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ABSTRACT

Two studies were conducted to evaluate the metabolic and reproductive effects of whole grains and high-fiber foods in metabolic syndrome and polycystic ovary syndrome. Insulin resistance is implicated in the pathology of both metabolic syndrome and polycystic ovary syndrome, and both conditions are associated with elevated levels of cardiovascular disease risk factors. Given the similar etiology of metabolic syndrome and polycystic ovary syndrome, whole grains and high fiber foods have been recommended for both conditions because they lower the glycemic response and improve insulin sensitivity. However, there is limited evidence from clinical trials as to the effectiveness of whole grains and high-fiber foods in the dietary treatment of these conditions. The purpose of the two clinical trials I conducted was to determine if including whole grains and high fiber foods in the diet reduces cardiovascular disease risk in metabolic syndrome, and lowers testosterone levels in polycystic ovary syndrome.

In the first study, obese men and women (25M, 25F) with metabolic syndrome were randomized to receive dietary advice to either avoid whole grain foods or to have all of their grain servings from whole grains for 12 weeks. All other dietary advice to achieve weight loss was the same for both groups. A fasting blood draw, 2-hour oral glucose tolerance test (OGTT), dual energy x-ray absorptiometry (DXA) scan, and biometric measurements were done at the beginning and end of the 12-week diet period.

Body weight decreased 3.7 ± 3.5 kg or 3.5% in the whole grain group (P<0.001) and 5.3 ± 5.2 kg or 4.8% in the refined grain group (P<0.001) (n.s. between groups). Waist circumference and body fat percentage also decreased in both groups from baseline (P<0.02). Despite a similar decrease in body weight, CRP levels decreased 38% in the
whole grain group, but were unchanged in the refined grain group \((P=0.007\) between groups). There were no other significant differences between groups with respect to changes in biometric measurements, lipids and lipoproteins, glucose tolerance, markers of inflammation and fibrinolysis, and sex hormones. Participants in the whole grain group but not the refined grain group increased their intake of dietary fiber and magnesium, and decreased their intake of saturated fat compared with baseline \((P<0.05)\). Participants in both groups reported a greater overall satisfaction with their diet compared with baseline \((P < 0.001)\), rated a greater sense of having a healthy lifestyle \((P < 0.001)\), and considered their families to be more approving of their diet \((P < 0.001)\). In addition, participants in the refined grain group had a lower preoccupation with food compared with baseline \((P = 0.002)\) and participants in the whole grain group rated their meal planning and preparation as more difficult \((P = 0.006)\).

The aim of the second study was to determine if varying meal composition affects postprandial testosterone levels in women with PCOS. I evaluated changes in testosterone, sex hormone binding globulin (SHBG), glucose, and insulin levels following a high-fat, Western meal (62% fat, 24% carbohydrate, 1g fiber) and a low-fat, high-fiber meal (6% fat, 81% carbohydrate, 27g fiber) in 15 women with PCOS using a randomized, 2x2 crossover design with a 7-day washout period. Blood samples were collected at baseline and at 30 minutes and every hour after each meal for six hours.

Testosterone levels decreased 27% within two hours after both meals \((P<0.001)\). However, the testosterone level and the free androgen index remained below pre-meal values for four hours after the low-fat, high-fiber meal \((P <0.004)\) and for six hours after the high-fat, Western meal \((P <0.004)\). Insulin levels were almost 2 fold higher at 30, 60,
and 120 minutes after the low-fat, high-fiber meal compared with the high-fat, Western meal (P <0.03). Glucose levels also were higher at 30 and 60 minutes after the low-fat, high-fiber meal compared with the high-fat, Western meal (P<0.003).

The findings from these two studies demonstrate that whole grains and high fiber foods have metabolic and reproductive effects in metabolic syndrome and polycystic ovary syndrome. The results from the first study demonstrate that weight loss can be achieved with a diet high in whole grains, similar to a conventional hypocaloric diet with refined grains, but there may be additional benefits to the cardiovascular risk profile. Specifically, I observed a reduction in c-reactive protein, an independent risk factor for cardiovascular disease, in participants consuming whole grains but not refined grains.

The finding of a prolonged reduction in postprandial testosterone levels following a high-fat, Western meal compared with a low-fat, high-fiber meal indicates that diet plays a role in the regulation of testosterone levels in women with PCOS, and that diet composition may be an important consideration in the treatment of hyperandrogenism in PCOS. Future studies examining larger cohorts for longer periods are necessary to determine the long-term health benefits of whole grains and high-fiber foods in metabolic syndrome and polycystic ovary syndrome.
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<th>Full Form</th>
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<tr>
<td>ATP III</td>
<td>Adult Treatment Panel III</td>
</tr>
<tr>
<td>AHA</td>
<td>American Heart Association</td>
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<tr>
<td>Apo</td>
<td>Apolipoprotein</td>
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<td>AUC</td>
<td>Area under the curve</td>
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<td>BP</td>
<td>Blood pressure</td>
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<tr>
<td>BMI</td>
<td>Body mass index</td>
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<td>kcal</td>
<td>Calorie</td>
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<tr>
<td>CAD</td>
<td>Coronary artery disease</td>
</tr>
<tr>
<td>CCK</td>
<td>Cholecystokinin</td>
</tr>
<tr>
<td>CDC</td>
<td>Centers for Disease Control</td>
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<tr>
<td>CHD</td>
<td>Coronary heart disease</td>
</tr>
<tr>
<td>CHO</td>
<td>Carbohydrate</td>
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<tr>
<td>CI</td>
<td>Confidence interval</td>
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<tr>
<td>DHEA</td>
<td>Dehydroepiandrosterone</td>
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<tr>
<td>DXA</td>
<td>Dual energy x-ray absorptiometry</td>
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<tr>
<td>ELISA</td>
<td>Enzyme-linked immunoassay</td>
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<tr>
<td>FAI</td>
<td>Free Androgen Index</td>
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<tr>
<td>FDA</td>
<td>Food and Drug Administration</td>
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<tr>
<td>FSH</td>
<td>Follicle-stimulating hormone</td>
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<tr>
<td>GCRC</td>
<td>General Clinical Research Center</td>
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<tr>
<td>GI</td>
<td>Glycemic index</td>
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<td>GnRH</td>
<td>Gonadotropin-releasing hormone</td>
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<tr>
<td>HDL-C</td>
<td>High-density lipoprotein cholesterol</td>
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<tr>
<td>HMG co-A</td>
<td>3-hydroxy-3-methyl-glutaryl coenzyme A</td>
</tr>
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<td>hs-CRP</td>
<td>High-sensitivity C-reactive protein</td>
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<td>IGT</td>
<td>Impaired glucose tolerance</td>
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<tr>
<td>IL</td>
<td>Interleukin</td>
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<td>ISI</td>
<td>Insulin sensitivity index</td>
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<td>IV</td>
<td>Intravenous</td>
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<tr>
<td>LDL-C</td>
<td>Low-density lipoprotein cholesterol</td>
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<td>LH</td>
<td>Luteinizing hormone</td>
</tr>
<tr>
<td>LPS</td>
<td>Lipopolysaccharide</td>
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<tr>
<td>MUFA</td>
<td>Monounsaturated fatty acid</td>
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<td>NCEP</td>
<td>National Cholesterol Education Program</td>
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<td>NDS</td>
<td>Nutrition Data System</td>
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<td>NEFA</td>
<td>Non-esterified fatty acids</td>
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<td>NHANES</td>
<td>National Health and Nutrition Examination Survey</td>
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<tr>
<td>NHLBI</td>
<td>National Heart, Lung, and Blood Institute</td>
</tr>
<tr>
<td>OGTT</td>
<td>Oral glucose tolerance test</td>
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<td>PAI-1</td>
<td>Plasminogen-activator inhibitor-1</td>
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<td>Abbreviation</td>
<td>Full Form</td>
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<tr>
<td>PCOS</td>
<td>Polycystic ovary syndrome</td>
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<tr>
<td>PPD</td>
<td>Peak particle diameter</td>
</tr>
<tr>
<td>PPAR-γ</td>
<td>Peroxisome proliferator-activated receptor gamma</td>
</tr>
<tr>
<td>PRO</td>
<td>Protein</td>
</tr>
<tr>
<td>PUFA</td>
<td>Polyunsaturated fatty acid</td>
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<tr>
<td>SAS</td>
<td>Statistical analysis software</td>
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<tr>
<td>SD</td>
<td>Standard deviation</td>
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<tr>
<td>SE</td>
<td>Standard error</td>
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<tr>
<td>SFA</td>
<td>Saturated fatty acid</td>
</tr>
<tr>
<td>SHBG</td>
<td>Sex hormone binding globulin</td>
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<tr>
<td>t-PA</td>
<td>Tissue plasminogen activator</td>
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<tr>
<td>TC</td>
<td>Total cholesterol</td>
</tr>
<tr>
<td>TNF-α</td>
<td>Tumor necrosis factor-α</td>
</tr>
<tr>
<td>TSH</td>
<td>Thyroid-stimulating hormone</td>
</tr>
<tr>
<td>US</td>
<td>United States</td>
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<tr>
<td>USDA</td>
<td>United States Department of Agriculture</td>
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<tr>
<td>VLDL</td>
<td>Very-low density lipoprotein cholesterol</td>
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CHAPTER 1
INTRODUCTION

Cardiovascular disease is the leading cause of death in the United States and being insulin resistant significantly increases one’s risk. Insulin resistance, or the inability of the body to respond properly to insulin, is also associated with chronic anovulation and infertility in reproductive age women with polycystic ovary syndrome. Improving insulin sensitivity with weight loss or insulin-sensitizing drugs lowers cardiovascular disease risk in overweight men and women, and improves ovulation and fertility in women with polycystic ovary syndrome. The premise of my thesis research was to test whether a diet high in whole grains and high fiber foods, which are frequently recommended to improve insulin sensitivity, lowers cardiovascular disease risk in men and women with metabolic syndrome and offers reproductive benefits to women with polycystic ovary syndrome.

Whole grains are naturally high in fiber, but also “package” antioxidants, phytoestrogens, and phytochemicals that are thought to be cardioprotective. Individuals who consume the most whole grains have a 30% reduced risk of cardiovascular disease in observational studies. Increased whole grain consumption is also associated with a reduced body mass index and decreased weight gain over time. However, few clinical studies have tested whether including whole grains into a hypocaloric diet increases weight loss and improves cardiovascular disease risk. I studied the effects of whole grain foods in
overweight and obese men and women with metabolic syndrome because this represents a population at increased risk of cardiovascular disease.

High-fiber diets are also effective in lowering testosterone levels in healthy men and women, and may confer benefits to women with polycystic ovary syndrome, since high testosterone levels are a hallmark trait. Other features of polycystic ovary syndrome include insulin resistance and a propensity towards increased risk of cardiovascular disease, thus suggesting common etiologies between cardiovascular disease and polycystic ovary syndrome. However, no studies have evaluated the effectiveness of a high-fiber diet in reducing testosterone levels in women with polycystic ovary syndrome.

