizing buffer. The detection limit for MDA, as per the manufacturer, is 0.1µM.

Dual energy x-ray absorptiometry (DEXA)

Although not a primary outcome measure in the present study, body composition was determined via DEXA. After the participant removed all extraneous metal objects, he or she was placed onto the QDR 4500W table in the supine position within the designated whole body scan area which is defined on the table mattress. The upper extremities were positioned palm down beside the thighs and care was taken to assure that the arms did not come in contact with the thigh; the feet of the participant were taped together. The DEXA scan was performed by a certified technician. Total and regional body fat percentages were computed in addition to fat and fat-free mass.

Dietary Records and Activity

During the initial lab visit a full explanation of dietary recording was provided to subjects, along with data collection forms. An overview of study procedures was also provided. All subjects were instructed to maintain their normal diet during week one of the study (prior to beginning the fast) and to record on data forms all food and drink consumed. During week 2 of the study, subjects began the Daniel Fast (traditional or modified) and were asked to record on data forms all food and drink consumed during week 4 of the study (the final week of the fast). Nutritional records from subjects before and during the fast period were analyzed for total calories, protein, carbohydrate, fat, and

a variety of micronutrients (Food Processor SQL, version 9.9, ESHA Research, Salem, OR). Subjects were instructed to maintain their normal physical activity habits during the entire study period. Subjects were given specific instructions regarding abstinence of alcohol consumption, in addition to the avoidance of strenuous exercise during the 48 hours immediately preceding the assessment days.

Compliance, Mood, Vitality, and Satiety

As an assessment of compliance, mood, vitality, and satiety, subjects were asked to complete a questionnaire related to their experience on the Daniel Fast (Appendix B). These data were based solely on subjects' self-report.

Statistical Analysis

Biochemical data were analyzed using a 2 (plan) x 2 (time) analysis of variance (ANOVA). Tukey post hoc tests were used as needed. Dietary data, anthropometrics, compliance, mood, vitality, and satiety data were analyzed using a one-way ANOVA. All analyses were performed using JMP statistical software (version 4.0.3, SAS Institute, Cary, NC). Statistical significance was set at $P \leq 0.05$. The data are presented as mean ± SEM.

Results

Twenty-nine subjects completed the study, 13 in the modified Daniel Fast diet and 16 in the traditional Daniel Fast diet. Subjects' descriptive characteristics are presented in Table 1. The main variables of interest were blood lipids, MDA, and CRP. We hypothesized that these variables would decrease to a similar extent between the two groups, with a greater reduction in HDL-C for subjects in the traditional Daniel Fast plan compared to the modified plan. In short, we observed no significant difference between the two dietary regimens on any of the main outcome variables. Although not significant,

there was a greater reduction in HDL-C in the traditional Daniel Fast group (13.3%) compared to the modified group (7.6%). A summary of our results are presented in Table 2.

Compliance, Mood, Vitality, and Satiety

All subjects completed the diet intervention as well as the pre and post assessments. Compliance was similar between groups (traditional: 96.0 ± 0.94% vs. modified: 91.4 ± 3.1%; $p = 0.13$). On a scale from 0-10, subjects rated their mood, vitality, and satiety during the diet. There were no condition x time interactions for the following variables ($p > 0.05$); "mental outlook" (traditional: 8.8 ± 0.3 vs. modified: 8.8 ± 0.3), "physical health and vitality" (traditional: 8.3 ± 0.5 vs. modified: 8.4 ± 0.5), and "feeling of satiety" (traditional: 8.8 ± 0.3 vs. modified: 8.3 ± 0.6). In both groups, "mental outlook" ($p = 0.03$) and "physical health and vitality" ($p = 0.006$) increased from pre to post ("mental outlook": 8.1 ± 0.5 to 8.8 ± 0.3 and 7.9 ± 0.3 to 8.8 ± 0.3 in the traditional and modified groups respectively, "physical health and vitality": 7.4 ± 0.4 to 8.3 ± 0.5 and 6.6 ± 0.5 to 8.4 ± 0.5 in the traditional and modified groups respectively).

Body weight and composition

No condition x time interactions were noted for body weight ($p = 0.98$), total body fat percentage ($p = 0.96$), or trunk body fat percentage ($p = 0.94$). Data are presented in Table 1. There was no significant difference in body weight from pre to post ($p = 0.68$) in the traditional group (pre: 81.7 ± 4.8 kg vs. post: 79.3 ± 4.9 kg) or in the modified group (pre 74.5 ± 6.1 kg vs. post: 72.4 ± 5.7 kg). Total body fat percentage was not significantly different ($p = 0.92$) from pre to post in the traditional group (pre: 35.7% ± 2.4 vs. post: 35.5 ± 2.5%) or in the modified group (pre: 33.8 ± 3.5% vs. post: 33.4 ±

3.6%). Similarly, trunk body fat percentage was not significantly different ($p = 0.94$) from pre to post in the traditional group (pre: 36.5 ± 2.5% vs. post: 36.2 ± 2.6%) or in the modified group (pre: 32.8 ± 3.7% vs. post: 32.0 ± 3.7%). Neither group experienced significant changes in fat mass ($p = 0.80$) or fat-free mass ($p = 0.70$) from pre to post.

Blood Lipids, Malondialdehyde, and C-Reactive Protein

No interactions, condition, or time effects were noted for TAG, LDL-C, HDL-C, VLDL-C, TC:HDL-C ratio, LDL-C:HDL-C ratio, or CRP ($p > 0.05$). Total cholesterol significantly decreased ($p = 0.02$) to a similar extent in the traditional group (16.7%) and modified group (14.2%) but no interactions or condition effects were noted ($p > 0.05$). A condition effect was noted for MDA ($p = 0.03$), with higher levels in the traditional group, but no significant interactions or time effects were noted ($p > 0.05$). A summary of our main outcome variables can be viewed in Table 2.

Dietary Data

Dietary Data are presented in Table 3. Intake between the groups was significantly different for grams of protein ($p = 0.009$), percentage of protein ($p = 0.001$), selenium ($p = 0.007$), and calcium ($p < 0.01$). Compared to the traditional group, the modified group consumed significantly more protein ($p = 0.04$), percentage of protein ($p = 0.02$), and Vitamin D ($p = 0.04$). During the diet, both groups consumed less kilocalories ($p = 0.0002$), grams of protein ($p = 0.002$), grams of carbohydrate ($p < 0.01$), sugar ($p = 0.0006$), grams of total fat ($p < 0.0001$), percentage of fat ($p = 0.006$), SFA ($p < 0.0001$), cholesterol ($p < 0.0001$), selenium ($p < 0.01$), and calcium ($p < 0.0001$), while consuming more grams of fiber ($p = 0.0008$), grams of soluble fiber ($p = 0.01$),

percentage of carbohydrate ($p = 0.018$), Vitamin A ($p < 0.001$), and Vitamin C ($p = 0.0005$).

Discussion

The results of the study indicate that there is no difference between a traditional Daniel Fast and a modified Daniel Fast, on selected biomarkers of health. We hypothesized that the diets would yield similar decreases in MDA, CRP, TC and LDL-C. In addition, we hypothesized that the traditional Daniel Fast group would experience a greater reduction in HDL-C. Although slightly different, both diets had similar effects statistically on all of the main outcome variables; blood lipids, MDA, and CRP.

Prior work with the Daniel Fast, along with data from the present investigation, indicates that a Daniel Fast dietary regimen is beneficial for cardiovascular and metabolic health [8, 9]. Our data indicate that these benefits are maintained when small quantities of animal products are added to a traditional Daniel Fast diet. In addition to the cardiovascular and metabolic benefits observed over a short period of time (21 days), the Daniel Fast has been shown to be well tolerated, with greater than 95% compliance [8, 9]. However, we have noted that the restriction of animal products makes the diet challenging and less appealing to many individuals. While compliance was similar between the diets during the present investigation, further research is still needed to compare long-term compliance and health benefits. Our goal in the present study was to determine if the addition of animal products to a traditional Daniel Fast would produce similar results as seen in the past, when followed for the same duration of study (3 weeks).

Total cholesterol significantly decreased in both groups: 16.7% in the traditional group and 14.2% in the modified group. Both diets demonstrated a similar non-significant decrease in LDL-C, 17.4% and 18.2% in the traditional and modified groups, respectively. Moreover, both diets resulted in similar non-significant reductions in VLDL-C, TAG, LDL-C:HDL-C ratio, and TC:HDL-C ratio, which is similar to what has been observed in healthy men undergoing a vegetarian diet or a vegetarian plus lean meat diet [42]. We hypothesized that the traditional Daniel Fast group would experience a greater reduction in HDL-C, which is common when undergoing a vegan based diet [31]. Furthermore, low cholesterol diets that include moderate consumption of lean meat have shown modest increases in HDL-C in healthy [42] and hypercholesterolemic [36, 43] subject populations. It appears as though lean meat may not be responsible for the rise in cardiovascular related diseases; rather, additives, preservatives, and the SFA associated with animal products and fast food may be the culprit.

In the present investigation, there was a non-significant decrease in HDL-C; 13.3% in the traditional group and 7.6% in the modified group. Earlier work with the traditional Daniel Fast demonstrated a significant reduction in HDL-C (14.5%) after 21 days of the diet [8]. As mentioned previously, adding small quantities of lean red or white meat to a cholesterol lowering diet helps maintain HDL-C in hypercholesterolemic men and women [36, 43]. Likewise, significant increases in HDL-C, and the LDL-C:HDL-C ratio have been observed in healthy middle aged males undergoing a combination of a vegetarian diet and 150 g/day of lean meat for one month [42]. While the results indicate no difference of statistical significance between the diets, when adding a small amount of lean meat (3 ounces) and skim milk (8 ounces) to an otherwise

strict vegan diet, less of a decrease in HDL-C is observed. Although not of statistical significance, the difference may have clinical relevance, in particular if the plan was carried out over a longer period of time and the changes were of greater magnitude. It is also possible that increasing the sample size may assist in uncovering findings of statistical significance.

Some studies suggest that the omega-6/omega-3 fatty acid ratio is important when considering changes in HDL-C. In healthy young adult males, a 3% increase in HDL-C was observed after three weeks of a low SFA diet (9% of total energy) with increased intake of omega-3 polyunsaturated fatty acids (PUFA) (5 g/day) [44]. In another study, Rajaram et al compared the effects of two diets rich in omega-3 PUFA and low in SFA (< 10% SFA) in mildy hyperlipidemic men and women, one from walnuts (42.5 g, 6 days/week) and the other from salmon (4 ounces raw, 2 twice/week) [37]. After four weeks, the fish diet resulted in a significantly greater increase in HDL-C. However, a more favorable change in the LDL-C:HDL-C ratio was observed in the walnut diet. In the present investigation, the modified Daniel Fast group experienced a slightly smaller decrease in HDL-C compared to the traditional diet. Noteworthy, was the change in the LDL-C:HDL-C ratio. Although not significant, a 15.7% decrease was observed in the modified group, compared to a 5.8% decrease in the traditional diet. The omega-6/omega-3 fatty acid ratio in the traditional and modified groups was 12.3 and 7.4 respectively. With careful review of the changes in blood lipids, perhaps the modified group benefited from the combination of omega-3 PUFA from plants and meat. Nonetheless, a favorable change in the ratio is more important when considering the

cholesterol carrying properties of the different lipoprotein particles, and may have clinical relevance.

In contrast to previous work with the Daniel Fast [9], in the present study MDA was not significantly impacted by either diet. Baseline MDA was significantly higher in the traditional Daniel Fast group, whereas the modified group had relatively low baseline levels of MDA (0.92 ± 0.14 $\mu mol \cdot L^{-1}$ vs. 0.62 ± 0.03 $\mu mol \cdot L^{-1}$, respectively). While there were no significant differences between the groups from pre to post, a 16% decrease in MDA was observed in the traditional group whereas a 3.1% increase in MDA was observed in the modified group. Other studies have also shown that adding lean meat to the diet does not negatively impact biomarkers of oxidative stress. In a cohort study including healthy lacto-ovo-vegetarians and omnivores, MDA was not different across the groups [29]. When 215 g/day of lean red meat was used to replace carbohydrate rich foods in middle aged men and women with high blood pressure, plasma F2-isoprostanes did not change [39]. While it appears that small amounts of lean meat/protein does not negatively impact biomarkers of oxidative stress, diets that incorporate increasing amounts of fruit and vegetable intake have demonstrated favorable changes in oxidative stress biomarkers. Furthermore, urinary excretion of 8-isoprostane $F_{2\alpha}$ was significantly decreased in healthy women undergoing a high fruit and vegetable diet for eight weeks (9.2 servings of fruit and vegetables per day) [45]. Similarly, 8-isoprostane $F_{2\alpha}$ has been shown to significantly decrease after only 14 days of a dietary intervention that increased fruit and vegetable intake to 12 servings per day. Interestingly, the authors noted no difference in urine concentrations of MDA [46]. Isoprostanes and MDA are the more prominent biomarkers of lipid peroxidation. However, other relatively stable end

products can be measured such as lipid hydroperoxides, α,β-unsaturated aldehydes, conjugated dienes, oxidized low-density lipoprotein, and 4-hydroxy-2-nonenal. In the present investigation, even though MDA did not change, it is possible that other biomarkers of oxidative stress were influenced. Using MDA as the exclusive biomarker of oxidative stress is a limitation of our study.