The aims of my thesis research are:

1. **To determine if a diet high in whole grains increases weight loss and improves cardiovascular disease risk compared with a diet high in refined grains in men and women with metabolic syndrome**

2. **To determine if there is a greater postprandial reduction in testosterone levels after a low-fat, high-fiber meal compared with a high-fat, Western meal in women with polycystic ovary syndrome.**

The results of these studies provide new information on the metabolic and reproductive effects of whole grains and high fiber foods that can be used to guide future research on the most effective diet composition for treating insulin resistant conditions.
CHAPTER 2
LITERATURE REVIEW

In 1988, Gerald Reaven identified a group of metabolic risk factors associated with insulin resistance that clustered together and increased risk of cardiovascular disease (CVD) (1). Reaven named this condition, characterized by reduced high-density lipoprotein cholesterol (HDL-C), elevated triglycerides, hypertension, and glucose intolerance “Syndrome X.” Follow-up experiments that showed insulin resistance to be a robust indicator of cardiovascular risk underscored the importance of insulin resistance, and Syndrome X was later renamed the insulin resistance syndrome (2). In clinical studies, the degree of insulin resistance is related to the magnitude of atherogenic dyslipidemia (elevated triglycerides, reduced HDL-C, and small, dense, low-density lipoproteins (LDL)), adhesion molecule expression, visceral obesity, and concentration of C-reactive protein (CRP) and plasminogen-activator inhibitor-1 (PAI-1) (3). Insulin resistance is also directly related to risk of CVD as well as surrogate markers of CVD including intima media thickness and coronary artery calcification (4-6).

Incidence and Pathogenesis of Coronary Heart Disease

Cardiovascular diseases are the leading cause of death worldwide. CVDs claimed the lives of an estimated 17.5 million people in 2005, representing 30% of all global deaths (7). Cardiovascular diseases include diseases of the heart and blood vessels, including stroke. Coronary heart disease (CHD) is the most common CVD, accounting for approximately 43% of deaths from CVD (7). CHD is caused by atherosclerosis, the
narrowing of the coronary arteries due to fatty build-ups of plaque. The term CHD encompasses any disease of the coronary arteries as well as the resulting complications such as chest pain and heart attack.

The understanding of the pathogenesis of CHD has evolved from a disorder of lipid accumulation to include a condition of chronic inflammation in the arteries (8). Inflammatory events characterize the pathophysiology of atherosclerosis at every stage; immune cells infiltrate early atherosclerotic lesions, their effector molecules accelerate the progression of these lesions, and inflammation evokes acute coronary syndromes (9). The degree of inflammatory activity and associated plaque instability, rather than the extent of arterial constriction, determines the likelihood of an acute coronary syndrome (10). In fact, in many cases there is less than a 50% blockage of the artery at the time of a heart attack (11).

Plaque rupture and thrombosis leading to an acute coronary syndrome (Figure 2.1) can be due to (a) erosion of the endothelial cells, which exposes thrombotic factors, (b) disruption of microvessels in the plaque, which can lead to hemorrhage or thrombosis in the plaque causing rupture, or (c) weakening and degradation of the fibrous cap leading to rupture (11). Each of these processes is accelerated by a state of inflammation. Currently, traditional risk factors as well as dietary and pharmaceutical treatments are being re-evaluated for their effects on inflammatory markers and processes.
Figure 2.1. Unstable fibrous plaque in atherosclerosis. Rupture of the fibrous cap or ulceration of the fibrous plaque can rapidly lead to thrombosis and usually occurs at sites of thinning of the fibrous cap that covers the advanced lesion (8).

Metabolic Syndrome as a Risk Factor for Cardiovascular Disease

Since the focus of CVD prevention has been on early intervention, the National Cholesterol Education Program (NCEP) Adult Treatment Panel (ATP) III introduced the clinical condition of metabolic syndrome into its guidelines and established criteria for diagnosis and instructions for treatment (12). The purpose of defining metabolic syndrome was to identify individuals at high risk for CVD and target them for early intervention. The diagnostic criteria for metabolic syndrome include the four risk factors initially identified by Reaven, as well as abdominal obesity, which is also directly related to insulin resistance and CVD risk (12). The presence of any three of these factors is necessary for diagnosis of metabolic syndrome (Table 2.1) (13). These criteria and their cut points, however, are still debated (14).
Table 2.1. ATP III criteria for clinical diagnosis of metabolic syndrome. The diagnosis of metabolic syndrome is made when three or more of these risk factors are present. Adapted from (15).

<table>
<thead>
<tr>
<th>Measure</th>
<th>Cutpoint</th>
</tr>
</thead>
<tbody>
<tr>
<td>Waist Circumference</td>
<td>≥ 102 cm in men</td>
</tr>
<tr>
<td></td>
<td>≥ 88 cm in women</td>
</tr>
<tr>
<td>Triglycerides</td>
<td>≥ 150 mg/dL or on drug treatment for elevated triglycerides</td>
</tr>
<tr>
<td>HDL-C</td>
<td>&lt; 40 mg/dL in men</td>
</tr>
<tr>
<td></td>
<td>&lt; 50 mg/dL in women or on drug treatment for reduced HDL-C</td>
</tr>
<tr>
<td>Blood Pressure</td>
<td>≥ 130 mmHg systolic blood pressure or ≥ 85 mmHg diastolic blood pressure or on antihypertensive drug treatment or history of hypertension</td>
</tr>
<tr>
<td>Fasting glucose</td>
<td>≥ 100 mg/dL or on drug treatment for elevated glucose</td>
</tr>
</tbody>
</table>

Metabolic syndrome is associated with approximately a two-fold greater risk of developing CVD and a three-fold greater risk of type 2 diabetes (16). It is prevalent, affecting 27% of adults age 20 and over in the United States, and prevalence increases with age and body weight (17). Because metabolic syndrome generally does not occur in the absence of obesity and physical inactivity, the first line clinical therapy to treat metabolic syndrome is lifestyle modification. Lifestyle modification includes improvements to diet and exercise habits along with behavioral changes (18). Exercise involves at least 30 minutes of physical activity five days per week. Behavioral modification consists of stress management, meal planning, reducing portion sizes, reading food labels, self-monitoring, and setting achievable goals. Dietary changes include limiting intake of saturated fat, cholesterol, and simple sugars, and increasing consumption of fruits, vegetables, and whole grains.
Polycystic Ovary Syndrome as a Component of Metabolic Syndrome

In addition to being a risk factor for CVD and type 2 diabetes, insulin resistance also plays a key role in the pathogenesis polycystic ovary syndrome (PCOS), one of the leading causes of infertility in reproductive age women. PCOS affects approximately 6-8% of women worldwide (19). According to the 2003 Rotterdam criteria, PCOS is diagnosed by having two out of three of the following: (a) clinical and/or biochemical signs of hyperandrogenism, (b) oligo- or anovulation, and (c) polycystic ovaries (20) (Table 2.2). Laboratory tests should also exclude other conditions that have a similar clinical presentation including congenital adrenal hyperplasia, androgen-secreting tumors, and Cushing’s syndrome.

In 2006, the Androgen Excess Society suggested modified guidelines based on a comprehensive literature review (Table 1.2) (21). These guidelines recommended that PCOS be diagnosed by the presence of (a) hirsutism and/or hyperandrogenemia, (b) oligo-ovulation and/or polycystic ovaries, and (c) exclusion of other androgen disorders. Other features associated with PCOS including insulin resistance and hyperinsulinemia, obesity, and an elevated LH:FSH ratio were not included in these criteria because they are common in disorders besides PCOS or are not observable in a large percentage of patients undergoing routine laboratory evaluations.

Table 2.2. Rotterdam and Androgen Excess Society criteria for defining PCOS (21).

<table>
<thead>
<tr>
<th>Rotterdam 2003 Criteria</th>
<th>Androgen Excess Society 2006 Criteria</th>
</tr>
</thead>
<tbody>
<tr>
<td>Include 2 of the following + exclusion of related disorders:</td>
<td>Include all of the following:</td>
</tr>
<tr>
<td>(1) Clinical and/or biochemical signs of hyperandrogenism</td>
<td>(1) Hyperandrogenism (hirsutism and/or hyperandrogenemia)</td>
</tr>
<tr>
<td>(2) Oligo- or anovulation</td>
<td>(2) Oligoanovulation and/or polycystic ovaries</td>
</tr>
<tr>
<td>(3) Polycystic Ovaries</td>
<td>(3) Exclusion of related disorders</td>
</tr>
</tbody>
</table>
**Elevated Androgens in PCOS**

In lean and obese women with PCOS, the degree of hyperandrogenemia, reflected by total or free testosterone levels, correlates positively with the magnitude of insulin resistance (22). Circulating total testosterone levels are the best hormonal correlate of the combined symptoms of polycystic ovaries and hyperandrogenemic chronic anovulation (23). Due to elevated androgen levels, women with PCOS often present with acne, hirsutism, and alopecia (male pattern baldness). Kitzinger et al. described PCOS as “the thief of womanhood” because women with PCOS often seek medical attention for infertility and hirsutism (24).

Hyperandrogenemia is thought to play a key role in the metabolic and reproductive abnormalities associated with PCOS. This is supported by observations that prenatal exposure to testosterone in female rhesus monkeys leads to symptoms in adulthood characteristic of PCOS including ovarian hyperandrogenemia, increased serum luteinizing hormone (LH) concentration, central obesity, and defective insulin secretion (25). In addition, follicles are most likely to mature in an environment with high estrogen and low androgen levels (26), so accelerated androgen production may inhibit follicle development.

**Elevated Luteinizing Hormone Pulsatility in PCOS**

Altered gonadotropin dynamics also play a role in the pathophysiology of PCOS. Both LH and follicle stimulating hormone (FSH) are secreted from the pituitary gland in a pulsatile manner and are under control of hypothalamic gonadotropin-releasing hormone (GnRH), also known as the “GnRH pulse generator.” Both lean and obese
women with PCOS have increased LH pulse frequency and amplitude, leading to increased 24-hour mean concentrations (Figure 2.2). This effect is believed to be largely due to elevated insulin concentrations, which stimulates LH secretion (27). LH regulates the synthesis of androgens in the theca cells of the ovaries, so when the concentration of LH increases relative to that of FSH, the ovaries preferentially synthesize androgen. Inhibition of follicular maturation occurs as a result of increased ovarian androgen and LH secretion and ovulation does not occur, leading to infertility in women with PCOS. Suppression of LH by GnRH analogs or by oral contraceptives reduces circulating androgen concentration and restores ovulation (28).

Figure 2.2. Luteinizing hormone (LH) dynamics in lean and obese women with and without polycystic ovarian syndrome (A) and integrated, 24-hour values of LH concentration, amplitude, and pulse frequency (B). (29).
Women with PCOS Have Cardiovascular Risk Factors Similar to Metabolic Syndrome

The metabolic profile of PCOS is comparable to that of metabolic syndrome, to the extent that PCOS has been proposed as Syndrome XX (30). Compared with age and body mass index (BMI) matched controls, women with PCOS frequently have an increased waist circumference, reduced HDL-C, and elevated total and LDL cholesterol, blood pressure, CRP, PAI-1, and concentration of small, dense LDL-C. Women with PCOS also have accelerated atherosclerosis, as indicated by increased coronary artery calcification (31, 32) and intima media thickness (33-35). Pregnant women with PCOS may also have an increased risk of pre-eclampsia (36, 37) and gestational diabetes (38, 39). However, there is little published work on whether diagnosis of PCOS increases morbidity or mortality from CHD (40).

The metabolic syndrome is present in a large percentage of women with PCOS. In a chart review of 106 patients seen over a 3-year period, Apridonidze et al. calculated that the prevalence of metabolic syndrome in women with PCOS was 43%, nearly 2-fold higher than that reported for age-matched women in the general population (Figure 2.3) (41). Women with PCOS and metabolic syndrome also had a significantly higher concentration of serum free testosterone and lower concentration of serum sex hormone binding globulin (SHBG) than women with PCOS without metabolic syndrome. Others have reported similar findings: Dokras et al. found that the age-adjusted prevalence of metabolic syndrome in 129 patients with PCOS was 47.3% (42) and Glueck et al found that out of 138 oligomenorrheic women with PCOS, the age-adjusted prevalence of metabolic syndrome was 46% (43).
Role of Insulin Resistance in Polycystic Ovary Syndrome

Like metabolic syndrome, insulin resistance is believed to play a key role in the pathogenesis of PCOS. Approximately 30% of women with PCOS have impaired glucose tolerance (IGT), defined as a 2-hour blood glucose ≥ 140 mg/dL after a glucose challenge, and 7.5% have type 2 diabetes compared with 14% and 0% of controls, respectively (44). In non-obese women with PCOS, 10.3% have IGT and 1.5% has type 2 diabetes. Both lean and obese women with PCOS are significantly more insulin resistant than age and BMI matched controls, and obese women with PCOS are approximately twice as insulin resistant as lean women with PCOS (Figure 2.4) (45). Among non-obese women, those with PCOS have lower insulin sensitivity than regularly cycling controls, and non-obese women with PCOS have a level of insulin resistance that approaches that of obese controls.
Figure 2.4. Insulin sensitivity in obese (Ob) and non-obese (Nob) women with PCOS and in cycling controls (NL) measured by the euglycemic clamp technique (45).

As described earlier, the degree of insulin resistance is positively correlated with androgen levels in women with PCOS. As insulin sensitivity decreases, there is a compensatory increase in insulin to maintain a normal glucose concentration. While muscle and adipose tissue become resistant to insulin, the ovary maintains a high level of response and continues to secrete androgens (46). Hyperinsulinemia contributes to hyperandrogenemia by (1) directly stimulating testosterone synthesis at the ovary, (2) indirectly stimulating testosterone synthesis by increasing secretion of LH at the pituitary gland, and (3) inhibiting production of SHBG at the liver (47) (Figure 2.5). In turn, high levels of androgens can inhibit follicular maturation (48). In women with PCOS, a reduction in testosterone levels by weight loss or pharmacologic agents leads to increased menstrual cycle frequency and as well as reduced acne and hair growth (49-52). Consequently, circulating androgen levels are a common target of treatment (23).
**Figure 2.5.** The hypothalamic–pituitary–ovarian axis and the role of insulin. The increased frequency of LH pulses in PCOS appears to result from an increased frequency of GnRH pulses. The relative increase in pituitary secretion of LH increases androgen production by ovarian theca cells. Insulin acts synergistically with LH to enhance androgen production. Insulin also inhibits hepatic synthesis of SHBG, the key circulating protein that binds to testosterone and thus increases the proportion of testosterone that circulates in the unbound, biologically available, or “free,” state. Solid arrows denote a higher degree of stimulation than dashed arrows (53).
Improving Insulin Resistance Ameliorates Abnormalities of Metabolic Syndrome and Polycystic Ovary Syndrome

Given the strength of the relationship between metabolic syndrome and polycystic ovary syndrome with insulin resistance, it is not surprising that improving insulin sensitivity by methods such as weight loss and insulin sensitizers improves abnormalities associated with both conditions.

Weight Loss

Weight loss is effective in improving clinical manifestations of metabolic syndrome including hypertension, glucose intolerance, and abdominal obesity. Weight loss decreases serum LDL-C and triglyceride levels, and increases in serum HDL-C are also typically seen when weight loss is sustained (54, 55). In the Diabetes Prevention Program, a lifestyle intervention in men and women with impaired glucose tolerance (2-hour glucose 140-199 mg/dL after a 75g OGTT and fasting glucose 95-125 mg/dL) designed to achieve 7% weight loss, the incidence of metabolic syndrome was reduced by 41% over 3 years compared with placebo (12). Of all 3,234 individuals participating in the Diabetes Prevention Program with and without metabolic syndrome, intensive lifestyle intervention significantly reduced triglycerides (avg 25 mg/dL), increased HDL-C (avg 1 mg/dL), and reduced the incidence of the proatherogenic LDL pattern B (56). In the Finnish Diabetes Prevention Study, which studied overweight and obese men and women with impaired glucose tolerance, a lifestyle modification resulting in a modest ~5% weight loss decreased the 7-year cumulative incidence of diabetes by 43% (57).
In obese women with PCOS, weight loss also reduces circulating androgens, increases SHBG, and increases ovulation frequency. In a small trial by Crosignani et al, 33 anovulatory overweight patients with PCOS, 27 of whom had oligomenorrhea, were prescribed a 1200 calorie diet (20% protein, 25% fat, 55% carbohydrate, 30g fiber) and advised to exercise at least once a week for 6-12 months (58). Twenty-five women (76%) lost at least 5% of their body weight and 11 of these women (33%) reached a 10% decrease in weight. Among the 27 patients with oligo-amenorrhea, 18 had a resumption of regular cycles and 15 experienced spontaneous ovulation.

In a study by Kiddy et al., 24 obese women with PCOS were treated for 6 months with a 1000 kcal, low-fat diet (59). Thirteen subjects lost more than 5% of their starting weight (range 5.9-22%) and in this group there was a marked increase in SHBG (23.6 ± 9.6 nmol/L pre-treatment, 36.3 ± 11.8 nmol/L post-treatment) and a reduction in free testosterone (77 ± 26 pmol/L pre-treatment, 53 ± 21 pmol/L post-treatment). Of the 13 women who lost greater than 5% of their starting weight, 9 of 11 that had amenorrhea or anovulatory menses at baseline showed an improvement in reproductive function, i.e. they either conceived (n=5) or experienced a more regular menstrual pattern. In contrast, in the group who lost less than 5% of their initial weight, only one of eight with menstrual disturbances had an improvement in reproductive function. Overall, dietary studies in women with PCOS involving caloric restriction consistently result in spontaneous resumption of menses and ovulation when accompanied by a weight loss of at least five percent of body weight (60).
Insulin Sensitizers

Insulin sensitizers including metformin and thiazolidinediones also improve metabolic and reproductive aspects of metabolic syndrome and PCOS. Metformin acts primarily by suppressing hepatic glucose production but also increases peripheral glucose uptake. Metformin has been shown to decrease cardiovascular events in men and women with type 2 diabetes mellitus (61). In the UK Diabetes Prospective Study, patients treated with metformin had a 42% reduction in diabetes-related death, a 39% reduction in myocardial infarction, and a 36% reduction in all-cause mortality compared with conventional treatment (diet alone).

In a meta-analysis of 7 randomized, placebo-controlled clinical trials in women with PCOS, five of which were double blind, BMI decreased 4%, fasting insulin decreased 27%, and androgen measures (free testosterone, free androgen index or total testosterone) decreased 20% after 1-6 months of 1.5-2g metformin/d (62). On average, one additional ovulation occurred every 5 months with metformin treatment. In another meta-analysis that included 13 randomized controlled studies where metformin was administered to 543 women with PCOS, patients taking metformin had an odds ratio for ovulation of 3.88 (95% CI: 2.25, 6.69) compared with placebo (63).

Thiazolidinediones are another class of anti-diabetic drugs that increase peripheral insulin sensitivity and decrease androgen levels in women with PCOS. Thiazolidinediones are selective ligands of the nuclear transcription factor peroxisome-proliferator-activated receptor gamma (PPAR-γ), a nuclear receptor that regulates gene expression. In the PROactive study comparing oral pioglitazone (titrated from 15 mg to 45 mg (n=2605) or matching placebo (n=2633), the hazard ratio of the composite of all-
cause mortality, non-fatal myocardial infarction, and stroke was 0.84 (95%CI 0.72-0.98, p=0.027) over an average of 34.5 months (64).

In women with PCOS, the most research has been done using troglitazone, however this drug was removed from the market in March 2000 due to hepatotoxicity. In a 3-month trial of 400 mg troglitazone/d in women with PCOS, free testosterone decreased 25–35% and SHBG increased by 25–66% (50, 65). In another multicenter study, three doses of troglitazone (150, 300, and 600 mg/d) or placebo were given for 44 weeks. Fifty seven percent of PCOS patients treated with the highest dose of troglitazone ovulated more than half of the time, compared with 12% in the placebo group (66). Several small studies have shown that the other thiazolidinediones, pioglitazone, and rosiglitazone have similar effects on glucose, insulin and reproductive parameters without causing hepatotoxicity.

**Potential Role of Dietary Fiber in the Treatment of Metabolic Syndrome**

Depending on its composition, diet has the capacity to increase or decrease insulin sensitivity, and thus has the potential to ameliorate metabolic syndrome. For example, a diet high in fat may contribute to metabolic syndrome by promoting insensitivity to insulin. In contrast, consuming foods high in fiber such as whole grains slows gastric emptying, reduces postprandial glucose and insulin levels, and can improve insulin sensitivity (67, 68). Fiber-rich foods are generally recommended for treatment of insulin resistant conditions like diabetes due to their ability to suppress postprandial elevations in glucose and insulin and increase satiety (69).
Dietary fiber is defined as a component of plant foods that cannot be digested in the human small intestine. Fiber is mainly the storage and cell wall polysaccharides of plants that cannot be hydrolyzed by human digestive enzymes (70). There are two types of dietary fiber. Soluble fiber dissolves or swells in water and is often metabolized (fermented) by bacteria in the large intestine. Insoluble fiber usually does not dissolve in water and is not metabolized by bacteria in the large intestine. Foods high in soluble fiber include oats, beans, peas, lentils, apples and pears. Foods high in insoluble fiber include whole-wheat breads, whole wheat cereals, rye, brown rice, cabbage, carrots, and Brussels sprouts (71).

The cholesterol lowering effects of dietary fiber are primarily attributed to soluble fiber (72). Soluble fiber dissolves in water and can form a gel that slows nutrient absorption after meals and reduces postprandial glucose and insulin secretion. Soluble fiber also lowers LDL-C, mainly by binding bile acids in the small intestine and interfering with their absorption. As a result, cholesterol is removed from the blood and converted into bile acids in the liver to replace the bile acids lost in the stool. Soluble fibers can also be fermented in the colon, which generates short chain fatty acids that can inhibit cholesterol synthesis (72).