The ongoing regulation of the production and quenching of free radicals is an essential process in biological redox signaling. Dysfunction in the body's ability to regulate RONS production has been implicated in the pathogenesis of many age related diseases; atherosclerosis, type II diabetes mellitus, and Alzheimer's disease to name a few [47]. The inability to scavenge increased RONS production has also been implicated in the early onset of systemic inflammation [48]. Dietary intake can affect the inflammatory response in the body, and this response has been linked to oxidative stress, insulin resistance, obesity, and CVD [49]. C-reactive protein in particular has been shown to be a reliable marker of systemic inflammation during the development of CVD [50], and any effect on this biomarker by the two diets are noteworthy.

There were no observed differences in CRP between the two diets. In a population with already low CRP levels (< 1.5 mg/L), the two diets demonstrated a trend for reducing CRP, both producing greater than 40% reductions. Although not statistically significant, this change may be clinically meaningful, especially due to the fact that the American Heart Association has identified CRP as an independent predictor for CVD risk [51].

Both groups in the present investigation increased dietary antioxidant intake, specifically Vitamin C and Vitamin A. A crossover study in healthy middle aged men

and women (~60 years old) undergoing a high-total antioxidant diet demonstrated significant decreases in CRP compared to a low-total antioxidant diet. In addition, no changes were observed in MDA following the dietary intervention [52]. The changes observed in the aforementioned study were similar to our findings in regards to the changes in CRP and MDA, indicating an improvement in systemic inflammation without changes in markers of oxidative stress. An investigation by Katcher et al studied the effects of a hypocaloric diet that included whole grains, fruits, vegetables, and small quantities of lean meat and low-fat dairy products in obese individuals with metabolic syndrome. Sharing many of the same characteristics as a modified Daniel Fast, the results of the study indicated a significantly greater reduction in CRP when including whole grains compared to refined grains [53]. Others have shown SFA be the most important variable contributing to CRP in healthy young adults [54]. This could explain a portion of the benefits observed in the present investigation, owing to the fact that both groups reduced dietary SFA to less than 8 grams during the intervention.

Recently, large cohort studies have delineated a relationship between red meat consumption, metabolic syndrome [55] and increased mortality [56]. Included in these cohort studies are processed meats, organ meats, and red meats. Indeed, the consumption of processed meats have been associated with coronary heart disease and diabetes, however, this relationship does not hold true for red meat alone (i.e. beef, hamburgers, lamb, pork, or game) [57]. Based on our investigation, as well as a review of the relevant literature, the effects of adding a moderate amount of lean meat (i.e. red meat, white meat, and/or fish) to a strict vegan diet produces similar results in health biomarkers as a strict vegan diet.

Conclusion

In conclusion, our results indicate that the addition of lean meat to a traditional Daniel Fast appears as beneficial as the vegan based Daniel Fast, if not yielding slightly more favorable results with regard to changes in HDL-C and certain lipid panel ratios. Both diets produced similar changes in all measured outcome variables. While these findings are interesting, future research is needed to assess the long-term health benefits of this plan, as well as the long-term compliance of individuals following this approach within a free living environment.

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APPENDIX A

TABLES

Table 1. Descriptive characteristics of men and women following a traditional and modified Daniel Fast for three weeks

Variable	Traditional		Modified	
Time	Pre	Post	Pre	Post
Number	16	16	13	13
Age (yrs)	42.4 ± 4.1	42.4 ± 4.1	41.7 ± 4.0	41.7 ± 4.0
Height (cm)	165.8 ± 2.0	165.9 ± 2.0	166.8 ± 2.2	166.8 ± 2.2
Weight (kg)	81.7 ± 4.8	79.3 ± 4.9	74.5 ± 6.1	72.4 ± 5.7
BMI ($kg \cdot m^{-2}$)	29.7 ± 1.6	28.8 ± 1.6	26.6 ± 1.8	25.9 ± 1.7
Waist (cm)	90.2 ± 3.8	87.8 ± 4.0	82.6 ± 5.2	80.9 ± 4.9
Hip (cm)	109.8 ± 3.1	107.6 ± 3.1	106.0 ± 3.0	104.3 ± 2.9
Waist:Hip	0.82 ± 0.03	0.82 ± 0.03	0.77 ± 0.03	0.77 ± 0.03
DEXA Total (%)	35.7 ± 2.4	35.5 ± 2.5	33.8 ± 3.5	33.4 ± 3.6
Fat-Free Mass (kg)	52.1 ± 3.3	50.6 ± 3.3	49.3 ± 4.1	48.2 ± 3.9
Fat Mass (kg)	29.6 ± 2.9	28.6 ± 2.9	26.4 ± 4.0	25.4 ± 3.9
DEXA Trunk (%)	36.5 ± 2.5	36.2 ± 2.6	32.8 ± 3.7	32.0 ± 3.7
Heart Rate (bpm)	69.0 ± 2.2	65.6 ± 2.1	69.3 ± 2.5	68.3 ± 2.6
Systolic Blood Pressure (mmHg)	115.1 ± 5.0	111.9 ± 5.2	105.6 ± 3.9	106.7 ± 4.2
Diastolic Blood Pressure (mmHg)	72.2 ± 3.3	71.9 ± 4.3	68.9 ± 2.8	64.1 ± 2.7
Aerobic Exercise ($hrs \cdot wk^{-1}$)	3.9 ± 1.0	NA	2.3 ± 0.4	NA
Years Aerobic Exercise	9.1 ± 3.2	NA	7.6 ± 2.3	NA
Anaerobic Exercise ($hrs \cdot wk^{-1}$)	0.9 ± 0.3	NA	0.9 ± 0.4	NA
Years Anaerobic Exercise	2.0 ± 0.8	NA	4.3 ± 1.9	NA

Values are presented as mean ± SEM.
No statistically significant differences noted ($p > 0.05$)

Table 2. Blood lipid, MDA, and CRP data of men and women following a traditional and modified Daniel Fast for three weeks

Variable	Traditional		Modified	
Time	Pre	Post	Pre	Post
Cholesterol ($mg \cdot dL^{-1}$)	186.75 ± 13.48	155.63 ± 9.74*	180.69 ± 11.82	155.08 ± 11.86*
Triglycerides ($mg \cdot dL^{-1}$)	101.00 ± 10.93	78.69 ± 6.65	89.54 ± 8.91	79.31 ± 10.03
HDL-C ($mg \cdot dL^{-1}$)	56.31 ± 3.80	48.81 ± 3.38	58.62 ± 4.22	54.15 ± 3.65
VLDL-C ($mg \cdot dL^{-1}$)	20.19 ± 2.17	15.75 ± 1.31	18.08 ± 1.78	15.85 ± 2.00
LDL-C ($mg \cdot dL^{-1}$)	110.25 ± 12.08	91.06 ± 8.42	104.00 ± 10.92	85.08 ± 9.85
LDL-C:HDL-C	2.08 ± 0.22	1.96 ± 0.19	1.97 ± 0.31	1.66 ± 0.23
TC:HDL-C	3.47 ± 0.25	3.32 ± 0.22	3.28 ± 0.34	2.97 ± 0.27
MDA ($\mu mol \cdot L^{-1}$)	0.92 ± 0.14**	0.76 ± 0.08**	0.62 ± 0.03**	0.64 ± 0.05**
CRP ($mg \cdot L^{-1}$)	1.36 ± 0.26	0.81 ± 0.24	1.45 ± 0.50	0.85 ± 0.28

Values are presented as mean ± SEM.
* Indicates significant difference from pre to post ($p = 0.02$)
** Indicates significant difference between groups ($p = 0.03$)
No other statistically significant difference noted ($p > 0.05$)

Table 3. Dietary data of men and women following a traditional and modified Daniel Fast for three weeks

Variable	Traditional		Modified	
Time	Pre	Post	Pre	Post
Kilocalories	2070.3 ± 211.9	1141.1 ± 74.2*	1977.5 ± 186.7	1523.0 ± 203.8*
Protein (g)***	69.8 ± 5.3**	35.9 ± 2.7*	73.4 ± 9.7**	66.4 ± 6.8*
Protein (%)***	13.9 ± 0.5**	12.6 ± 0.7	15.1 ± 1.6**	18.5 ± 1.2
Carbohydrate (g)	256.6 ± 24.5	176.3 ± 13.0*	264.8 ± 31.7	206.2 ± 33.3*
Carbohydrate (%)	50.3 ± 2.0	62.3 ± 2.5	52.7 ± 2.3	53.9 ± 4.0
Fiber (g)	19.9 ± 2.0	26.8 ± 2.0*	18.5 ± 2.0	30.8 ± 4.4*
Fiber-soluble (g)	0.9 ± 0.35	2.4 ± 0.42*	1.3 ± 0.6	2.6 ± 0.8*
Sugar (g)	86.6 ± 9.7	53.9 ± 6.5*	110.4 ± 18.5	62.5 ± 7.4*
Fat (g)	84.2 ± 11.5	37.0 ± 3.9*	68.5 ± 8.0	43.8 ± 6.0*
Fat (%)	35.6 ± 1.7	28.5 ± 2.2*	31.5 ± 2.5	26.3 ± 2.1*
SFA (g)	25.9 ± 3.8	6.4 ± 0.7*	20.2 ± 2.6	7.4 ± 1.1*
Monounsaturated Fat (g)	21.3 ± 4.8	11.3 ± 1.8	15.3 ± 2.6	16.0 ± 2.4
PUFA (g)	9.7 ± 1.9	6.4 ± 0.8	9.2 ± 1.6	8.7 ± 1.2
Trans Fat (g)	3.1 ± 1.9	0.1 ± 0.1	1.7 ± 0.6	0.3 ± 0.2
Omega 3 (g)	0.6 ± 0.1	0.4 ± 0.1	0.5 ± 0.2	0.7 ± 0.1
Omega 6 (g)	7.8 ± 1.9	4.9 ± 0.7	5.9 ± 1.0	5.2 ± 0.7
Cholesterol (mg)	258.0 ± 38.0	12.3 ± 5.0*	219.0 ± 25.3	68.3 ± 11.5*
Vitamin C (mg)	44.9 ± 5.1	112.1 ± 21.7*	59.0 ± 12.0	109.7 ± 18.2*
Vitamin E (mg)	6.1 ± 1.2	5.4 ± 0.9	4.9 ± 0.9	7.2 ± 1.3
Vitamin A (RE)	208.8 ± 78.7	781.1 ± 155.8*	204.1 ± 49.5	584.3 ± 165.6*
Selenium (µg)	40.7 ± 5.2**	21.4 ± 2.8*	52.4 ± 8.5**	41.2 ± 5.4*
Calcium (mg)	583.6 ± 91.8**	251.4 ± 18.7*	700.2 ± 46.1**	464.4 ± 56.1*
Vitamin D (IU)***	22.3 ± 10.6	5.5 ± 2.7	32.8 ± 11.3	59.0 ± 15.1

Values are presented as mean ± SEM.

* Indicates significant difference from pre to post ($p < 0.05$)

** Indicates significant difference between groups ($p < 0.05$)

*** Indicates significant interaction ($p < 0.05$)

No other statistically significant difference noted ($p > 0.05$)

APPENDIX B

EXTENDED LITERATURE REVIEW

Fasting/Dietary Restriction

Investigations involving the consumption of food and the effects on biochemical parameters of health have been of great interest to researchers over the past several decades. Food is an essential part of the human existence and therefore, a lack of intake can lead to malnutrition and death. On the contrary, over-consumption of food, coupled with a lack of physical activity, results in the excess storage of body fat and weight gain. The latter statement is seen as one of the driving forces behind many health-related deaths in developed countries. This has led many investigators to seek the understanding of how dietary intake impacts human health.