A high-fiber diet (10-20g/1000 kcal) may prevent the carbohydrate-induced hypertriglycerideremia and conversion to an atherogenic phenotype that can result from a low-fat diet (73). In type 2 diabetics, a 6-week eucaloric diet high in fiber (25 g/d soluble fiber, 25 g/d insoluble fiber) lowered the area under the curve for 24-hour plasma glucose and insulin concentrations by 10 percent and 12 percent, respectively, compared with a lower fiber diet (8g/d soluble fiber, 16g/d insoluble fiber) with the same energy and
macronutrient content (73). The high-fiber diet also reduced plasma total cholesterol concentration by 6.7 percent, triglyceride concentration by 10.2 percent, and very-low-density lipoprotein cholesterol concentration by 12.5 percent.

Potential Use of Whole Grains as a Treatment for Metabolic Syndrome

The health benefits of fiber rich foods may also come from protective components other than fiber. Whole grains, in particular, are high in fiber but also “package” of many cardioprotective components including antioxidants, phytoestrogens, and phytochemicals (74). In epidemiological studies, men and women who consume the most whole grains (~3 servings per day) have approximately a 30% reduced risk of coronary heart disease and ischemic heart disease independent of variables such as age, BMI, smoking, alcohol intake, multivitamin use, aspirin use, physical activity, type of fat intake, and postmenopausal hormone use (75-77). The prevalence of metabolic syndrome also is lower among individuals who consume the most whole grains (78) and increased whole grain consumption is associated with reduced weight, abdominal obesity, and insulin resistance, which are all associated with lower incidence of metabolic syndrome (79-81).

Whole Grains

A whole grain contains all of the naturally-occurring parts and nutrients of the entire grain seed. A whole grain consists of three layers: the bran, germ, and endosperm (Figure 2.6) (82). The bran is the coarse outer layer that protects the other two parts of the kernel. The bran contains B vitamins (thiamin, niacin, riboflavin, and pantothenic acid), minerals (iron, zinc, magnesium, potassium, phosphorus, copper, manganese,
selenium), fiber, some protein, and phytochemicals. The germ is the embryo that, if fertilized by pollen, will sprout into a new plant. It contains minerals, unsaturated fats, B vitamins, and antioxidants. The endosperm is the energy supply for the embryo, and makes up approximately 80% of the grain. The endosperm is predominantly starch (50-75%), with some protein (8-18%) and contains relatively few vitamins, minerals, or phytochemicals and little fiber.

**Figure 2.6.** Layers of a whole grain

The most common grains are wheat, rice, and corn, which together comprise at least 75% of the world’s grain production (83). Other whole grains include oats, rye, barley, triticale, sorghum, amaranth, buckwheat, quinoa, and millet. The FDA defines a whole grain as a product with at least 51% of ingredients by weight as whole grains.
Although grains account for 25% of energy consumption in the United States, it is estimated that 95% of grains are refined (84). The Department of Health and Health Services nutrition objectives for 2010 promote consumption of three servings of whole grain foods per day to reduce risk of chronic disease (85); however the average American consumes 1 serving of whole grains per day (86). A USDA survey reported that 29% of individuals sampled consume no whole grains, 36% consume less than one per day and 8% eat the recommended three servings per day (86).

Generally, grains in developed countries including the United States are subject to milling, heat extraction, cooking, parboiling or other techniques to optimize flavor, color, texture, appearance, and longevity (79, 84). In the refining process, the nutrient-rich bran and germ layers are separated from the starchy endosperm and the endosperm is ground into flour. Since most of the nutrients are contained in the bran and germ layer of the grain, fiber, vitamins, minerals, phytoestrogens, antioxidants and other beneficial components are lost (Figure 2.7). Although refined grains in the United States are fortified with B vitamins and iron, refined grains lack their naturally occurring vitamins, minerals, antioxidants, fiber and phytochemicals (87).
Figure 2.7. Percentage of nutrients lost and supplemented in refined grains compared with whole wheat (88)
Protective Components of Whole Grains in Addition to Fiber

Fat and Associated Constituents

Fat accounts for an average of 3.6% of the total caloric content of whole grains. The lipid composition is high in unsaturated fatty acids, especially linoleic acid. The lipid fraction also contains plant sterols and tocotrienols, which have hypocholesterolemic effects in animal studies (89). Whole grains could contribute >200 mg/d of plant sterols in the average Western diet (90). This amount is less than what is needed for significant cholesterol reduction, however the contribution from whole grains is additive to the rest of the diet. Tocotrienols are concentrated in the bran and are structurally similar to tocopherols. They are antioxidants and can inhibit cholesterol synthesis by reducing activity of hydroxymethylglutaryl (HMG) CoA reductase (90).

Antioxidants

Humans are continually exposed to oxidative stress in the form of free radicals, which arise from normal metabolic processes and from external sources, including smoking, electromagnetic radiation, and air and water pollutants. Whole grain foods have a high antioxidant capacity relative to fruits, vegetables and refined grains (Figure 2.8) (91). The majority of antioxidant activity in whole grains is concentrated in the bran. Whole grains contain many antioxidants including tocopherols, tocotrienols, selenium, phenolic acids, lignans, and phytic acid. Although the role of antioxidant supplementation in protecting against CHD has been questioned, prospective epidemiological studies suggest that intake of antioxidants from dietary sources is protective against CHD (92).
**Figure. 2.8.** The average antioxidant activity for different foods. Averages compared are from analysis of 3 melons, 20 vegetables, 12 fruits, 2 white breads, 1 rice cereal, 3 corn cereals, 2 whole grain breads, 3 whole grain oat cereals, 3 whole wheat cereals, 2 whole grain cereals with raisins, and 5 berries.

### Average Antioxidant Activity

<table>
<thead>
<tr>
<th>Food Type</th>
<th>Average Antioxidant Activity (TE/100 grams)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Melons</td>
<td></td>
</tr>
<tr>
<td>Common Vegetables</td>
<td></td>
</tr>
<tr>
<td>Common Fruits</td>
<td></td>
</tr>
<tr>
<td>White Bread</td>
<td></td>
</tr>
<tr>
<td>Rice Cereal</td>
<td></td>
</tr>
<tr>
<td>Corn Cereals</td>
<td></td>
</tr>
<tr>
<td>100% Whole Grain Bread</td>
<td></td>
</tr>
<tr>
<td>Whole Grain Oat Cereals</td>
<td></td>
</tr>
<tr>
<td>Whole Grain Wheat Cereals</td>
<td></td>
</tr>
<tr>
<td>Whole Grain Cereal/Raisins</td>
<td></td>
</tr>
<tr>
<td>Common Berries</td>
<td></td>
</tr>
</tbody>
</table>

### Lignans

Lignans are one of three main groups of plant compounds classified as phytoestrogens (the other two are isoflavonoids and coumestans) (93). They are the building blocks of lignin in the plant cell wall. The plant lignans secoisolariciresinol and atiresinol are converted by bacteria in the human gut to the mammalian lignans enterolactone and enterodiol where they are absorbed and enter circulation. Some lignans have potent antimicrobial, antifungal, antiviral, and antioxidant properties, suggesting they play a role in plant defense against pathogens and pests (94). Lignans also have anti-tumor and anti-inflammatory actions, which may be protective against
cancer in humans. Enterolactone has been proposed as a biomarker for whole grains. However seeds, especially flaxseed, are rich sources in lignans, which limits the utility of enterolactone as a biomarker.

Anti-Nutrients

An anti-nutrient is a substance that interferes with the utilization of one or more nutrients by the body. Anti-nutrients found in grains include phytic acid, phenolic compounds, digestive enzyme (protease and amylase) inhibitors, and hemagglutinins (89).

Phytic acid is concentrated in the bran layer of grains (95). It chelates with various metals, including copper and iron, which suppresses the production of free radicals. This may contribute to the antioxidant effects of whole grains. In animal models, including phytic acid in the diet reduced colon cancer incidence (96) and increased colonocyte apoptosis and cell differentiation (97). Phenolic compounds, including ferulic acid, p-coumaric acid, and syringic acid, are also in the outer layer of grains and have antioxidant capacities (95). Some phenolic compounds protect the plant against disease and have antimicrobial and antifungal properties (89). Protease inhibitors make up 5-10% of the water soluble protein in grains and are concentrated in the endosperm and germ. They have been shown to reduce the risk of breast and colon cancers in animals (95).
**Relationship Between Whole Grains and Body Weight**

Reports from observational studies suggest that intake of whole grains is inversely associated with body weight. Whole grain intake was inversely related to BMI in the Framingham Heart Study (78, 98) and the Iowa Women’s Health Study (75). In the Nurses’ Health Study (n=74,091), women who consumed the most whole grains (1.62 servings/1000 kcal) weighed 0.9 kg less than women who consumed the least whole grains (0 servings/d) at baseline. An increased intake of whole grains was associated with less weight gain over 12 years (mean weight gain of 1.58 kg every 2-4 years in the lowest quintile vs. 1.07 kg in the highest quintile) (81). Likewise, in the Health Professionals Follow-up Study (n = 28,082) each 40g increment in whole grains was associated with 0.49 kg less weight gain over 8 years (99).

A recent randomized, clinical trial by Melanson et al. reported a similar degree of weight loss in overweight and obese men and women on 6-month weight-management programs with and without whole grain cereals (100). In this study, participants were randomized to one of three 24-week programs: [1] exercise only, [2] exercise plus a hypocaloric diet with 1-2 fiber-rich whole grain cereals/d, or [3] exercise plus a hypocaloric diet without whole-grain cereals. Participants had weekly counseling visits for the first 12 weeks and had no weekly visits for the following 12 weeks to assess sustainability of the diet without active support. The hypocaloric diet with whole grain cereals group ate two meals containing a high-fiber whole grain cereal (7.7g fiber/serving) for the first 12 weeks, and one meal containing the cereal for the next 12 weeks, in addition to following instructions to restrict energy intake using the Exchange Lists for Meal Planning. The hypocaloric diet without whole grains group was told to
avoid cereal, and was also given instructions to restrict energy intake using the Exchange Lists. Body weight decreased in the hypocaloric diet groups with and without whole grain cereals by 5.6kg and 6.2kg, respectively, but weight loss between the two groups did not differ significantly.

Whole grain foods and other high-fiber foods are usually less energy dense and more filling than refined grain foods (79, 101, 102). Whole grain foods are high in soluble fiber, which slows the digestion and absorption of food so satiety signals are stimulated for longer. For example, cholecystokinin (CCK), a satiety hormone produced in the small intestine, is increased after consumption of high-fiber meals as compared to isocaloric low-fiber meals (79, 103). Finally, soluble fiber lessens postprandial glucose and insulin concentrations, which is linked to a reduced rate of the return of hunger and subsequent energy intake (79, 104). No clinical research has directly examined whether a hypocaloric diet high in whole grains enhances weight loss and improves CVD risk factors in individuals with metabolic syndrome.