Significant attention has been given to specific dietary regimens and their potential role in extending lifespan, as well as in preventing disease. Typically, dietary regimens involve the restriction of calories or the restriction of certain nutrients. Caloric restriction (CR) and alternate day fasting (ADF) are two dietary regimens that seek to restrict the overall intake of calories—either on a daily basis or on an intermittent basis. The restriction of specific components or nutrients from the diet is commonly referred to as dietary restriction (DR). Several religiously motivated fasts have been investigated and fall into these categories. The religiously-motivated Daniel Fast, a stringent vegan diet that has been investigated recently and noted to result in multiple health-related benefits [1, 2], falls into this category of DR. Other religiously motivated fasts include Islamic Ramadan, which consists of a fasting period from sunrise to sunset (a form of ADF), and Greek Orthodox Christianity fasting (GOC), which is also a variant of DR.

Caloric Restriction

Caloric restriction has been extensively studied in rodents, monkeys, and several other species in the animalia kingdom. Many of these studies, which are typically carried out with 20-40% restriction of *ad libitum* feeding [3], have demonstrated a plethora of health-related benefits with regards to increasing lifespan [4]. Some of the more prominent benefits are as follows: improved cardiovascular health, increased insulin sensitivity and glucose regulation, decreased oxidative damage, favorable changes in lipid profiles, and reduced production of inflammatory mediators [4, 5]. As a result, CR has been shown to reduce the onset of age-related diseases such as cardiovascular disease (CVD), diabetes, and cancer [6, 7].

The favorable effects of CR on cardiovascular health can be attributed to the following: lowering of resting blood pressure (BP) and heart rate (HR), improved ventricular function, improved HR and BP response following exercise, increases in HR variability, and increased flow mediated vasodilation [4]. Moreover, 12 months of 20% CR in humans has been shown to lower coronary heart disease (CHD) risk factors by favorably altering blood lipids [8]. Specifically, and along with weight loss, CR induces its favorable effects by reducing plasma total cholesterol (TC), triglycerides (TAG), low-density lipoprotein cholesterol (LDL-C), and total cholesterol/high-density lipoprotein cholesterol (TC/HDL-C) ratio [8].

There have been many hypotheses regarding the life-extending effects of CR and the mechanisms involved. One that has gained much support over the last few decades is the oxidative stress hypothesis [9]. Markers of oxidative stress, such as, malondialdehyde (MDA), superoxide ($O_2^{\bullet-}$), and hydrogen peroxide (H_2O_2) have been

shown to decline following a CR regimen [9]. A review of investigations in rodents supports the notion that CR lowers resting levels of oxidative stress, delays the oxidant damage associated with age, and delays loss of membrane fluidity [10]. A reduction in energy intake attenuates the production of RONS, sometimes referred to as free radicals (radicals), that are available to damage proteins, lipids, and deoxyribonucleic acid (DNA). As lipids and protein make up an integral part of the plasma membrane, maintaining this barrier is vital for the functionality of cells, organs, and organisms as a whole. Along with attenuated oxidative stress, CR has also been shown to reduce inflammation by reducing circulating levels of leukocytes and other inflammatory markers such as tumor necrosis factor alpha (TNF-α) and C-reactive protein (CRP) [8, 11].

One hypothesis attributes the health-related benefits of CR to an increase in the metabolic rate. An increased metabolic rate causes a rise in the production of mitochondrial RONS, which then up-regulates the production of radical-scavenging enzymes as a defense mechanism [12]. The adaptive response of an increased antioxidant defense system is speculated as one of the mechanisms responsible for the anti-aging effect of CR [12]. While the specific mechanisms concerning CR are yet to be fully determined, the effects of CR on health-related parameters are remarkable as evidenced above. However, compliance to such a plan can be difficult, in particular when a higher percentage of calories are removed from the diet (e.g., > 20%). Many regimens that offer similar health benefits have been proposed in place of CR due to the strictness of the diet. Some of these diet regimens are developed with the hopes of promoting long-term compliance and lifestyle changes.

Alternate-Day Fasting

Due to the rigorous demands of a CR regimen that restricts caloric intake on a daily bases, other dietary intervention strategies have gained attention in the scientific community. Alternate-day fasting (ADF) is another form of caloric restriction; however the regimen differs from CR because restriction occurs every other day. ADF regimens generally involve alternating days of *ad libitum* feeding followed by a fasting day on which food is withheld or reduced. Two forms of ADF exist: A true ADF regimen where no calories are allowed on the "fast" day [13] and a modified ADF where subjects are typically restricted to 25% of baseline energy requirements [14].

Studies that involve ADF are mostly carried out in rodents and humans. In a recent review, ADF investigations on rodents have reproduced some of the same favorable effects as CR with regards to increasing lifespan, improving CVD risks, as well as improving variables related to diabetes [15]. Human studies are in their infancy and have only begun to shed light on the favorable effects of ADF. Regarding cardiovascular health, ADF in obese individuals has been shown to lower HR and systolic BP, but not diastolic BP [14]. However, changes in BP are not seen in healthy normal weight individuals [16]. Like CR, ADF favorably alters CVD risk factors by improving ones blood lipid profile [17], decreasing markers of oxidative stress and inflammation [18], and improving glucoregulatory function [19].

Regarding the effects of ADF on glucoregulatory health, fasting levels of plasma glucose and insulin are not affected; however, it does appear that insulin sensitivity increases in as little as two weeks, as measured by the euglycemic-hyperinsulinemic clamp technique [19]. Insulin sensitivity following ADF has been shown to be gender

specific. Heilbronn et al (2005) noted a decrease in glucose clearance following a test meal in healthy females after 3 weeks of ADF. This decrease was not observed in men, but more importantly, there insulin concentrations significantly decreased following the meal indicating improved insulin sensitivity [13].

An ADF dietary regimen has been shown to improve blood lipids by decreasing plasma TC, LDL-C, TAG, while having no effect on HDL-C [20]. Others have found gender specific changes in TAG and HDL-C following an ADF regimen (i.e. only women had an increase in HDL-C and only men a decrease in TAG) [16]. Moreover, 8 weeks of an ADF regimen has been shown to reduce LDL-C while also reducing the proportion of small dense LDL particles and increasing the proportion of large LDL particles [17].

Reduced markers of oxidative stress, as measured by protein carbonyls, nitrotyrosine, and 8-isoprostanes, have been observed in overweight adults with moderate asthma following 8-weeks of an ADF regimen. This investigation also noted significant reductions in TNF-α and ceramides indicating a reduced inflammatory state. In addition, similar reductions in CRP, TC, LDL-C, and TAG were noted between both groups undergoing a CR or ADF diet [18]. While ADF and CR have been demonstrated to increase lifespan [4] and help prevent the age-induced onset of disease [15], protein restriction has produced similar results. This has led some to conclude that protein restriction could be responsible for some of the health-related benefits of the aforementioned diets [21].

Dietary Restriction

Dietary restriction (DR) is a dietary regimen that restricts certain components of the diet, usually macronutrients, without necessarily restricting overall caloric intake. Protein restriction (PR) is a form of DR and has shown to induce favorable health-related benefits in mammals [22]. Investigations on PR usually reduce protein intake by 40-85%, some with varying degrees of caloric restriction (reviewed in [21]), and others in which protein is replaced with carbohydrates/fats (reviewed in [22]). According to a review by Pamplona et al (2006), increases in lifespan have been found in almost every study with regards to mammals and PR. However, the effects of PR on life extension was approximately half (20%) of what is typically found in CR (40%), indicating that PR could be responsible for 50% of the life-prolonging effects of CR [21].

There is an increasing body of evidence that indicates that the amino acid most responsible for the life-extending effects of PR is methionine—known as methionine restriction (MR) [23]. In particular, MR (by 40-80%) in rats has been shown to inversely correlate with maximum lifespan potential [22]. Compared to control groups, MR in rats has also been shown to decrease biomarkers of oxidative stress [24], decrease mitochondrial RONS [24], and improve glucoregulatory function by decreasing serum glucose, insulin, and IGF-1 [25]. Some of these benefits are not observed with the restriction of lipid and or carbohydrates [26], and therefore, the central focus remains on elucidating the life-extending mechanisms of PR.

There remains much to be understood with regards to MR in humans. Vegetarian diets tend to be low in methionine content and studies have consistently demonstrated improvements in blood lipids and decreased age-associated oxidative stress (reviewed in

[27]). With regards to blood lipids, vegetarian diets are associated with reductions in TC, LDL-C, very low-density lipoprotein cholesterol (VLDL-C), TAG, and little to no decreases in HDL-C [27]. Vegetarian diets have little effect on oxidative stress in women (age: 20-40 years) when compared to age-matched non-vegetarians [28, 29]. However, there does appear to be some efficacy in lowering age-related oxidative stress levels in older individuals (age 60-70), as measured by oxidized purines, oxidized pyrimidines, and conjugated dienes of fatty acids [28]. A recent study using a stringent variant of a vegan diet (Daniel Fast) has noted a reduction in oxidative stress biomarkers [1].

In addition to the above changes with MR, data appear to favor reduced circulating inflammatory mediators; however, there are conflicting findings. Investigations have noted significantly lower CRP in vegetarians when compared to age-matched non-vegetarians [30, 31]. However, others have found no difference in plasma CRP, between vegetarians and omnivores (age: ~37) [29]. One investigation also indicated reduced concentrations of serum insulin-like growth factor-1 (IGF-1) in moderately protein restricted (0.95g/kg of body weight per day) [32]. Interestingly, long-term (1 and 6 years) CR in humans does not reduce serum IGF-1 [32], and this is contrary to the ~40% reduction shown in rodent studies [33]. More importantly, decreased IGF-1 concentrations have been associated with increased lifespans in rodents [34]. Many of the favorable outcomes associated with a vegetarian diet are analogous to the effects of a Daniel Fast [1, 2], and it is possible that MR may account for a large portion of these observed benefits.

Religious Fasting

Ramadan

The Islamic Ramadan fasting period is similar to a modern day ADF regimen, consisting of alternate feeding and fasting periods over the course of the 24 hour day. During the holy month of Ramadan, people of the Muslim faith refrain from eating or drinking from sunrise to sunset, and then feed *ad libitum* from sunset to sunrise for 28-30 days [35]. Very little agreement exists on various outcome measures of health following Ramadan fasting (reviewed in [36]). Only one study has investigated the effects of Ramadan fasting on oxidative stress, and this study found reduced levels of MDA in erythrocytes with no changes in serum MDA or plasma protein-bound carbonyls in healthy men and women [37]. During Ramadan fasting in young healthy men and women, IL- 6 and CRP have been reported to significantly decrease when compared to basal levels [38]. Additionally, significant reductions in fasting blood glucose, the homeostatic model assessment for insulin resistance, and TNF-α have been noted in obese and healthy males [39].

Heterogeneous findings have been reported on for the following: systolic and diastolic blood pressure, TAG levels, glucose levels, HDL-C, LDL-C, and LDL-C/HDL-C ratio. It is evident that these findings were potentially mixed due to a number of confounding variables. Some of these variables include different population types, different amounts of fasting time, and differences between food choices and eating habits of different cultures (reviewed in [36]). It is recommended that future research consider these confounding variables when designing a study in order to better understand the effects of Ramadan fasting on these particular health-related markers.

Greek Orthodox Christianity

Three periods during the year comprise the GOC fasting regimens: the Nativity fasting period, Easter Lent, and the Assumption. Together, these periods restrict dietary consumption for 180-200 days of the year. During the fasting periods, the diet can be considered a form of DR and a variant of vegetarianism, consisting largely of bread, fruits, nuts, legumes, vegetables, and seafood [40]. While a diet with such a profile appears to be healthy, the effects of GOC fasting periods on some health biomarkers are conflicting. Decreases in TC and LDL-C have been noted following periods of fasting [40, 41]. However, findings are conflicting with regards to TAG, HDL-C, TC/HDL-C ratio, and blood pressure (reviewed in [36]).

The three GOC fasting periods contain different dietary instruction with regards to meat, fish, and olive oil; which could explain for some of the variance seen between studies. The effect of GOC fasting periods on oxidative stress and inflammatory markers has not been addressed. While more work needs to be done in addressing each fasting period individually, there does appear to be parallelism between GOC fasting periods and vegetarianism. The Biblically-based Daniel Fast shares similarities with GOC in some way, and will be elaborated on at the conclusion of this literature review.

Blood Lipids

The major lipids in the blood are cholesterol, TAG, and phospholipids. Due to the insoluble nature of these lipids they are packaged into complexes of lipid and protein (lipoproteins) for transport in the blood. These particles have important biological roles, including but not limited to, energy storage, cell membrane integrity, and cell signaling (reviewed in [42]). Many countries are experiencing the adverse effects from these

particles in the form of developed CVD, as CVD is the main causes of death in the United States and other industrial nations [43]. While epidemiologic studies define lipid disorders as independent risk factors for CVD [44], intervention studies have shown that treating these abnormalities decrease the risk for cardiovascular events [45].

Lipid panels are blood measurements that attempt to assess the likelihood of developing coronary heart disease (CHD). Levels of HDL-C, LDL-C, VLDL-C, TC, and TAG are analyzed to give a measurement of the amount of each particle that is present in the blood. This section of the review will discuss each variable in the lipid panel, as well as the TC/HDL-C ratio and the LDL-C/HDL-C ratio. In addition, this section will be concluded with a discussion regarding the methods available to improve one's lipid profile, such as pharmacological, nutraceutiacal, exercise, and dietary interventions.

Low-Density Lipoproteins

Low-density lipoprotein cholesterol functions to carry serum cholesterol (~60-70% of total serum cholesterol) from the liver to peripheral tissues. When levels of LDL-C are in excess, the smaller particles are more prone to penetrate into the arterial wall, which then exposes them to oxidation via radicals in the blood (reviewed in [46]). Atherosclerosis is characterized by the hardening of the vessel due to buildup of fatty material. An early step in the progression of atherosclerosis is the inflammatory immune response due to the oxidation of LDL-C in the arterial wall (reviewed in [46]).

The National Institute of Health (NIH) recommends that serum LDL-C be less than 100 mg/dL for optimal cardiovascular health. The mean level of serum LDL-C among U.S. adults is 120 mg/dL, and 70.9% of this population has serum LDL-C levels above the recommended 100 mg/dL [47]. Low-density lipoprotein cholesterol has been

identified by the Adult Treatment Panel (ATP) III of the National Cholesterol Education Program (NCEP) as the major atherogenic lipoprotein and the primary target for cholesterol-lowering therapy [48]. High LDL-C is a modifiable risk factor in the development of heart disease, and a reduction of this lipoprotein would be favorable in reversing the risk of cardiovascular related events (e.g., myocardial infarctions and strokes) [47].

Lower LDL-C levels should be of greatest importance to individuals who are seeking to improve cardiovascular health. With regards to a target LDL-C level, less than 100 mg/dL would be optimal for cardiovascular health, but individuals will differ based on the number of risk factors for CHD [48]. Due to the high risk associated with elevated LDL-C and cardiovascular related events [49], there have been many investigations focused on lowering serum LDL-C levels, some of which report greater benefit than others [50]. While pharmaceutical therapy takes a more aggressive approach in achieving the reduction of LDL-C, diet regimens have also shown to produce favorable outcomes with less adverse side effects [51].

High-Density Lipoproteins

High-density lipoprotein cholesterol (HDL-C) makes up 20-30% of the total serum cholesterol and a high level is strongly and inversely associated with risk for CHD. Serum HDL-C levels lower than 40 mg/dL is classified as low in men and lower than 50 mg/dL is low in women [52]. There has been some evidence suggesting that HDL-C can protect against atherosclerosis mainly due to its role as a reverse cholesterol transport lipoprotein [53]. During the process of reverse cholesterol transport, excess cholesterol from the arterial wall's foam cells are returned to the liver for excretion (reviewed in

[54]). In addition, HDL-C has shown to inhibit thrombosis, prevent oxidation of LDLs with its antioxidative activity, and protect the endothelium against inflammation by increasing nitric oxide (NO) bioavailability (reviewed in [55]).

Low HDL-C concentration has been identified as an independent risk factor for the development of CHD [48]. Low HDL-C levels also correlates with the presence of other atherogenic factors such as elevated serum TAG, small remnant LDL particles, as well as signs of insulin resistance [48]. It is estimated that greater than 40% of coronary events occur in individuals with less than 40 mg/dL HDL-C [55]. Considering this finding, a level greater than 40 mg/dL HDL-C would be favorable when considering optimal cardiovascular health.

High-density lipoprotein cholesterol possesses enzymes that can prevent the formation of inflammatory mediators. In addition, HDL-C inhibits the expression of adhesion molecules and migration of monocytes into the subendothelial space [56]. Due to the inflammatory role in the development of atherosclerosis [46], and the anti-inflammatory properties of HDL-C [57], raising HDL-C has developed as a therapeutic target to complement LDL-C lowering therapies [48]. The development of drugs aimed at increasing HDL-C is a fast growing area of research [50]. It has still yet to be seen whether or not this approach translates into the reduction of cardiovascular related events, however, there is an increasing amount of evidence that supports this [55, 56, 58].

Triglycerides, Very Low-Density Lipoproteins, and Non-High-Density Lipoproteins

Triglycerides are transported in the blood as chylomicrons or VLDL-C. Very low-density lipoprotein cholesterol makes up ~10-15% of the total serum cholesterol and is a TAG-rich lipoprotein that also contains cholesterol esters in the lipid core. The

formation of VLDL-C is ultimately dependent on the availability of cholesterol and TAG as a substrate [59]. In the peripheral tissue, lipoprotein lipase acts on the TAG rich VLDL-C, extracting free fatty acids (FFA) for the uptake and storage of TAG. Due to its large size, VLDL-C particles rarely enter into the arterial wall. However, hepatic lipase and lipoprotein lipase hydrolyzes TAG in the lipid core rendering a VLDL remnant that is predominantly cholesterol esters with similar atherogenic characteristics as LDL-C (reviewed in [51]).

Resting TAG levels greater than 150 mg/dL has been recognized as less than optimal by the ATP III of the NCEP [48]. This value is 50 mg/dL lower than what was recommended in the previous panel indicating an increasing awareness of the health risk associated with elevated TAG. Elevated TAG (greater than 200 mg/dL) is associated with an increase in atherogenic lipoproteins and increases the risk of CVD beyond that of which LDL-C predicts alone [48]. Therefore, non-HDL-C is a secondary target of therapy in individuals with TAG greater than 200 mg/dL [44]. It has been postulated that hypertriglyceridemia promotes cholesteryl ester exchange via the cholesteryl ester transfer protein (CETP). High serum TAG increase the transfer rate of cholesteryl ester from HDL-C to VLDL-C, resulting in low serum HDL-C and the formation of small dense LDL-C—which increases ones vulnerability to plaque formation (reviewed in [59]).

A normal level of VLDL-C is less than or equal to 30 mg/dL and therefore a target goal for non-HDL-C is 30 mg/dL higher than LDL-C. The National institute of Health identifies elevated VLDL-C remnants as a risk factor for the development of

atherosclerosis due to specifically cited evidence that shows an increased risk of CHD with increasing levels of TAG-rich lipoproteins [48].

Non-HDL-C, the sum of VLDL+LDL-C, is a better predictor for CVD risk assessment when TAG are elevated above 200 mg/dL. It appears as though LDL-C alone does not adequately assess the risk of CVD when TAG are elevated due to the strong correlation between VLDL-C and atherogenic remnant lipoproteins [44]. Some data actually favor the use of non-HDL-C over LDL-C in clinical evaluation of risk [48]. Apolipoprotein B (Apo B), which is the main lipoprotein in LDL-C, is associated with all forms of atherogenic lipoproteins and therefore it is also a strong predictor for CHD [48]. When TAG are elevated, non-HDL-C (including VLDL-C) can serve as a secondary target of therapy [48].

Total Cholesterol and Cholesterol Ratios

Total cholesterol is the amount of all lipoproteins present in the blood. Currently it is recommended that TC be less than 200 mg/dL [48]. Statistics indicate that individuals with higher cholesterol levels have more cardiovascular related diseases than those with lower levels [48, 60]. Moreover, clinical trials have indicated that a 1% reduction in TC reduces CHD by 2% [49]. There is an apparent relationship between TC and LDL-C due to the simple fact that the majority of lipoprotein particles in the blood exist as LDL-C [55]. Therefore, it would be beneficial for individuals to reduce TC while maintaining HDL-C levels when considering health from a cardio-protective standpoint.

The TC/HDL-C ratio has been characterized an emerging lipid risk factor. It can be used as a better predictive measure for classifying individuals at risk for CVD when

compared to TC, LDL-C, and HDL-C alone. However, ATP III of the NCEP does not indicate the TC/HDL-C ratio as a primary or secondary target of therapy; rather, it is used as a predictor for lipid risk assessment. This risk can be attributed to the nature of the lipoprotein particles associated with each number in the ratio. In short, the ratio reflects two powerful components of risk by considering the atherogenic properties related to TC and the anti-atherogenic properties of HDL-C [48, 49]. Similarly, the LDL/HDL-C ratio is equally effective in predicting cardiovascular risk [49]. This can be credited to the close relationship between LDL-C and TC. As stated earlier, LDL-C and TC values are closely related due to the fact that LDL-C makes up the majority of TC [55]. However, when the TAG concentrations exceed 300 mg/dL, the LDL/HDL-C ratio may underestimate the magnitude of lipoprotein abnormalities [49].

Improving Blood Lipids

Pharmaceuticals

Pharmaceutical therapy is available for individuals with elevated blood lipid levels who are at a high risk for developing atherosclerotic disease. Drugs such as statins (HMG-CoA reductase inhibitors) are first line therapy treatments and have been shown to reduce LDL-C in the range of 18-55% and TAG 7-30% by inhibiting synthesis of cholesterol (reviewed in [51]). With marginal increases of HDL-C, ranging from 7-30%, the lowering of LDL-C from statins appears to be the major factor in the reduction of cardiovascular mortality [48, 50, 61].

Cholesterol absorption inhibitors are a class of drugs that interfere with dietary cholesterol uptake into the intestinal wall to lower blood lipids [62]. According to a recent meta-analysis, Ezetimibe in particular, when paired with statin therapy, has been

shown to more aggressively reduce LDL-C to help patients reach their cholesterol goal [62]. Like cholesterol absorption inhibitors, bile acid sequestrants can also be used in addition to statin therapy to help reduce LDL-C an additional 12-16% [48]. Nicotinic acid (Niacin) on the other hand is the most effective pharmacologic method for increasing HDL-C (15-35% with 1.5-3.0g/day) while also showing a 5-25% reduction of LDL-C and 20-50% reduction in TAG (reviewed in [50, 51, 61]). Fibric acids (Fibrates) are another widely used lipid-modifying pharmaceutical that demonstrates less significant improvements in both HDL-C (15-35% increase) and LDL-C (5-20% reduction) but are predominantly used to lower TAG (20-50%) via the up-regulation of lipoprotein lipase [51, 61].

A healthy lifestyle that involves adequate exercise and optimal nutrient intake is an alternative to lowering blood lipids without the use of pharmacological interventions. These types of changes are regarded as therapeutic lifestyle changes by the NCEP [48]. Exercise and diet can allow for better maintenance of weight and body composition with a reduction in total body fat and visceral body fat. In addition, dietary supplements have become another alternative that many people have turned to for blood lipid management.

Dietary Supplements

One non-pharmacological intervention that has received much interest is the use of nutraceuticals. Nutraceuticals are foods that have shown to provide health benefits and can be used as supplementation for the treatment of disease [63]. The following has been demonstrated to improve one's lipid profile with regard to LDL-C: Vitamin E (8-42% reduction), guggulipid (5-12% reduction), ginseng (10% reduction), probiotics (5-8% reduction), sesame oil (10%), red yeast rice (22-32% reduction), and pantethine (20-27%)

[58]. The following nutraceuticals have shown efficacy in elevating HDL-C: pantethine (8.4% increase), red yeast rice (0-20% increase), policosanol [58]. While supplements can assist in the modification of blood lipids, macronutrient intake affects them as well.