**Cardiovascular Disease Markers and Their Relationship with Whole Grains and Fiber**

*C Reactive Protein (CRP)*

Of the inflammatory biomarkers, CRP is perhaps the most widely studied. CRP is an acute phase protein (t½ = 19 hours) that increases during systemic inflammation (105). CRP is secreted primarily by the liver in response to interleukin (IL)-6. IL-6 is released from monocytes, macrophages, and adipose tissue (~25%) and is stimulated by IL-1β and TNF-α, which are secreted during a proinflammatory response. In response to tissue
injury, CRP levels rise within 4-6 hours and increase exponentially, doubling every 8-9 hours and peaking at several hundred fold within 24-48 hours (106). CRP levels return to normal when normal tissue structure and function is restored. In individuals at risk of atherosclerosis, CRP is minimally elevated (<10 mg/L).

Blood levels of CRP predicts future coronary events in individuals without CHD and predicts recurrent ischemia and death in individuals with stable and unstable angina and those who present with an acute coronary syndrome or myocardial infarction (107). CRP is a robust CVD risk factor because it adds prognostic information at all LDL-C levels and at all Framingham risk scores (Figure 2.9) (108). In addition, CRP is an easily assessed risk factor because it has a long half-life, does not have a circadian cycle, has a standardized assay, and has accepted normal ranges and screening guidelines from the American Heart Association (AHA) and Centers for Disease Control (CDC) (109). CRP is also a reliable biomarker irrespective of sex, age, blood pressure, lipid levels, and smoking status.

**Figure 2.9.** CRP provides prognostic information at all levels of LDL cholesterol and at all levels of the Framingham Risk Score. Adapted from (108).
The primary function of CRP is not established, but CRP may play a role in atherosclerosis by increasing adhesion molecule expression, inhibiting nitric oxide synthase, and enhancing LDL-C uptake by macrophages (110). However, some in-vitro studies have recently been called into question due to possible contamination of CRP with lipopolysaccharide (LPS) or azide (111). Several investigators have reported that recombinant CRP, which is generated by a time consuming process involving purification of malignant ascites, and commercial CRP that has been purified do not induce inflammation compared with commercial CRP that is produced by recombinant techniques using predominantly Escherichia coli (111-114).

The American Heart Association recommends that if a person’s cardiovascular risk score, judged by global risk assessment, is low (the possibility of developing CVD is <10% in 10 years), a test for CRP is not necessary (115). If the risk score is in the intermediate range (10-20% in 10 years), a CRP value can help predict a cardiovascular and stroke event and direct further evaluation and therapy. However, the benefits of such therapy based on this strategy remain uncertain. A person with a high risk score (>20% in 10 years) or established heart disease or stroke should be treated intensively regardless of CRP levels. Global risk assessment uses information from the Framingham Heart Study and is based on age, gender, total and LDL cholesterol, systolic blood pressure, and smoking status. Individuals with CRP < 1.0 mg/L are at low risk of developing CVD. If CRP is between 1.0 and 3.0 mg/L, a person is at average risk. If CRP is higher than 3.0 mg/L, a person is at high risk (107).

A handful of studies have investigated the relationship between whole grain and fiber intake with CRP. In 902 diabetic women in the Nurse’s Health study, there was a
significant trend towards reduced CRP with increasing quintiles of whole grain intake ($P = 0.03$). The concentration of CRP was 18% lower in the highest quintile of cereal fiber compared with the lowest, but there were no associations with fiber from fruit and vegetables or total fiber. There was a fairly small, not statistically significant, inverse correlation between whole grain intake and CRP in a random sample of 468 women from the Nurse’s Health Study II and 468 men from the Health Professionals Follow-up Study (116). CRP was 9.4% lower in the highest versus lowest quintile ($P=0.32$) after adjusting for diet and lifestyle factors.

Other observational studies have reported an inverse association between dietary fiber intake and glycemic load with CRP (117-119). The authors of many of these studies have hypothesized that the reduced CRP concentration is due to lower day-long glycemia. In support of this, there was a significant association between 2-hour glucose with CRP in the Insulin Resistance Atherosclerosis Study ($n=1625$) after adjusting for age, sex, ethnicity and clinical center ($r = 0.27$, $P<0.0001$) (120). However, a crossover clinical trial by Jenkins et al. found that 3-month supplementation with 19 g/d of cereal fiber did not affect significantly affect CRP levels compared with a 4 g/d fiber supplement in 23 men and women with type 2 diabetes (121).

**Plasminogen-Activator Inhibitor Type 1 (PAI-1)**

PAI-1 is a fast-acting inhibitor of plasminogen activation and the primary inhibitor of the fibrinolytic system (122). Fibrinolysis is the cascade of enzymatic reactions that results in the degradation of fibrin, a protein involved in blood clotting. As depicted in **Figure 2.10**, PAI-1 inhibits conversion of inactive plasminogen to active
plasmin, a fibrin-degrading protease, by binding and inactivating t-PA and urokinase plasminogen activator. This means that PAI-1 promotes clot formation, which could increase fibrin deposition and promote formation of a thrombus (123).

**Figure 2.10.** Activation and inhibition of the fibrinolytic pathway. Tissue plasminogen activator (t-PA) circulates in plasma as a complex with plasminogen-activator inhibitor type 1 (PAI-1) in a 1:1 ratio. The fibrin clot provides the surface on which the reactions occur. Plasminogen is activated by t-PA or urinary-type plasminogen activator (u-PA). Plasminogen, t-PA, and fibrin form a ternary complex that promotes the formation of plasmin and the subsequent lysis of cross-linked fibrin into low-molecular-weight fragments (fibrin-degradation products). PAI-1 also binds to fibrin and, when bound, retains its inhibitory activity against t-PA. From (123).
PAI-1 is released into the bloodstream by the liver, vascular endothelial cells, platelets, and adipose tissue where it is present in its active, unstable form ($t^{1/2} = 30 \text{ minutes}$) (124) or, more likely, complexed with t-PA or vitronectin (123). Plasma PAI-1 has a circadian variation that peaks in the early morning, coincident when there is a higher probability of acute coronary events (125, 126). The normal concentration of PAI-1 in plasma is 6-80 ng/mL (125).

Several clinical studies have reported a strong correlation between circulating PAI-1 levels and cardiovascular events and mortality (127-131). One large observational study found that increased PAI-1 predicts a myocardial infarction and sudden cardiac death, but not after adjustment for BMI, triglycerides and HDL-C (132). PAI-1 is increased in obese men and women with metabolic syndrome and type 2 diabetes (122), especially in those with an abdominal body fat distribution (133-135). In obese men and women, visceral adipose tissue expresses five times more PAI-1 than subcutaneous adipose tissue (136). Changes in PAI-1 during weight loss correlates with changes in visceral but not subcutaneous fat (134). PAI-1 decreases after weight loss (137) and following use of insulin-sensitizing drugs like metformin (138) and troglitazone (139, 140).

No observational studies to date have evaluated the relationship between whole grain intake and PAI-1. A diet high in fiber is associated with reduced PAI-1 after adjusting for anthropometric, lifestyle, and metabolic factors (141). In a randomized, crossover study by Turpeinen et al., 40 healthy subjects (18M, 22F) consumed wholemeal rye bread or low-fiber wheat bread as part of their habitual diet for four weeks (142). There were no significant differences between the two periods in PAI-1. Sundell
et al. reported a 50% decrease in PAI-1 in 11 healthy subjects following supplementation with 10g oat husk/d for two weeks (143). Enriching the diet with guar gum (10g 3x/d for 6 weeks), a soluble fiber, also decreased PAI-1 activity compared with placebo (144). Jarvi et al. compared a 24-day controlled eucaloric low versus high glycemic index diet with identical energy and macronutrient content in 5 women and 15 men, and found that PAI-1 activity decreased 54% on the low GI diet but was unchanged on the high GI diet (145). Possible mechanisms for the reduction in PAI-1 could be via reduced plasma insulin or from a decrease in hepatic synthesis due to lower triglyceride-rich lipoproteins (145).

**Insulin Resistance**

Insulin resistance is a physiological condition where the biological effects of insulin are attenuated (146). There are many metabolic actions that are affected in insulin resistant conditions. Insulin regulates glucose metabolism by inhibiting hepatic glucose production and stimulating glucose uptake, particularly in skeletal muscle. Insulin resistance leads to impaired suppression of endogenous glucose production and to reduced insulin–stimulated glucose uptake after meals. When peripheral tissues become insulin resistant, day-long glucose concentrations do not necessarily rise initially because the pancreas secretes additional insulin, which is known as compensatory hyperinsulinemia. Insulin also regulates lipid metabolism by stimulating lipoprotein lipase activity, reducing hepatic VLDL production, and inhibiting lipolysis and increasing lipogenesis in adipose tissue. Resistance to insulin increases hepatic VLDL production,
and thus circulating serum triglycerides. It also increases lipolysis, resulting in increased flux of non-esterified fatty acids (NEFA) to the liver and skeletal muscle.

A high intake of whole grains is associated with reduced insulin resistance (78, 80, 98) in observational studies. Two randomized controlled clinical trials also report that whole grains improve insulin sensitivity (67, 147). The first study by Pereira et al. was a controlled feeding trial with two 6-week feeding periods (67). The menus in both diets were identical except that in the whole-grain diet, equal quantities of whole-grain items were substituted for refined-grain products. Following the whole grain diet, fasting insulin was 10% lower (P = 0.03) and insulin sensitivity, measured by euglycemic hyperinsulinemic clamp, was significantly greater than after the refined grain diet (67).

In a second study, Jang et al. isocalorically replaced refined rice with whole grain and legume powder as a source of carbohydrate in a meal (147). Patients with coronary artery disease (CAD) were randomly assigned to receive a whole-grain meal or a refined grain meal daily for 16 weeks. In the whole-grain group, serum concentrations of glucose and insulin decreased by 24% and 14%, and the area under the curve for insulin and glucose during an oral glucose tolerance test was significantly reduced in non-diabetic individuals in the whole grain group but not the refined grain group (147). The results of these studies suggest diet high in whole grains may confer benefits in an insulin resistant population.

**LDL Particle Size**

There are at least seven subspecies of LDL-C, ranging from the large, lipid enriched LDL-I to the small lipid depleted LDL-IVB (148); each with a different size,
density, and composition. The amount of small LDL is independent of total LDL-C concentration (149). *In-vitro* studies suggest that small LDL particles are more atherogenic than larger LDL because they have increased oxidative susceptibility, reduced receptor-mediated clearance, increased transport into subendothelial space, and bind tighter to arterial proteoglycans (149). In fact, in most (150-155) but not all (156, 157) studies, a small LDL particle diameter and/or increased concentration of small dense LDL predicted risk of CVD.

The size of LDL particles in humans has a bimodal distribution that can be divided into a buoyant and dense phenotype. A predominance of buoyant, large or medium sized LDL is designated pattern A, and a predominance of small, dense LDL particles is designated pattern B. 85-90% of individuals can be characterized as LDL pattern A or B; the others have an intermediate phenotype (158). The prevalence of LDL pattern B is 30% in adult men, 5-10% in and premenopausal women and 15-25% in postmenopausal women (159-161). In a study of 178 overweight or obese men without hypertension or diabetes, 47% of the cohort and 75% of the men with metabolic syndrome had a pattern B lipid phenotype (149).

There is a strong interrelationship between changes in small, dense LDL with changes in triglyceride and HDL-C levels. Triglycerides are frequently elevated when there is increased adiposity and insulin resistance due to increased hepatic production of VLDL and reduced catabolism and clearance of VLDL and chylomicron particles (149). Triglycerides in VLDL can be transferred to HDL and LDL via cholesterol ester transfer protein (CETP). The triglyceride-enriched LDL and HDL are then processed by lipases, which reduces the size of LDL and HDL particles and increases HDL clearance. Thus, in
many cases LDL particle size does not predict CVD risk independent of triglycerides, HDL-C, and the total:HDL-C ratio (150, 151, 153-155).

The prevalence of LDL pattern B in men and premenopausal women is strongly related to the percent of dietary carbohydrate in the diet (Figure 2.11) (162). Studies that have varied carbohydrate while maintaining either fat or protein content have demonstrated that the LDL response from carbohydrate restriction is mainly due to a change in carbohydrate and not due to a change in dietary fat or protein (163, 164). Krauss et al. also demonstrated that weight loss reduces the expression of a pattern B phenotype regardless of carbohydrate content of the diet (164). This is consistent with findings of other studies that have demonstrated that weight loss increases LDL-PPD (165-167).

**Figure 2.11.** Relationship between the prevalence of LDL pattern B to the percent of dietary fat and carbohydrate in short-term feeding studies (3-6 wks). Shaded circles: men (n=615), open circles: women (n=72). Shaded bars indicate the prevalence of pattern B predicted for individuals consuming diets with average US carbohydrate and fat intakes (162).
Two studies have examined the effect of dietary fiber supplementation on LDL size. Davy et al. randomly assigned 36 overweight men age 50-75 to consume oatmeal or whole wheat cereal providing 14 g dietary fiber/d for 12 weeks. The concentration of small LDL was reduced from 1.77 to 1.47 mmol/L in the oat group, whereas it increased from 1.01 to 1.61 mmol/L in the whole wheat group (p = 0.01) (168). There was a trend towards an increased concentration of large LDL in the oat group compared with the whole wheat group (p = 0.08) that was strongly correlated with changes in triglycerides. The authors hypothesized that these differences could be due to the differing types of fiber (soluble vs. insoluble) in the cereal products. In contrast, Behall et al. found that supplementation with β-glucan from barley for 5 weeks in men and women significantly reduced the concentration of large LDL relative to a Step 1 diet, with no changes in small or medium LDL fractions (169).

Androgens

Reduced testosterone and SHBG levels in men is correlated with several CVD risk factors including an atherogenic lipid profile, hypertension, insulin resistance, increased visceral fat mass, and a proinflammatory state (170-172). In clinical studies, treatment of abdominally-obese men with physiological levels of testosterone significantly improves insulin resistance, and reduces visceral fat volume, total cholesterol and triglycerides, and diastolic blood pressure (173). These observations are supported by animal studies. Rats become severely insulin resistant when castrated, but their insulin sensitivity is restored by physiological testosterone replacement (196).
Likewise, testosterone administration ameliorates atherosclerosis in castrated male rabbits (174, 175).

Long-term observational studies on the relationship between testosterone levels and CAD in humans, however, are inconclusive. Testosterone levels were lower in patients with coronary artery disease (CAD) compared with healthy controls in 16 out of 32 cross sectional studies (172). However, in three prospective cohort studies, there was no correlation between baseline testosterone levels and subsequent development of fatal or nonfatal CAD, stroke, or heart failure after adjusting for potential confounders (176-178).

In men, a low-fat, high-fiber diet significantly reduces plasma testosterone levels in long-term dietary studies (6-10 weeks). Dorgan et al. conducted a controlled feeding study in 43 healthy men aged 19-56 to evaluate the effects of fat and fiber consumption on sex hormones in men (179). Subjects were randomly assigned to a low-fat, high-fiber (19% fat, 61g fiber/day) diet or an isocaloric high-fat, low-fiber diet (41% fat, 26g fiber/day) for 10 weeks in a crossover design. Mean plasma levels of total testosterone were 13% higher after the high-fat, low-fiber diet, compared with the low-fat, high-fiber diet. Similar results were reported in a recent study by Wang et al. in which 39 healthy men age 50-60 were studied while consuming their usual high-fat, low-fiber diet (38% fat, 19g fiber) and after eight weeks on an isocaloric low-fat, high fiber diet (14% fat, 32% fiber) (180). After switching over to the low-fat, high-fiber diet, mean serum total testosterone levels fell 12%. Serum free testosterone levels also were 10% lower on the high-fiber diet than on the low-fiber diet.
The effect of androgens on CVD risk in women is uncertain. As described earlier, women with PCOS have more CVD risk factors than age and BMI matched controls. However, in a 19 year follow-up of 651 postmenopausal women, serum levels of total and bioavailable testosterone did not differ among women with and without a history of CAD at baseline, and did not predict cardiovascular death or death from ischaemic heart disease (181). Likewise, in a 20 year retrospective survey at the Amsterdam Gender Dysphoria Clinic, 293 female to male transsexuals age 17-70 treated with oral testosterone undeconate or intramuscular testosterone every 2 weeks had no excess CVD mortality or morbidity compared with the general population of Dutch women (182). In female rats, testosterone administration reduces whole body insulin sensitivity, which improves when hyperandrogenemia is corrected (197). Treating female ovariectomized monkeys with testosterone for 24 months increased the size of atherosclerotic plaques in the coronary artery compared with untreated controls. However, the coronary artery diameter was enlarged and endothelium dependent acetylcholine vasodilator responses were enhanced, suggesting there may also be cardiovascular benefits (183).

In long-term studies, a low-fat, high-fiber diet is effective in reducing testosterone levels in healthy women. In the Diet and Androgens (DIANA) study, 104 postmenopausal women with high plasma levels of testosterone (T > 38 ng/dL) followed a 4.5 month diet where they [1] increased phytoestrogen consumption by eating more soy products, fruits and vegetables and [2] reduced insulin levels by increasing omega-3 and monounsaturated fatty acids, reducing intake of sugar and refined carbohydrates and increasing unrefined cereals, legumes and vegetables (184). At the end of the study period, plasma testosterone levels decreased 20% and estradiol decreased as well.
Similarly, in a controlled feeding study by Goldin et al., 48 healthy women with normal menses consumed a typical American diet for four weeks followed by an 8-10 week diet that was 20-25% or 40% fat with 12 or 40g of fiber/d (185). At the end of the study, there was a significant decrease in testosterone (up to 12%) and SHBG for individuals on a high-fiber or low-fat diet. The magnitude of decrease was greater when fat and fiber were lowered simultaneously.

**Postprandial Effects of a Meal on Testosterone Levels**

*A Meal Reduces Testosterone Levels In Men*

Several studies in men have reported a postprandial reduction in total and free testosterone, however the effect of diet composition is inconsistent. In a study by Volek et al. in 11 healthy men, total and free testosterone levels declined 22% and 23%, respectively, 1 hour after a fat rich meal (1300 kcal, 11% CHO, 3% PRO, 86% FAT (52g SFA, 59g MUFA, 12g PUFA). Total and free testosterone levels remained significantly below baseline levels for the entire 8-hour postprandial period (186). Meikle et al. found that a fat-containing milk-shake (800 kcal, 34% CHO, 9% PRO, 57% FAT) reduced total and free testosterone levels at 2, 3, and 4 hours after a meal in 8 men, whereas there was no change in these parameters after a nonnutritive carbonated drink and a mixed carbohydrate and protein milk-shake (800 kcal, 73.3% CHO, 25.5% PRO, 1.2% FAT) (187). In contrast, Habito and Ball observed that mean testosterone levels were significantly reduced 15-22% 2 hours after a 400 kcal low-fat tofu and lean meat meal (49% CHO, 29% PRO 20% FAT) in 15 healthy men, but not after an isocaloric
higher-fat meal with animal fat or safflower oil (21% CHO, 24% PRO, 54% FAT) (188). The mechanism of this decrease is unknown.

**A Meal Reduces Testosterone Levels in in Women with PCOS**

Parra et al. studied 13 healthy ovulatory women and 6 women with PCOS to determine the effect of a meal on free testosterone levels and the role of pancreatic insulin secretion (189). On day 1 all women ingested a 725 kcal breakfast (725 kcal, 55% CHO, 14% PRO, 31% FAT) and blood samples were collected every 30 minutes for 3 hours. On day 2, women with PCOS had the breakfast with a simultaneous 90 minute IV infusion of epinephrine (6ug/min) and propranolol (80ug/min). Epinephrine stimulates the alpha adrenergic system, inhibiting pancreatic insulin release, which is potentiated by the beta blocker propranolol. Free testosterone levels rose in healthy women (4.7 ± 0.6 pmol/L to 6.6 ± 0.6 pmol/L at 30 minutes) whereas it was reduced in women with PCOS. In five of the six women with PCOS, free testosterone decreased an average of 62% at 90 minutes. However, in the sixth woman there was a steady 80% rise. On day 2, glucose increased and insulin was 56-84% lower than day 1 in the first 90 minutes. However serum free testosterone levels still declined progressively (24-65% from fasting) in all women.

In the discussion of this paper, the authors mention that the increase in androgens in healthy women was unexpected, as other studies report a decrease in serum androgens following an oral glucose load in normal ovulatory women (190-192). They also discuss that the lack of relationship between serum insulin and androgen concentrations is
consistent with other studies that do not show a rise in androgens following oral IV 
glucose-induced hyperinsulinemia or during exogenous insulin infusion (191, 193-195).

Research Goals

My thesis research seeks to (1) determine the effects of a hypocaloric diet high in 
whole grains on weight loss and changes in CVD risk factors in men and women with 
metabolic syndrome, and (2) to evaluate the effects of a low-fat, high-fiber meal and a 
high-fat, low-fiber meal on postprandial testosterone levels in women with PCOS.

As described above, an increased intake of whole grain foods is associated with a lower body weight and reduced incidence of CVD in observational studies. However, 
there is limited research from clinical trials on the effect of whole grain foods on weight loss and subsequent changes in CVD risk factors. Since overweight and obesity affects 65% of adults in the United States (198), determining dietary components that promote weight loss could positively impact public health by reducing incidence of chronic diseases associated with increased body weight including type 2 diabetes and cardiovascular disease.