Exercise

Regular aerobic exercise has shown to elicit improvements in HDL-C, TAG, and LDL-C. Specifically, aerobic exercise at moderate to high intensity for greater than 12 weeks has been shown to increase HDL-C 4.6%, and decrease TAG and LDL-C by 3 and 5% respectively (reviewed in [64]). However, these changes are dependent on baseline lipid measures, exercise intensity, and duration. There exist confounding findings in many of the studies reviewed, and this is attributed to the different methodologies and variations in subject types. A meta-analysis regarding progressive resistance training for greater than four weeks indicates significant reductions in LDL-C, TAG, non-HDL-C, TC, and TC/HDL-C ratio by 6.1, 8.1, 8.7, 5.5, and 0.5 mg/dL respectively [65]. Exercise has also been shown reduce the onset of type 2 diabetes mellitus (T2DM) by 56%; more effective than pharmaceuticals such as metformin and troglitazone [58]

Nutrients

Due to the increase in cardiovascular related deaths around the world, an abundance of research has focused on the effects of nutrient intake on blood lipid parameters. Specifically, investigators have sought to determine the effects of adding or removing components from the diet, both macro- and micro-nutrients. There is an increasing amount of evidence that indicates improvements in blood lipid parameters when substituting saturated fatty acids (SFA) for polyunsaturated (PUFA) and monounsaturated fatty acids (MUFA) [66, 67]. When compared to a corresponding SFA

diet equal in fat quantity (30-33%), replacing ~9% of SFA with either PUFA or MUFA, leads to reductions in TC (19 and 12%), LDL-C (22 and 15%), TC/HDL-C ratio (6 and 8%), TAG (15% for both), and HDL-C (14 and 4%) after two and a half weeks of dietary intervention [66]. Furthermore, studies have shown a 42% reduction in CHD risk when replacing 5% of energy from SFA with MUFA and PUFA [68]. Additionally, studies have demonstrated favorable results, with regard to elevating HDL-C, when incorporating n-3-polyunsataurated fatty acids and low-GI carbohydrates [58, 61]. Conversely, some believe n-3-PUFA to mainly influence the lowering of VLDL-C and therefore TAG as well, while having minimal influence on LDL-C and HDL-C [51]. On the contrary, evidence indicates that trans-fatty acid rich diets increase LDL-C and decrease HDL-C, significantly increasing the risk factor for cardiovascular events [68].

The glycemic index indicates the effects of a food on blood glucose concentrations. The type of carbohydrate, with regards to their glycemic index, has been suggested to be a relevant determinant of CHD risk in some studies; with an increased risk from high glycemic refined carbohydrates [67]. In addition, a phenomenon known as carbohydrate-induced hypertriglyceridemia has given rise to research regarding the efficacy of lowering carbohydrate consumption. A carbohydrate-rich diet has shown to increase TAG as well as VLDL-C, more so in overweight individuals (BMI $\geq$ 28 kg/m^2) than in normal weight individuals (BMI $\leq$ 28 kg/m^2) [69]. Similarly, a recent review favors the replacement of SFA with PUFA and MUFA over the replacement of SFA with higher carbohydrate intakes; with lower LDL-C and HDL-C from the former and increased TAG, smaller LDL-C particles, and reduced HDL-C from the latter [67].

Similarly, others cite evidence for a low-carbohydrate dietary approach that reduces TAG and LDL particle size while increasing HDL-C [70].

With regards to protein consumption, a protein rich diet (30% protein, 41% carbohydrate, and 29% fat) for 10-weeks has been shown to significantly reduce TC, LDL-C, and TAG , while having no effect on HDL-C [71]. A 6-week protein rich diet (25% daily kcal requirements, ~50% plant sources) in pre-hypertensive adults found similar results in all of the aforementioned variables, however, a significant reduction in HDL-C (1.3 mg/dL) was observed [72]. A recent meta-analysis indicates that a diet rich in soy protein containing isoflavones results in the decreases of TC (3.77%), LDL-C (5.25%), TAG (7.27%), and an increase in HDL-C (3.03%). Data from this meta-analysis also indicates that reductions in TC, LDL-C, and TAG occurs in short term intervention trials, whereas increases in HDL-C were only observed in interventions lasting greater than 12 weeks [73].

Aside from macronutrients, other components have also shown to influence blood lipids. It is known that increases in dietary cholesterol and fat results in increases in plasma cholesterol levels [74]. Recent reviews indicate that the addition of the following foods to the diet has been associated with declines in LDL-C: vegetables, grains, plant sterols, viscous fiber, soy protein, and nuts. Specifically, dietary fiber intake, when included with a diet low in SFA and cholesterol, lowers LDL-C (5-10%) [58, 61]. Studies involving healthy, as well as hypercholesterolemic subjects have shown that the intake of nuts lowers TC, LDL-C, and the LDL-C/HDL-C ratio. Moreover, epidemiological studies found an 8.3% reduction in risk of death from CHD for each weekly serving of nuts (reviewed in [68]). While changes regarding blood lipids remain

an area of focus for improving the health of individuals in many developed countries, oxidative stress is another area that has garnered a great deal of attention.

Oxidative Stress

Free radicals are atoms, molecules, or ions that have unpaired electrons [75]. The formation of electrons pairs is more energetically favorable and therefore, molecules with unpaired electrons are more reactive. The reactivity of such molecules, specifically those derived from oxygen and nitrogen, has been shown to play an important role in biological processes. Reactive oxygen and nitrogen species (RONS) are products of normal cellular metabolism. These reactive products facilitate redox homeostasis through cellular signaling molecules that are necessary for specific initiation and/or signal transduction pathways [76]. The redox state of a cell is very tightly regulated and depends on the rate of RONS production, as well as their removal by an intricate antioxidant defense system that includes, but is not limited to, superoxide dismutase (SOD), glutathione (GSH), catalase, alpha lipoic acid, and coenzyme Q10 (CoQ10) [76]. In short, RONS participate in various redox-regulatory mechanisms, but when RONS production exceeds antioxidant defense systems, oxidative damage to molecules such as DNA, proteins, lipids and carbohydrates can occur. This oxidative insult is termed oxidative stress [77].

Causes of Oxidative Stress

Oxidative damage affects cellular lipids, DNA, and proteins in tissue and occurs at certain levels in all animals. Many investigations suggest that steady-state oxidative damage to macromolecules occurs to a greater extent with increasing age in organs like the liver, heart, and brain (reviewed in [21]). Enzymes such as, nicotinamide adenine dinucleotide phosphate (NAD(P)H) oxidase, NO synthases, cytochrome P450 oxidase,

xanthine oxidase, and cyclooxygenase have been shown to be a source of radical production, as well as various mediators, cytokines, and growth factors [78]. In addition, RONS production can be acutely increased due to stressors in the form of environmental pollutants (e.g., cigarette smoke, ozone, nitrogen dioxide) [77], acute physical exercise [79], and the metabolism of food [80]. Feeding-induced oxidative stress, which is referred to as "postprandial oxidative stress", has been shown to occur following lipid [81, 82], protein [82], and carbohydrate [83] consumption.

Postprandial oxidative stress

Following feeding, glucose and FFA metabolism results in the formation of $O_2^{\bullet-}$ radicals via leakage of electrons from the mitochondrial electron transport chain (ETC). During the metabolism of a high-calorie meal, rich in carbohydrates and/or lipids, the processing of substrates through the ETC results in a highly reduced inner mitochondrial membrane [84]. The result is the excessive supply of electrons to complex II, causing a backing up of electrons at complex-I in the ETC. Electron leakage and the ensuing formation of $O_2^{\bullet-}$ following feeding is believed to predominantly occur at complex-I; however, evidence also indicates electron leakage and $O_2^{\bullet-}$ generation to a lesser degree at complex-III [85]. Superoxide formation leads to a shift in the redox environment through the formation of H_2O_2. This disruption in homeostasis and direct interaction with the $O_2^{\bullet-}$ radical leads to the activation of several pro-inflammatory transcription factors [86]. This aforementioned pathway has been proposed as a mechanism responsible for acute decreases in insulin sensitivity, leading to a hyperglycemic/hypertriglyceridemic state.

During conditions of hyperglycemia and hypertriglyceridemia, following the consumption of a meal, the vasculature appears to be vulnerable to oxidative damage.

This is in part due to the endothelium's lack of cellular machinery that restricts the entry of substrate within the vasculature [87]. During high carbohydrate feedings, glucose enters the endothelium via a non-insulin dependent mechanism, resulting in endothelial intracellular hyperglycemia. It has been hypothesized that the lack of specific mechanisms in endothelial cells whereby glycemic control can be obtained, results in endothelial dysfunction through the generation of RONS and the subsequent downstream effects (reviewed in [88]). Superoxide generation within the vasculature triggers the migration of phagocytic cells to the endothelial surface, which then promote secondary RONS production and activation of inflammatory transcription factors (reviewed in [89, 90]). To summarize, postprandial RONS production can lead to the activation of transcriptional factors that favor the production of pro-inflammatory cytokines. In addition, inflammatory cytokines induced from stress leads to the formation of RONS. This cyclical process, which is reviewed in [78], can play a role in the pathogenesis of disease due to the deleterious effects on the endothelial cells.

Measuring Biomarkers of Oxidative Stress

Biomarkers are molecules that can be measured and evaluated as an indicator of normal biological processes. One way to measure the degree of oxidative stress in the body is through the analysis of biomarkers in biological tissue and urine [91]. Some of the RONS capable of oxidizing biomolecules include: NO, $O_2^{\bullet-}$, peroxyl radicals ($ROO^{\bullet}$), peroxynitrite anion ($ONOO^{--}$), H_2O_2, and hydroxyl radicals ($OH^{\bullet}$). The concentration of RONS production can be directly measured by electron spin resonance (ESR) spectroscopy. Spin traps, which are molecules that react with primary radical species to give longer lived secondary radical species can be used to help measure RONS

production [92]. Electron spin resonance (ESR) spectroscopy is currently the only direct measure of oxidative stress analysis *in vivo*. This method measures the magnetic properties of unpaired electrons and their molecular environment. This method however, is less than ideal and is not commonly used in research. Indirect measures of oxidative stress include the end products of protein oxidation/nitration, DNA oxidation/nitration, and lipid oxidation. While RONS are typically short-lived and relatively unstable, the measurement of more stable molecular end products resulting from oxidative damage allows for an indirect measurement of oxidative stress. Aside from measuring RONS, the total antioxidant capacity (TAC) can be used to represent oxidative stress changes *in vivo*.

Protein oxidation/nitration

Damage to proteins can occur directly via RONS production, or indirectly via secondary products. Individual oxidized amino acids can be measured to detect protein oxidation/nitration; however, two common biomarkers used are protein-bound carbonyls and nitrotyrosine [93]. Protein carbonyls are generated by direct oxidation of amino acid residues or via secondary reactions with primary oxidation products of lipids and sugars. Recently reviewed in [94], the cleavage of peptide bonds, the oxidation of glutamyl residues, and metal-catalyzed oxidation of protein side-chains render highly reactive carbonyl derivatives. Some of the common methods for the detection of carbonylated proteins include: spectrophotometric 2,4-dinitrophenylhydrazine (DNPH) assay, spectrophotometric DNPH assay coupled to protein fractionation by high-performance liquid chromatography (HPLC), and one- or two-dimensional electrophoresis and Western blot immunoassay (reviewed in [93]).

Nitration of the amino acid tyrosine results in the formation of 3-nitrotyrosine, which is another viable biomarker to measure protein oxidation/nitration [95]. A variety of methods are available to quantify the presence of 3-nitrotyrosine in blood plasma: enzyme-linked immunosorbent assay (ELISA), HPLC with electrochemical detection, mass spectrometry-based assays, gas chromatograph-tandem mass spectrometry [93]. While it has been reported that only $ONOO^{--}$ will nitrate tyrosine residues under physiological conditions, others have shown multiple pathways for the formation of 3-nitrotyrosine [92]. In addition, recent reviews have raised concern over the quantification of 3-nitrotyrosine, due to varied plasma concentrations of free and protein-bound 3-nitrotyrosine in healthy subjects [93]. The downstream effects of protein structure modification include inhibition of enzymatic and binding activities, increased susceptibility to aggregation and proteolysis, increased or decreased uptake by cells, and altered immunogenicity. In addition, oxidative damage to proteins can contribute to secondary damage to DNA repair enzymes and DNA polymerases (reviewed in [91]).

DNA oxidation/nitration

The highly reactive $OH^{\bullet}$ is one of the main contributors to DNA damage, (reviewed in [96]). Oxidized DNA bases and strand breaks can be measured to quantify oxidative damage to DNA. Oxidation products of the DNA base guanine, specifically 8-hydroxy-2'-deoxyguanosine (8OHdg), is the most commonly used biomarker due to its mutagenic characteristics reflected in its coding properties (reviewed in [97]. The measurement of oxidative damage to DNA can be quantified by HPLC with electrochemical detection, gas chromatograph-mass spectrometry, liquid chromatograph mass spectrometry, and antibody based techniques (reviewed in [91, 98]. Biological

samples are typically obtained from blood serum, lymphocytes, myocardium, liver and urine. However, none of the analytical methods identify where the oxidative DNA damage is located [93]. Measurement of 8OHdg in urine is advantageous because it can be used to assess whole-body oxidative DNA damage, however, it has been speculated that the concentration of a single product may not truly reflect rates of oxidative damage to DNA [98].

Lipid Oxidation

Lipid peroxidation is characterized by the oxidation of lipids in the plasma membrane. Lipid peroxidation can be initiated by the removal of a hydrogen atom from a carbon side chain. This leads to the formation of a carbon centered lipid radical that mostly reacts with O_2, forming a $ROO^{\bullet}$. Peroxyl radicals can abstract hydrogen from adjacent fatty acid side chains, particularly unsaturated fatty acids with weak carbon/hydrogen bonding, thereby initiating the chain reaction of lipid peroxidation [99]. Lipid peroxidation damages the degree of fluidity through the plasma membrane which can play a role in the impairment of normal cellular functions. Many methods can be used to detect and measure lipid peroxidation, but the use of blood plasma/serum, breath condensates, and whole organs are typical (reviewed in [100]). Some of the more prominent biomarkers of lipid peroxidation include MDA and F_2-IsoPs. However, other relatively stable end products can be measured such as lipid hydroperoxides, α,β-unsaturated reactive aldehydes, conjugated dienes, oxidized low-density lipoprotein, 4-hydroxy-2-nonenal, and 2 propenal (acrolein) (reviewed in [91]).

Malondialdehyde is formed when RONS react with unsaturated lipids [99]. In addition, MDA reacts to form protein and DNA adducts that behave mutagenically

(reviewed in [101]). The most common method used for measuring MDA is the thiobarbituric acid (TBA) test. The TBA test is frequently carried out under acidic conditions at ~100°C, resulting in a condensation reaction that gives rise to a high absorbtivity adduct (TBA_2-MDA) that can be spectophotometrically analyzed at an absorbance/fluorescence of 532/553 nm (reviewed in [100, 101]). The measurement, which is usually reported as "TBA reacting substances" (TBARS), indicates the degree of lipid peroxidation, however this method has been faulted for its lack of specificity and the ability of TBA to react with other compounds other than MDA [102].

F_2-isoprostanes (F_2-IsoPs) are a group of prostaglandin-like compounds that are generated by radical induced peroxidation of arachidonic acid, an omega-6 fatty acid in the phospholipids of cell membranes [103]. The detection of F_2-IsoPs can be quantified in all human fluids and tissues, but human plasma and urine are the most common methods for quantifying F_2-IsoPs. Recent reviews indicate that the quantification of F_2-IsoPs in urine and plasma give highly accurate indices of radical mediated lipid peroxidation *in vivo* [91, 104]. Several methods for analyzing F_2-IsoPs exist (reviewed in [104]), but mass spectrometric assays are advantageous because they are more sensitive/specific. In the field, mass spectrometry is considered the gold standard for F_2-IsoPs quantification; however, this method is labor-intensive and requires significant expenditures on equipment.

Total Antioxidant Capacity

Plasma total antioxidant capacity (TAC) reflects the synergistic activity of antioxidants and can be used to help evaluate the effects of different treatments (e.g., diets) on plasma redox status. This characteristic of the test allows for a "bigger picture"

of *in vivo* oxidative stress, when compared to measuring specific antioxidants/RONS. Blood TAC measurements, preferably plasma, can be obtained by quantifying the amount of RONS scavenged from a known quantity of a given RONS (reviewed in [105]). Data suggests that dietary TAC may help estimate the risk in developing metabolic syndrome features [106]. Other data demonstrate that TAC is a potential marker of diet quality in healthy subjects [107]. Antioxidant capacity can be measured using at least two different types of assays: hydrogen atom transfer reaction based assays and single electron transfer reaction based assays. The electron transfer reaction based assays are more commonly used, this group includes assays such as the Trolox Equivalent Antioxidant Capacity Assay (TEAC), the ferric ion reducing antioxidant power assay (FRAP), the N,N-dimethyl-p-phenylenediamine assay, and the Cu(II) reduction capacity assay (reviewed in [108]. Two common hydrogen atom transfer reaction based assays are the oxygen-radical absorbing capacity (ORAC) and the total radical-trapping antioxidant parameter (TRAP). These two methods measure the ability of an antioxidant to quench radicals by hydrogen donation [109]. Both assays use peroxyl radicals as the oxidant and measures the radical chain breaking capacity of the sample [110]. While TAC appears to be a useful concept, these methods have been criticized for yielding different results based on the conditions in which the assays are run [108], and therefore caution needs to be used when analyzing the results.

Implications for Health and Disease

As mentioned previously, the production of RONS is an essential biological process that aids in cellular redox homeostasis and signal transduction pathways [76]. It is conceivable that high levels of RONS production is detrimental and is one of the

underlying mechanisms in the pathophysiology of many illnesses/diseases [76, 78, 88, 90]. As such, the role of oxidative stress on the progression of disease will be discussed in further detail.

Insulin Resistance

Oxidative stress can damage surrounding biomolecules, inhibit the systematic functions of lipids, proteins, and DNA, and induce low grade systemic inflammation. Evidence has demonstrated proportional increases in oxidative stress and feeding-induced hyperglycemia/hypertriglyceridemia. During times of high calorie/fat feeding, these oxidative insults promote a decrease in insulin sensitivity in adipose and muscle cells, as shown in [111]. This state of insulin resistance is derived from the formation of $O_2^{\bullet-}$ following feeding, possibly as a defense mechanism of the cell to prevent further damage from the metabolism of substrate. One proposed mechanism for this insulin resistant response is the translocation of glucose transporter type 4 (GLUT 4) from the plasma membrane [87]. Following postprandial oxidative stress, mitochondrial production of $O_2^{\bullet-}$ is reduced due to the inhibition of intracellular FFA oxidation [112]. Studies have shown that prolonged low-levels of oxidative stress impair insulin mediated GLUT 4 translocation, ultimately leading to impaired glucose tolerance [113]. Insulin resistance has been implicated in the pathogenesis of several diseases, most notably, CVD [88] and diabetes [114]. Routine exposure to high calorie, high-fat, high simple sugar meals may impart adverse effects on metabolic function in such a way as to increase disease risk. Dietary modification to favor lower calorie intake, devoid of SFA and processed food, may be responsible for a reduction in disease risk.

Cardiovascular Disease and Diabetes

The beta cells (β-cells) of the pancreas and endothelial cells of the vasculature are unable to modulate substrate uptake like adipose and muscle tissue, and are therefore exposed to hyperglycemic/hypertriglyceridemic states. The inability of β-cells and endothelial cells to modulate substrate uptake leads to additional RONS production, which ultimately promotes an increased risk for diabetic complications [114]. Studies indicate that prolonged exposure to elevated glucose, FFA, or a combination of both, lead to β-cell dysfunction and endothelial dysfunction [114]. The aforementioned state is associated with an increase in $ONOO^{--}$. Peroxynitrite is cytoxic because it oxidizes sulfhydryl groups in proteins, initiates lipid peroxidation, and can disrupt many signal transduction pathways [115]. The production of $ONOO^{--}$, through the overproduction of NO and $O_2^{\bullet-}$, results in acute endothelial dysfunction, enhancing the risk for CVD [116]. In addition, it has been suggested that hyperglycemia activates several major biochemical pathways that influence vascular complications including: advanced glycation end products (AGEs) and receptors for AGE (RAGE) [117], protein kinase C (PKC), the polyol pathway, and hexosamine pathways [118].

The β-cells of the pancreas have low levels of antioxidant enzymes that would play a key role in scavenging RONS [119]. Therefore, an increase in oxidative damage leads to impaired insulin production and secretion. The Common Soil Hypothesis is based off of evidence that points towards oxidative stress as the "common" driving force behind T2DM and CVD [87]. While oxidative stress is a common denominator in the steps leading towards diabetes and CVD, it appears as though insulin resistance is one of the initiating physiologic responses from oxidative stress that leads to the progression of

disease. This can be inferred when looking at evidence that associates a hyperglycemic/hypertriglyceridemic state with an insulin resistance response and the subsequent effects on the endothelial and β-cells. These are all key characteristics in the development of T2DM [114] and CVD [116].

Reducing Oxidative Stress

Pharmaceuticals

The link between oxidative stress, T2DM, the metabolic syndrome, and CVD is widely accepted in the scientific community (reviewed in [120]). Additionally, recent evidence indicates that many drugs used to treat symptoms of the metabolic syndrome have strong intracellular antioxidant activity and can be used to attenuate oxidative stress. These drugs include calcium channel blockers, glitazones, thiazolinediones, angiotensin-converting enzyme (ACE) inhibitors, statins, and angiotensin II type 1 receptor blockers (reviewed in [87, 121]). A growing body of evidence suggests that oxidative stress is one of the underlying factors in the pathogenesis of many diseases [88, 114, 122], therefore it is of no surprise that the aforementioned drugs additionally treat disease by working on pathways that influence the production of RONS [123].

Dietary Supplements

From a dietary supplement perspective, the effect of antioxidants on attenuating oxidative stress is most widely investigated. Research indicates potential benefits from micronutrients that are associated with natural products. Some of which include popular antioxidant-rich foods such as fruits, vegetables, legumes, olive oil, red wine, green tea, and nuts (reviewed in [124]). Supplements that include lycopene, beta-carotene, alpha-carotene, bixin, lutein, and paprika carotenoids have been shown to lower oxidative stress

[125]. Additionally, one study demonstrated vitamin C supplementation to be as effective at enhancing serum TAC as strawberry and spinach consumption [126]. Supplemental vitamin C, vitamin E, and carotenoids appear to have the most benefits with regards to their antioxidant effects, working synergistically to quench free-radicals (reviewed in [127]). However, some studies fail to demonstrate a reduction in oxidative stress biomarkers from antioxidant supplementation, leaving some to conclude insufficient evidence (reviewed in [128]).

Exercise

It has been postulated that part of the beneficial effects of exercise are attributed to the acute increase in RONS production; specifically RONS generated via intracellular mitochondria [12]. The motive of such a claim comes from evidence that indicates increases in RONS production and oxidative stress biomarkers during both acute aerobic [129] and anaerobic [130] exercise. Based on the concept of hormesis/mitohormesis, there is an optimal level of RONS production from exercise that is beneficial to health [131]. Chronic exercise training and the repeated exposure of increased RONS production leads to an increase in endogenous antioxidant activity, perhaps an adaptation to protect during subsequent bouts of physical activity [132]. However, overtraining and chronic high-intensity exercise is associated with a production of RONS that is overwhelming to the antioxidant defense system, thereby resulting in oxidative damage and increased risk for disease [133].

Nutrients

The consumption of food in the form of lipid [81, 82], carbohydrate [83], protein [82], as well as a mixed meal [80] increases the production of RONS. The severity of

oxidative insult can vary with regards to the subject population and the meal composition used. For example, aging can influence RONS production (reviewed in [134]) and insulin sensitivity [135], potentially leading to confounding findings following macronutrient consumption.

The rise in TAG following lipid consumption seems to correlate with the degree of oxidative stress observed in healthy individuals [136], with exacerbated responses observed in diseased individuals [137]. In relation, there is evidence that demonstrates a decrease in postprandial oxidative stress in patients with the metabolic syndrome, following a high MUFA diet (20% fat from MUFA) when compared to a high SFA diet and a low-fat/high carbohydrate diet [138].

Carbohydrate consumption (75g glucose tolerance test) in insulin resistant individuals results in a significant elevation of oxidative stress, as measured by F_2-IsoPs [139]. However, increases in oxidative stress in healthy people following carbohydrate ingestion do not occur [81, 140]. Protein, when compared to both lipid and carbohydrate consumption, results in the lowest increase in $O_2^{\bullet -}$ production [82]. Furthermore, evidence indicates that when protein is added to a high-carbohydrate [141] and high-fat meal [142], the result is a decrease in glycemia and lipemia; both of which cause increases in RONS production [90].

Aside from macronutrients, an abundance of research has gone into understanding the role of micronutrients on attenuating oxidative stress. In this regard, antioxidant vitamins are most extensively studied and include: ascorbic acid (Vitamin C), tocopherol (Vitamin E), and carotenoids. These exogenous antioxidants are obtained by diet/supplementation and can play a role in the inhibition of oxidative damage

Vitamin C can best be obtained from fruit and vegetable sources, as well as supplements (drinks, pills, etc.). The antioxidant properties of vitamin C is characterized by the ability to scavenge $O_2^{\bullet-}$, H_2O_2, hypochlorite, $OH^{\bullet}$, $ROO^{\bullet}$, and singlet oxygen (O_2) (reviewed in [127]). Data indicate that vitamin C supplementation in smokers and non-smokers may or may not decrease oxidative stress as measured by TBARS and MDA [128]. However, large doses of vitamin C (2000 mg/day/) for five days in heavy smokers (30+ cigarettes/day) resulted in a significant reduction in the urinary excretion of F_2-IsoPs [143]. This finding provides strong support for the antioxidant effects of vitamin C, given that F_2-IsoPs are considered to be one of the more reliable markers of oxidative damage. *In vitro* trials have demonstrated a regenerating effect of vitamin C reducing the tocopheroxyl radical back into the radical scavenging α-tocopherol molecule (reviewed in [144]). In theory, this regenerative activity of vitamin C allows for the scavenging of RONS into relatively unreactive products thus preventing oxidative damage. However, *in vivo* studies have shown that acute intake of Vitamin C (500 mg) alone, or in combination with Vitamin E (400 IU), has no protective effects on DNA damage or lipid peroxidation in healthy individuals [145].