The aim of my first study was to determine whether including whole grain foods into a hypocaloric diet results in a greater reduction in body weight and improvement in CVD risk factors compared with a diet containing only refined grains. Since whole grains are a good source of fiber and other cardioprotective compounds, my hypothesis was that consuming whole grain foods would enhance weight loss and improve CVD risk factors compared with refined grains.
The primary outcome of this study was weight loss since weight loss is associated with improvements in all aspects of metabolic syndrome. The secondary outcomes were changes in lipids, markers of inflammation and fibrinolysis, blood pressure, waist circumference, androgens, and glucose tolerance - all of which are associated with increased CVD risk. According to a recent estimate, metabolic syndrome is present in approximately 27% of the U.S. population and brings with it a two-fold increased risk of cardiovascular disease and three-fold increased risk of type II diabetes. Thus, this study may offer evidence of an effective dietary treatment for a condition that is highly prevalent.

As described above, women with PCOS tend to have greater insulin resistance and elevated levels of CVD risk factors compared with age and BMI matched controls. In women with PCOS, diet is an effective means of reducing testosterone levels when accompanied by weight loss of at least five percent of body weight. In light of this, there is current interest in whether a particular diet composition is most effective in lowering testosterone levels.

The aim of my second study was to determine the effect of varying the fat and fiber content of meals on postprandial testosterone levels. I compared the effects of a low-fat, high-fiber meal versus a high-fat, Western meal in 15 women with PCOS. The primary outcome was testosterone concentration since lowering testosterone levels is associated with improved menstrual cyclicity and fertility in this population.

The two clinical trials that I have completed provide new and important information on the effects of whole grains and high-fiber foods on reproductive and metabolic aspects of metabolic syndrome and PCOS. I anticipate that the information
gained from these studies will provide the basis for future research on the role of diet composition in the treatment of insulin resistant conditions including PCOS and metabolic syndrome. Ultimately, the knowledge gained from these and future studies may contribute to the development of dietary guidelines for treatment of insulin resistant conditions and prevention of the costly co-morbidities associated with them.
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CHAPTER 3

THE EFFECTS OF A WHOLE GRAIN ENRICHED HYPOCALORIC DIET ON CARDIOVASCULAR DISEASE RISK FACTORS IN MEN AND WOMEN WITH METABOLIC SYNDROME

Introduction

In 2004, the Centers for Disease Control and Prevention (CDC) estimated that as many as 64 million Americans, or 27% of the United States population, has metabolic syndrome (1). Metabolic syndrome is characterized by a cluster of cardiovascular disease (CVD) risk factors including abdominal obesity, elevated blood glucose, dyslipidemia, and high blood pressure, and carries an increased risk of type II diabetes and CVD (2-4). Lifestyle changes such as diet, weight loss, and exercise are the first line of treatment for metabolic syndrome (5), however the optimal diet composition is debated (6).

Whole grain foods are recommended for prevention of CVD because they contain many cardioprotective compounds including dietary fiber, trace minerals, phytoestrogens, and antioxidants (7). In observational studies, an increased intake of whole grain foods is associated with decreased development of metabolic syndrome (8) and mortality from CVD (9). Increased whole grain consumption also is inversely related to CVD risk factors including body weight, abdominal obesity, and insulin resistance (10-12). Based on evidence that dietary patterns high in whole-grain products and fiber are associated with increased diet quality and decreased risk of CVD, the American Heart Association
now recommends that at least half of grain intake come from whole grains (13). Similar recommendations are made in the 2005 Dietary Guidelines for Americans and by the American Diabetes Association (14, 15).

Although numerous groups have made recommendations to increase whole grain intake, there is limited information from randomized, clinical trials on whether including whole grains in a hypocaloric diet increases weight loss. Whole grains are believed to have a beneficial effect on body weight because they are usually less energy dense and more satiating than refined grain foods (10). However, a recent study by Melanson et al. reported similar reductions in body weight in overweight and obese men and women following a 24-week diet with and without whole grain cereals (16). Changes in other cardiovascular risk factors were not examined.

The aim of the present study was to determine if including whole grain foods into a hypocaloric diet enhances weight loss and improves CVD risk factors. I hypothesized that there would be a greater reduction in body weight and improvement in CVD risk factors in individuals consuming whole grains. The free-living design of this study better predicts if a diet high in whole grains will reduce CVD risk in the general population.

Methods

Participants

Fifty obese men and women (25M, 25F) with metabolic syndrome age 20 to 65 were recruited to participate. Men and women were eligible if they had a body mass index (BMI) $\geq 30$ kg/m$^2$ and at least three out of five National Cholesterol Education Program Adult Treatment Panel III criteria for metabolic syndrome (17). These criteria
were defined as: [1] triacylglycerol $\geq 150$ mg/dL, [2] HDL-C $< 40$ mg/dL in men or $< 50$ mg/dL in women, [3] fasting glucose $\geq 100$ mg/dL, [4] systolic blood pressure $\geq 130$ mmHg and/or diastolic blood pressure $\geq 85$ mg/dL, and [5] waist circumference $\geq 102$ cm in men or $\geq 88$ cm in women. Participants were excluded if they had been diagnosed with type I or II diabetes, CVD, cancer, any other serious medical condition, or were using any medications known to affect glucose, insulin, cholesterol, or reproductive hormones. Individuals were also excluded who smoked, drank more than 2 alcoholic beverages/d, consumed a diet high in whole grains ($>3$ servings/d), or were pregnant or lactating. The study was conducted in accordance with the guidelines of The Pennsylvania State University Institutional Review Board, and all participants gave written informed consent.

**Study Design**

I used a randomized, open-label, parallel-arm study design where participants received dietary advice to either avoid whole grain foods (i.e., the refined grain group) or to have all of their grain servings each day from whole grain foods (i.e., the whole grain group) for 12 weeks. Participants were assigned to either a whole grain or refined grain hypocaloric diet using a stratified randomization scheme. The stratification factors used in the randomization were gender (male, female) and BMI status (BMI $< 40$ kg/m$^2$, BMI $\geq 40$ kg/m$^2$). The study opened to accrual in September of 2005 and completed enrollment in August of 2006.

A registered dietitian met individually with each participant at baseline to discuss the dietary intervention and provided educational materials to facilitate understanding and
adherence. Participants in the whole grain group were given a target number of daily whole grain servings, either 4, 5, 6, or 7 servings/d, based on the number of grain servings recommended in the 2005 Dietary Guidelines for Americans for their energy needs (15). Energy needs were calculated using the Mifflin equation (18) with an activity factor of 1.3, minus 500 to account for the calorie deficit during weight loss. Participants in the whole grain group were given a list and description of whole grain foods to help them identify foods to include in their diet and were encouraged to select foods that had a whole grain food listed as the first ingredient. To ease the transition, participants in the whole grain group were advised to consume three servings of whole grain foods for the first two weeks of the study and then increase to their target number of daily whole grain servings for the remaining ten weeks. Participants in the refined grain group also were given a list of whole grain foods and asked not to consume any of these foods during the study period.

In addition to the instruction on whole grain servings, participants in both groups were asked to eat five servings of fruit and vegetables, three servings of low-fat dairy products, and two servings of lean meat, fish or poultry/d, as recommended in the 2005 Dietary Guidelines for Americans. The target macronutrient composition for all participants was 55% carbohydrate, 30% fat, with emphasis on unsaturated fats, and 15% protein. All participants were encouraged to engage in moderate physical activity at least three times a week for 30 minutes per session and were instructed to avoid dietary supplements throughout the study period. Participants in both groups were told that their aim was to lose at least 1 pound/wk for the duration of the study.
To increase compliance, participants were instructed to keep track of their daily food intake and exercise in a diary log that was provided, and to record their weight at home weekly. Every other week, participants visited the study site and reviewed their diet records with a dietitian on a one-on-one basis. During this time, the dietitian presented an educational lesson that explained the rationale for the dietary guidelines used in the study, and offered nutritional guidance, encouragement, and suggestions for improvement. The participant’s weight, blood pressure, and waist circumference were also recorded. At the end of each visit, participants selected two foods to take home containing either refined grains or whole grains, depending on their randomized assignment. On the weeks that participants did not come in for a study visit, they were contacted by phone or e-mail by a dietitian to discuss their progress and address any concerns or questions. A fasting blood draw, 2-hour oral glucose tolerance test (OGTT), dual energy x-ray absorptiometry (DXA) scan, and biometric measurements were done at the beginning and end of the 12-week diet period at The Pennsylvania State University General Clinical Research Center (GCRC) at University Park, PA.

**Long-term Follow-up**

Each participant will be contacted at 9 months following completion of the study and asked to come to the GCRC for a follow-up visit. As part of the follow-up, participants are asked to complete a 3-day diet recall, diet satisfaction questionnaire, and physical activity questionnaire and come to the GCRC for a fasting blood draw and measurement of their weight, blood pressure, waist circumference, and body fat percentage. At the end of the study period, all participants were encouraged to continue
their dietary regimen and exercise routine. However, participants in the refined grain group were told that they can eat whole grain foods during the follow-up period.

**Dietary Assessment**

At baseline and every 4 weeks, participants kept a detailed, 3-day diet record. A dietitian reviewed the 3-day diet record with each participant and shared the diet analysis with him or her at their next bi-weekly visit. Diet records were analyzed using Nutrition Data System (NDS) for Research Software (Version 2005, Nutrition Coordinating Center, University of Minnesota, Minneapolis) by a dietitian trained in using NDS. If a whole grain food was not listed in the NDS database, an appropriate whole grain substitution with a similar macronutrient and dietary fiber content was selected. Grain servings were defined as per the 2005 Dietary Guidelines for Americans as 1 slice of bread, 1 oz. of ready-to-eat cereal, and ½ cup of cooked cereal, rice or pasta. A grain product was identified as a whole grain if a whole grain was listed as the first ingredient on the food label. A food was categorized as some whole grain if a whole grain appeared anywhere else on the food label. Grain products that contained no whole grain ingredients were identified as a refined grain.

A validated diet satisfaction questionnaire was administered at baseline and at the end of the 12-week study period (19). This 45-item questionnaire evaluated seven issues that affect diet satisfaction including ease of meal planning and preparation, convenience, cost, impact on family dynamics, sense of having a healthy lifestyle, presence of negative feelings such as deprivation or embarrassment, and preoccupation with food. The available responses to questions were arranged on a 5-point Likert scale from 1 =
“strongly disagree” to 5 = “strongly agree”. A score for each of the seven sections as well as a global score for overall diet satisfaction was calculated by averaging the responses to the questions in the respective sections.

Clinical Measurements

Waist circumference was measured according to guidelines from the National Heart, Lung, and Blood Institute (NHLBI) (20). Weight was measured in light clothing without shoes using an electronic Detecto CN20 scale. Blood pressure was measured using an Omron automatic blood pressure monitor. Participants remained comfortably seated with legs uncrossed for at least five minutes before blood pressure was measured. Three blood pressure measurements were taken at least one minute apart and the second and third readings were averaged. A DXA scan was performed at baseline and at the end of the study to assess body composition and total body bone mineral density using a Hologic QDR-4500W (Hologic Corp., Waltham, MA). Each participant underwent a total-body scan at baseline and at the end of the study using fan-beam mode. Proper operation of the x-ray subsystem was verified daily using a spine phantom and tissue composition calibration was performed once/wk on a tissue equivalent phantom.