Vitamin E refers to a group of fat-soluble molecules, tocopherols and tocotrienols (each of alpha, beta, gamma, and delta). The most biologically active form of Vitamin E and the most extensively studied is α-tocopherol, which is abundant in wheat germ oil, sunflower oil, safflower oil, nuts, and green leafy vegetables. The ability of α-tocopherol to scavenge RONS and herby protect from lipid peroxidation is the hypothesized antioxodiant mechanism. Vitamin E (280 mg/day for 10 weeks) has been shown to significantly reduce lipid oxidative damage in smokers and non-smokers, as measured by

TBARS [146]. Vitamin E supplementation in hypercholesterolemic patients at 600 mg/day for 14 days rather than 100 mg/d resulted in a reduction in lipid peroxidation (measured by F_2-IsoPs). In vivo data is lacking pertaining to the regeneration of Vitamin E from the tocopheroxyl radical via reduction by ascorbate. However, it appears as though Vitamin E mainly scavenges lipid $ROO^{\bullet}$ to yield lipid hydroperoxides and a tocopheroxyl radical, acting as a radical "chain breaker" in lipid peroxidation (reviewed in [144]).

Carotenoids are organic pigmented compounds, responsible for the green, yellow, red, and orange colors that are associated with the beneficial properties of fruits and vegetables to prevent disease. Carotenoids of different structures can be found in many fruits and vegetables including: carrots (alpha- and beta-carotene), tomatoes (lycopene), citrus fruits (β-cryptoxanthin), spinach (lutein), and maize (zeaxanthin). These phytochemicals are known to exert antioxidant effects by scavenging O_2, tricholromethyperoxyl radicals, and $ROO^{\bullet}$. Several *in vitro* and *in vivo* studies have demonstrated the antioxidant properties of beta-carotene and lycopene, and there is a growing body of evidence associated with lycopene and the reduction of disease risk factors (reviewed in [147]). By acting as an antioxidant and reducing oxidative damage, it seems reasonable that adequate consumption of carotenoids can help prevent the progression of many diseases.

Evidence indicates that the consumption of a wide variety of micronutrients associated with antioxidant properties could possibly be beneficial in attenuating oxidative damage by up-regulating the antioxidant defense system. In addition to oxidative stress, inflammation is also known to play a role in the pathogenesis of disease.

Inflammation

Inflammation is initiated in the body as a response mechanism to certain stimuli. The inflammatory response is associated with an increase in blood plasma and leukocytes to an "injured area", in an attempt to rid the body of foreign substances or repair damaged cells [148]. One cause of inflammation in the body is the inability to modulate cellular redox homeostasis, which has been demonstrated in the aging process [149]. As such, aging has been associated with an increase in inflammation due to an age related functional decrease in RONS scavenging antioxidant enzymes. A decline in the ability to effectively respond to increased RONS production shifts the intracellular redox balance. A shift in redox homeostasis initiates a cascade of inflammatory responses that is driven by the activity of NF-κB; a redox sensitive transcription factor responsible for oxidative damage induced inflammation (reviewed in [149]). In addition to aging, dietary intake can affect the inflammatory response in the body, and this response has also been linked to oxidative stress, insulin resistance, obesity, T2DM, and CVD [150].

Measuring Biomarkers of Inflammation

White blood cell count (WBC) is the most fundamental clinical measure of inflammation, as it represents cells of the immune system that are responsible for fighting infectious disease and foreign material. Studies have shown WBC to be a predictor of decreased insulin sensitivity, as well as the development of T2DM [151], CHD, and CVD [152]. However, with the growth of technology, and our understanding of disease, there are a number of inflammatory molecules becoming prevalent in the pathogenesis of age related diseases. Two of the more common methods used for measuring these inflammatory molecules include the use of ELISA techniques and multiplex assays.

Until recently, an ELISA format limited the analysis of a sample to one type of protein/cytokine at a time (reviewed in [153]). Advances in ELISA technology has allowed for multiple proteins to be measured within a single sample. This multiplex ELISA procedure provides accurate and reproducible results, with improved sensitivity and economy when compared to the single ELISA format [154].

The term cytokine is used to describe a large family of immunomodulating/cell signaling protein molecules that play a role in intracellular communication. During endothelial injury, cytokines act as signaling molecules that mediate the migration of leukocytes, cause activation of the acute phase response, and initiate the release of associated inflammatory mediators from the liver (reviewed in [155]). Inflammatory cytokines such as Interleukin-6 (IL-6), IL-8, IL-1β, TNF-α, and CRP are some of the main pro-inflammatory cytokines that initiate the response to a specific stressor/injury [156].

The inflammatory molecules IL-6 and TNF-α, stimulate the synthesis of the acute phase reactants which consists of CRP, amyloid-A, and fibrinongen [157]. While TNF-α and IL-6 have been shown to be associated with recurrent [158] and future [159] coronary events respectively, CRP has been identified as the most reliable assessment of inflammation in clinical practice [48, 160]. C-reactive protein is an early acute phase reactant that responds to active infection and acute inflammation. Recently reviewed in [156], CRP has been shown to correlate most strongly with obesity/visceral fat deposition, but additional studies have shown correlations with markers of insulin sensitivity, TAG, and HDL-C. C-reactive protein < 1, 1-3, and > 3 mg/L, corresponds to low, moderate, and high CVD risks [161]. Traditional assays of CRP were not sensitive

enough to monitor changes in chronic inflammation, and therefore, the development of high-sensitivity CRP assays have made detection of chronic inflammation possible [162].

Implications for Health and Disease

It is widely accepted that obesity, among other environmental factors, is linked to diseases such as hypertension, CVD, dyslipidemia, and T2DM [163]. Mechanisms that relate the pathogenesis of these diseases to obesity are still being elucidated, however, an increase in inflammation is a common feature of many age related chronic diseases [163]. Elevation of the pro-inflammatory molecule TNF-α has been shown to play a role in systemic insulin resistance by interfering with receptor signaling [164]; a potential link between obesity, insulin resistance, and diabetes. Moreover, TNF-α has been shown to be an independent predictor in CHD and CVD events and total mortality among men [165].

Inflammation has been shown to play a role in pathways involving insulin mediated responses [164, 166]. It has been postulated that exposure to a fat-rich diet causes an increase in inflammation in the hypothalamic area involved with leptin and insulin signaling, leading to impaired control of food intake and energy expenditure (reviewed in [166]). Other data demonstrates similar mechanistic actions, as seen from the effects of TNF-α mediated disturbances in insulin signaling pathways of myocytes and brown adipocytes [167]. These data demonstrate the correlation of inflammation with all of the following variables; obesity, local inflammatory mediators, insulin resistance, CVD, and T2DM. Even more, recent work has indicated that chronic inflammation with elevated serum concentrations of TNF-α, IL-1, IL-6, and CRP are

associated with the progression from normolycemia to insulin resistance [168, 169]—important factors in the development of T2DM and CVD [150].

Interluekin-6, which is also linked with the development of insulin resistance, as well as future cardiovascular events, is released by adipose tissue and acts as a mediator of inflammation [170, 171]. Additionally, studies have shown that older sedentary adults have higher levels of TNF-α, CRP, and IL-6 compared to age-matched active individuals [172]. Moreover, CRP has been found to be an independent predictor of cardiovascular events [160]. A recent review indicates that CRP is not only a biomarker in the development of atherosclerosis, but according to *in vitro* findings, CRP is involved in the pathogenesis through a number of mechanisms (reviewed in [156]).

In accordance, the molecular inflammation hypothesis of aging associates age related obesity (specifically greater visceral fat) with increased systemic inflammation. As mentioned previously, and reviewed in [149], there is an age associated decrease in antioxidant defense enzymes and this further underlies the inflammatory process, specifically due to a lack of control over redox homeostasis. This unresolved low-grade systemic inflammation is believed to be the culprit for many age related diseases, such as, CVD, cancer, diabetes, metabolic syndrome, dementia, osteoporosis, and arthritis (reviewed in [134]). These data presented are strong evidence for the role that inflammation plays in aging as well as its implications for health and disease. With that said, reducing inflammation would be ideal for optimal health, and this will be discussed in the next section.

Reducing Inflammation

Pharmaceuticals

There is a growing body of evidence indicating that statins not only act as lipid lowering drugs, but they also act in an anti-inflammatory manner that is lipid-independent (reviewed in [173]). Studies have shown that the use of statins (cerivastatin—0.4 or 0.8 mg/day for 8 weeks) results in a 24.5% reduction in mean CRP levels [174]. Others have noted decreases in CRP up to 37% in healthy individuals (CRP > 2.0 mg/L) following treatment with rosuvastatin [175]. Metformin is used as first-line therapy for the treatment of T2DM, and has been shown to reduce CRP by 7 and 14% in men and women with impaired glucose tolerance [176], and by 26% in individuals recently diagnosed with T2DM [177]. An investigation by Kahn et al demonstrated a > 40% reduction in CRP within 6 months of treatment with rosiglitazone in diabetics [177]. Rosiglitazone is in the thiazolinedione class of drugs, which are known for their insulin sensitizing effects. Additionally, thiazolinediones have been shown to reduce inflammation in a mechanism that is independent of their effects on glycemic control [178]. While drugs have shown efficacy in reducing inflammation, an increasing interest in dietary supplements has led investigators to study if and how these supplements affect inflammation.

Dietary Supplements

Outside of pharmacological therapy, the use of nutraceuticals and phytochemicals (chemical compounds in plants) has been extensively studied and has revealed promising results in their abilities to reduce inflammation (reviewed in [179]). Phytochemicals, mostly flavones and flavonols, have shown to possess anti-inflammatory effects by

inhibiting TNF-α gene expression and inhibiting NFκB activity [180]. Supplementing fish oil that is rich in omega-3 PUFAs for 12 week has been shown to significantly reduce plasma TNF-α in healthy males and females [181]. However, a recent meta-analysis indicated no effects from fish-oil supplementation on markers of CRP and TNF-α [182]. Curcuma longa, which is commonly referred to as turmeric, exerts its anti-inflammatory effects by reversing insulin resistance, hyperglycemia, hyperlipidemia, and other symptoms linked to inflammation (reviewed in [183]). Additionally, *in vitro* studies have demonstrated the ability of curcumin to decrease the expression of inflammatory cytokines IL-6, IL-1β, and TNF-α [184]. A recent review by Aggarwal (2010) describes other spice nutraceuticals that have demonstrated the ability to modulate inflammatory pathways including: capsaicin, gingerol, cinnamaldehyde, piperine, and fenugreek [183]. While there are many prospective supplements/nutraceuticals associated with anti-inflammatory benefits, the majority of this evidence stems from animal and *in vitro* studies [179]. With more research and a greater understanding of these molecules *in vivo*, the use of these supplements/nutraceuticals could prove to be efficacious for the treatment of many inflammatory- related diseases.

Exercise

Obese individuals are at higher risks for developing cardiovascular related diseases, partly because of an increase in systemic inflammation [185]. Cross-sectional studies indicate an association between physical inactivity and systemic inflammation in healthy middle-aged and older adults in the United States [186]. Evidence indicates that exercise would benefit this population due to the associated anti-inflammatory effects seen with chronic exercise (reviewed in [150]). It has been proposed that exercise

induced pro-inflammatory cytokines triggers the up-regulation of anti-inflammatory cytokines following exercise [187]. Individuals at risk for ischemic heart disease who engage in a variety of exercises (2.5 hours/week) for six months show a significant reduction in the pro-inflammatory cytokine TNF-α as well as a 35% reduction in CRP [188]. A cross-sectional study by Colbert et al (2004) indicated that higher levels of exercise are associated with lower levels of CRP, TNF-α, and IL-6 [172]. While exercise has been investigated as an option for reducing inflammation, investigations involving nutrient intake is as important, if not more important.

Nutrients

Evidence indicates that long chain SFAs contribute to inflammation through a variety of mechanisms. One hypothesis associates a fat-rich diet, specifically SFAs [189], with an impaired ability to modulate leptin and insulin signaling in the hypothalamus. This hypothesis is based off of studies that demonstrates SFA induced inflammatory damage to local networks of neurons in the hypothalamus, leading to an impaired ability to modulate energy homeostasis and the development of obesity (reviewed in [166]). An investigation by Arya et al (2006) indicated that SFAs were the most important nutrient that contributed to increases in CRP in healthy adolescents and young adults [190]. It was also noted that for every 1% decrease in calories of SFA consumed, a decrease in serum CRP by 0.14 mg/L was shown [190].

A recent review of literature indicates that there is a lower risk in the development of inflammatory related diseases with a lower omega-6/omega-3 fatty acid ratio intake [191]. PUFA intake, specifically omega-3 PUFA, has been shown to inversely correlate with plasma levels of soluble TNF receptors, as well as CRP, albeit to a lesser degree (p

= 0.08). These associations were demonstrated in the omega-3 PUFAs, Eicosapentaenoic acid (EPA) and Docosahexaenoic acid (DHA), but not α-Linolenic acid (ALA)—commonly found in vegetable oils [192]. The dietary intake of MUFAs has been shown to be inversely associated with plasma CRP and IL-6 in healthy middle aged men and women [193]. These findings are in line with other data, in which a high MUFA diet (> 20% total fat) significantly reduced IL-6 and CRP in young overweight men and women (average age of 28 years) [194]. It appears as though one could favorably alter inflammation, specifically CRP, IL-6, and TNF-α, if one were to increase MUFA intake, decreases SFA intake, and consume a lower omega-6/omega-3 fatty acid ratio.

The LIPGENE study investigated the effects of a 12 week dietary intervention on CRP and F_2-isoPs in 486 volunteers with the metabolic syndrome. This study included four separate dietary approaches: two high-fat diets (38% energy), one rich in SFA and one in MUFA, and two low-fat high complex carbohydrate diets, one supplemented with omega-3 fatty acids and one with oleic acid. Interestingly, this study found no significant differences in pre- and post-intervention values of CRP and F_2-isoPs [195]. A recent investigation by Johnstone et al (2011) was a well-controlled, randomized, cross-over design study in obese men and women that compared a 4-week low carbohydrate diet rich in MUFA (30% protein, 4% carbohydrate, and 66% fat) versus a 4-week moderate carbohydrate diet (30% protein, 35% carbohydrate, and 35% fat). The study noted a significant reduction in TNF-α and IL-10 in both groups. Also, the moderate carbohydrate diet decreased IL-10 concentrations significantly more than the low-carbohydrate diet [196]. Regarding carbohydrate type (i.e. high versus low glycemic), a low glycemic index diet is associated with a lower concentration of CRP when compared

to a high glycemic index diet [197]. In addition, studies have demonstrated a significant reduction in CRP when obese men and women consume a 12-week hypocaloric diet in which all of their grains are derived from whole grains [198].

A 52-week energy restricted/high-protein diet (30% energy) has been shown to significantly reduce CRP levels in obese individuals with hyperinsulinemia [199], which is in line with other findings [200, 201]. Protein in the form of soy and soy isoflavones have been shown to have little effect on CRP, TNF-α, and IL-6 (reviewed in [202]). However, there is also data that demonstrate beneficial effects of soy products on inflammation following 8 weeks of soy-based nutritional supplementation. This was demonstrated in end-stage renal disease patients on hemodialysis by the inverse relationship of total blood isoflavones and CRP, and a ~47% reduction in CRP. It is noteworthy that the CRP levels in the subjects were high (median 18.2 mg/L); nonetheless, the anti-inflammatory effects of isoflavones appeared to benefit this population [203].

A recent cross-sectional study on the relationship between dietary intake and inflammatory markers in young boys and girls indicated no relationship between total flavonoid intake and markers of CRP, IL-6, and TNF- α. However, total fruit and vegetable intake was significantly and inversely correlated with CRP, IL-6, and TNF-α—CRP was inversely associated with intakes of fruit and vitamin C, TNF-α was inversely associated with carotene, and IL-6 was inversely associated with intakes of legumes, vegetables, beta-carotene, and vitamin C [204]. This study indicates the beneficial effects of fruit and vegetable intake at a young age. Furthermore, the acute intake of

Vitamin C (1g) and Vitamin E (800 IU) has been shown to significantly reduce the meal induced rise in CRP in T2DM patients following a fat-rich calorie dense meal [205].

C-reactive protein was significantly reduced following 2 weeks of a high TAC diet versus a low TAC diet in healthy men and women. Also, during the high TAC diet, intake of Vitamin C and E were significantly higher [206]. However, other data does not support this association [207]. It is noteworthy to mention that the former study [206] was specifically comparing the effects of two different diets on biomarkers of inflammation, while the later [207] was a cross-sectional study that measured the correlation between the dietary intake of micronutrients and inflammation. One interesting finding from the investigation of de Oliveira et al (2011), was that a higher intake of heme-iron was positively associated with plasma CRP concentrations [207]. While animal products are common sources of heme-iron, this association was independent of red meat, poultry, and fish, and was likely attributed to a combination of the three. Other cross-sectional studies have found that as the consumption of red-meat increases, so too does dietary iron, plasma CRP, and the risk of metabolic syndrome [208].

The Daniel Fast, which is characteristically a high TAC diet devoid of animal products and lower in protein as compared to traditional diets [1, 2], has been shown to reduce CRP from 3.15 to 1.6 mg/L in 21 days (~49%) [1]. Although this reduction was not statistically significant, it could prove to have clinical relevance. Furthermore, the Daniel Fast promotes the consumption of fruits, vegetables, and whole grain products, all of which constitute that of a vegetarian diet and are emphasized in a recent expert panel on vegetarian nutrition [209].

Daniel Fast

The Daniel Fast is a Christian based fast that has been derived from biblical text. The Daniel Fast is commonly practiced at the turn of a New Year; however one may partake in the fast at any time. In Daniel 1:8-14, Daniel asked the chief official for permission to have only vegetables to eat (or pulse in some translations, which means "food grown from seed") and water to drink, instead of the royal food and wine. In Daniel 10:2-3, Daniel refrained from choice foods for 21 days, which coincided with a period of mourning. From these two passages, a modern-day Daniel Fast has been developed and can be described as a form of DR and a variation of veganism. The Daniel Fast allows for the *ad libitum* consumption of fruits, vegetables, whole grains, nuts, seeds, legumes, and oil. Animal products, refined foods, white flour, preservatives, additives, sweeteners, flavorings, caffeine, and alcohol are prohibited during this fast.

The Daniel Fast, like many plant-based diets [210], has demonstrated favorable outcomes on blood lipids [1]. A 21-day Daniel Fast is associated with significant reductions in TC, LDL-C, and HDL-C [1]. The TC/HDL-C ratio, TAG, and VLDL-C did not change during this intervention. However, recently completed unpublished work with the Daniel Fast has demonstrated a reduction in the LDL-C/HDL-C ratio and TC/HDL-C ratio, in addition to similar findings for the other blood lipids. While the reductions in blood lipids in just 21 days are favorable, the fast is also associated with a decrease in HDL-C, which is unfavorable from a cardio-protective standpoint. Reductions in HDL-C are common in low-fat vegetarian diets, but this reduction is not associated with poor cardiovascular health (reviewed in [210]). The latter statement is relevant to the Daniel Fast as well, due to the striking similarities between the two diets.

A 21-day Daniel Fast has also been shown to significantly reduce markers of oxidative stress and increase antioxidant capacity in healthy men and women (35 ± 1 years of age), as measured by MDA and TEAC respectively [2]. Additionally, this investigation noted a trend for the lowering of H_2O_2. However, recent unpublished data from a 21-day Daniel Fast did not corroborate this finding, as there was no significant reduction in MDA in healthy men and women (33.9 ± 2.1 years of age) with low baseline levels of MDA. Regarding inflammation, there was a trend for lower CRP levels (~40% reduction), as well as a significant reduction in the white blood cell count [1].

Another plausible explanation for the benefits associated with the Daniel Fast could be due to the effects of protein restriction (as well as the SFA that coincides with consumption of certain animal proteins), as protein consumption has been shown to significantly decrease during times of fasting [2]. Additionally, MR may account for a large portion of the benefits observed with the Daniel Fast due to the abstinence of meat from the diet and the relatively low methionine content of a plant based diet [23]. However, the strictness of a Daniel Fast diet may make compliance and sustainability difficult if adopted for a long period of time. Therefore, including a small amount of animal products may be reasonable, in particular if such inclusion does not adversely impact the positive findings in a significant way. Indeed, evidence indicates that moderate amounts of lean meat are beneficial to total nutritional intake and can still be used to adequately reduce blood cholesterol, albeit to a lesser extent than a pure vegetarian diet [211, 212]. Moreover, the addition of some animal protein may allow for maintenance of HDL-C [213-215]. If so, such maintenance of this cholesterol fraction

might offset any untoward effect that the animal protein may have on other health-related biomarkers.

Our prior work with the Daniel Fast has generated many favorable results. As it appears, many of the other aforementioned dietary regimens provide a certain degree of health-related benefits as well. However, it goes without saying that many people have had trouble adopting these dietary regimens and incorporating them into their everyday life. The *ad libitum* nature of the Daniel Fast makes it a good candidate for dietary adherence. Prior investigations with the Daniel Fast have noted a greater than 95% compliance rate [1, 2]; however these studies only lasted three weeks. Based on subject comments, long-term compliance without animal protein may not be high, and therefore a modified approach must be considered for long-term compliance. However, before incorporating a long-term diet with unknown effects, we first must ensure that this modified approach can produce similar results as those seen in a traditional 21-day Daniel Fast.

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APPENDIX C

DANIEL FAST QUESTIONNAIRE

Please answer the following questions as accurately and honestly as possible. Provide as much detail as you feel necessary. Upon completion, a research assistant will review your responses with you in order to provide clarification where necessary.

1. Please rate your **compliance** to the fast as a percentage (0-100%) based on food choices, use of additives, preservatives, flavorings, caffeine, etc. Provide any necessary remarks for us to better understand reasons behind your compliance. Were there one or two items in particular that lead to your non-compliance, if this was the case?

2. Please rate your overall **mental outlook and mood** during the period of the fast on a scale of 1-10 (1 = as low as possible; 10 = as high as possible). Were there any outside influences that factored into your overall mood or can you accurately state that your mood was influenced primarily by the fast?

3. Please rate your overall **physical health and vitality** during the period of the fast on a scale of 1-10 (1 = as low as possible; 10 = as high as possible). Were there any outside influences that factored into your overall physical health and vitality or can you accurately state that your physical health and vitality was influenced primarily by the fast?

4. Please rate your overall **level of satiety** (feeling of fullness in relation to food intake) during the period of the fast on a scale of 1-10 (1 = as low as possible; 10 = as high as possible).

APPENDIX D

INSTITUTIONAL REVIEW BOARD APPROVAL

THE UNIVERSITY OF MEMPHIS

Institutional Review Board

To:	Richard Bloomer, Tyler Farney, Rick Alleman, Innocence Harvey, Greg Cantrell, and Kelley Hammond Health and Sport Sciences
From:	Chair, Institutional Review Board For the Protection of Human Subjects irb@memphis.edu
Subject:	Comparison of a traditional and modified Daniel Fast on biochemical and anthropometric markers of health (051311-702)
Approval Date:	May 18, 2011

This is to notify you of the board approval of the above referenced protocol. This project was reviewed in accordance with all applicable statuses and regulations as well as ethical principles.

Approval of this project is given with the following obligations:

1. At the end of one year from the approval date, an approved renewal must be in effect to continue the project. If approval is not obtained, the human consent form is no longer valid and accrual of new subjects must stop.
2. When the project is finished or terminated, the attached form must be completed and sent to the board.
3. No change may be made in the approved protocol without board approval, except where necessary to eliminate apparent immediate hazards or threats to subjects. Such changes must be reported promptly to the board to obtain approval.
4. The stamped, approved human subjects consent form must be used. Photocopies of the form may be made.

This approval expires one year from the date above, and must be renewed prior to that date if the study is ongoing.

Brian Schilling

Digitally signed by Brian Schilling
DN: cn=Brian Schilling, o=Univeristy of Memphis, ou=Institutional Review Board Chair, email=bschlng@memphis.edu, c=US
Date: 2011.05.18 14:23:05 -05'00'

Chair, Institutional Review Board
The University of Memphis