Blood samples were collected in the morning after a 12-hour fast. A nurse inserted an IV catheter into a vein, and a fasting blood sample was drawn into 10mL syringes and transferred into Vacutainer tubes (VWR Scientific Products, West Chester, PA). An OGTT was then performed with a 75 g oral glucose challenge. Blood samples were taken at 30-minute intervals for two hours for measurement of glucose and insulin.
Serum and plasma were separated by centrifugation for 15 minutes at 1,465 \times g (3,200 rpm) and 4°C and aliquoted into 0.5 – 2mL cryovials and stored at −80°C until analyzed.

**Biochemical Analysis**

Glucose was measured by the glucose oxidase technique using a Yellow Springs Instruments 2300 Glucose Analyzer. Insulin was measured by radioimmunoassay (Linco, St. Charles, MO) (21). Total cholesterol, HDL-C, and triacylglycerol were measured by Quest Diagnostics using an automated chemistry analyzer (Olympus AU-5400). LDL-C concentrations were calculated using the Friedewald equation (22). Measurements of mass concentrations of LDL-C subfractions were performed by analytic ultracentrifugation as described elsewhere (23). Nondenaturing polyacrylamide gradient gel electrophoresis with lipid staining of plasma was performed as described previously for determination of peak LDL particle diameter (24).

IL-1β, IL-6, TNF-α, and hs-CRP were measured by Enzyme Linked Immunoassays (ELISAs) developed by the Cytokine Core Laboratory of the Pennsylvania State University GCRC (25). PAI-1 was measured using the Zymutest PAI-1 Antigen ELISA (Hyphen BioMed, France). Apolipoproteins A-I (apo A-I) and B (apo B) were measured by immunoturbidometric assays (24). All of the immunoassays used in these studies had inter-assay CV’s of less than 10% at the median level.

**Statistical Analyses**

The study was designed to detect a 2 kg difference in weight loss at the end of the 12 week trial between the two hypocaloric diet groups. I assumed a dropout rate of 20%
and a common standard deviation of 1.8 kg resulting in a standardized effect size of 2/1.8=1.1. On the basis of these assumptions, I needed to enroll 50 participants for the study to have a power of 92% for a two-sided test with a type I error rate of 0.05 to detect a 2 kg difference between the two hypocaloric diets at the end of the 12 week trial. Area under the glucose and insulin curves during the OGTT were calculated using the trapezoidal rule (21). An insulin sensitivity index (ISI) was calculated according to the method of Matsuda and DeFronzo (26).

Two-sample t-tests were used to test for differences between the diet groups in subjects’ characteristics measured at baseline. Linear mixed-effects models were fit to assess the changes in weight, biometric, biochemical, dietary intake, and diet satisfaction parameters within and between the two hypocaloric diet groups over the course of the 12 week study (27). The linear mixed-effects model is an extension of the traditional analysis of variance model that accounts for the within- and between-subject correlation inherent in longitudinal trials. Furthermore, the model allows adjustment for the baseline value of the outcome of interest, which was done when assessing changes in weight, biometric and biochemical parameters. If necessary to meet modeling assumptions such as normality, the outcome variable was transformed. The degrees of freedom for the mixed-effects models were adjusted using the method of Kenward and Roger (28). P-values and 95% confidence intervals were adjusted using Bonferroni’s procedure to account for multiple testing within each outcome assessed. The linear relationship between the change (postdiet–prediet) in any two continuous outcomes was quantified using Pearson's correlation coefficient. Data were analyzed following the intention to
treat principle and all hypotheses tests were two-sided. All analyses were performed using SAS software (version 9.1; SAS Institute Inc., Cary, NC).

Results

Twenty-five men and 25 women age 24 to 63 yrs were randomized into the whole grain (12M, 13F) and refined grain (13M, 12F) groups (Figure 3.1). The baseline characteristics of the study participants are listed in Table 3.1. Systolic blood pressure and the percentage of the LDL-III subclass were significantly higher in the refined grain group at baseline ($P = 0.03$ for both), but there were no other significant differences at baseline between the diet groups. Forty-eight participants classified themselves as White, one as African American, and one as Hispanic. Forty-seven of the 50 participants (94%) completed the study. One male and one female in the refined grain group withdrew due to a change in job schedule and for family reasons, respectively. One female in the whole grain group withdrew due to an inability to adhere to the diet.

Participants in the whole grain group increased their intake of whole grain foods to approximately 5 servings/d whereas participants in the refined grain group decreased their intake to less than 0.2 servings/d (Figure 3.2). The primary source of whole grain foods for participants in the whole grain group was bread and rolls (2-2.5 servings/d) with other sources from ready-to-eat cereal, brown rice, oatmeal, pasta, salty snacks (crackers, snack chips, popcorn), and snack bars (each 0.5-1 serving/d) (Figure 3.3). Participants in the whole grain group also significantly reduced their intake of refined grains to 1.5 servings or less per day ($P <0.001$), whereas participants in the refined grain
group maintained their intake of refined grain foods at approximately 5 servings per day (Table 3.2).

Body weight decreased -3.7 kg or -3.6% in the whole grain group \( (P < 0.001) \) and -5.2 kg or -4.9% in the refined grain group \( (P < 0.001) \) (Figure 3.4). There were no significant differences in weight loss between groups at any time point.

Mean CRP concentration decreased 38% in the whole grain group, but there was no change in mean CRP concentration in the refined grain group (Figure 3.5). CRP concentration was decreased in 18/24 (75%) of the participants who completed the study in the whole grain group compared with 12/23 (52%) of those who completed the study in the refined grain group. When comparing only participants that had a reduction in CRP, the average percent decrease in CRP was 45% in the whole grain group and 26% in the refined grain group \( (P < 0.01) \) (Figure 3.6). Although CRP was correlated with BMI at baseline \( (r = 0.46, P < 0.001) \), the change in CRP concentration did not correlate with weight loss \( (r = -0.07, P = 0.66) \). There were no significant differences in the percent change of lipids, lipoproteins, and glucose and insulin measures between subjects categorized having as a high or low CRP level based on a median split (Figure 3.7). There were no significant differences between groups in changes in the concentrations of IL-1, IL-6 or TNF-\( \alpha \). However, 32-53% of the samples were below the level of detection for these assays.

Changes in biometric measurements, lipids and lipoproteins, glucose and insulin measures, and PAI-1 are listed in Table 3.3. Waist circumference and body fat percentages were decreased in both groups compared with baseline \( (P < 0.02) \). However, there was a trend towards a greater decrease in percent fat in the abdominal region in the
whole grain group ($P = 0.08$) (Table 3.6). Systolic blood pressure decreased in the refined grain group compared with baseline ($P = 0.003$) but not the whole grain group. The area under the curve for insulin after the OGTT decreased 10% in the whole grain group compared with 2% in the refined grain group (n.s.). There were no significant differences between groups in changes in waist circumference, body fat percentage, or blood pressure. The frequency of the metabolic syndrome criteria did not significantly change within or between diet groups.

Total, LDL, and HDL cholesterol were significantly decreased from baseline in the whole grain group ($P < 0.05$) but not in the refined grain group. Apo A-I and PAI-1 were decreased compared with baseline in the refined grain group only ($P = 0.03$ and $P = 0.01$, respectively). The changes in Apo A-I and PAI-1 were correlated with weight loss ($r = 0.54$, $P < 0.001$ and $r = 0.43$, $P = 0.003$). There were no significant differences between groups in changes in triacylglycerol, total, LDL, or HDL cholesterol, LDL subclasses, or PAI-1. There were also no significant differences within or between groups in glucose or insulin concentrations during the OGTT at any time point (Figure 3.8).

Analyses of energy and nutrient intake from 3-day recalls administered at baseline, and week 4, 8, and 12 are shown in Table 3.4. Energy intake decreased from baseline in the refined grain group at weeks 4, 8, and 12 ($P < 0.001$), but the reduction in energy intake was not significant in the whole grain group. Carbohydrate intake increased in the whole grain group at weeks 4 and 8 ($P < 0.05$) and protein intake increased in the refined grain group at weeks 4 and 12 compared with baseline ($P < 0.04$). Participants in the whole grain group decreased their intake of total and saturated fat by
18% and 25%, respectively compared with baseline \((P < 0.02)\) and increased their intake of total, insoluble, and soluble fiber by 50%, 47%, and 52%, respectively \((P < 0.001)\). Magnesium intake was higher in the whole grain group throughout the study period compared with the refined grain group \((P < 0.001)\). Sodium and added sugar intake were decreased in the refined grain group at most time points compared with baseline \((P < 0.02)\), but there were no significant changes in these measures in the whole grain group.

Ratings of diet satisfaction at baseline and week 12 are listed in Table 3.5. At week 12, participants in both groups had a greater overall satisfaction with their diet compared with baseline \((P < 0.001)\), rated a greater sense of having a healthy lifestyle \((P < 0.001)\), and considered their families to be more approving of their diet \((P < 0.001)\). At the end of the study, participants in the refined grain group had a lower preoccupation with food compared with baseline \((P = 0.002)\) and participants in the whole grain group rated their meal planning and preparation as more difficult \((P = 0.006)\). There were no significant differences within or between groups in ratings of cost of the diets or presence of negative feelings compared with baseline.

**Discussion**

Since obesity and CVD are prevalent health problems worldwide, researchers have been interested in the effectiveness of different dietary patterns to decrease body weight and improve other CVD risk factors. I hypothesized that individuals who included whole grains into their diet would have a greater reduction in body weight and improvement in CVD risk factors. However, I observed a similar degree of weight loss in men and women with metabolic syndrome consuming whole grains versus refined
grains. Despite similar weight loss, there was a greater reduction in CRP, an important predictor of cardiovascular events, in the whole grain group compared with the refined grain group. Participants in the whole grain group also increased their intake of dietary fiber and magnesium and decreased their intake of saturated fat compared with baseline.

Whole grain foods are thought to have a beneficial effect on body weight due to their increased fiber content, which decreases energy intake and body weight when supplemented in clinical trials (29). The finding of equivalent weight loss when whole grains are incorporated into a hypocaloric diet is in agreement with a recent study by Melanson et al. that reported a similar degree of weight loss in overweight and obese men and women on 6-month weight-management programs with and without whole grain cereals (16). In this study, body weight decreased in the hypocaloric diet groups with and without whole grain cereals by 5.6kg and 6.2kg, respectively, but weight loss between the two groups did not differ significantly. Since participants in our study and the study by Melanson et al. were given additional advice to achieve weight loss (i.e. increase intake of fruits and vegetables, decrease fat intake, and decrease portion sizes) beyond eating whole grain foods, the results of neither study can establish a direct cause-effect relationship between whole grain intake and weight loss. A weight loss study manipulating only the source of grains would be necessary to conclusively establish whether whole grains affect weight loss. Our results do indicate that people can lose weight on a whole grain enriched diet, similar to a conventional hypocaloric diet with refined grains, while benefiting from a greater reduction in CRP.

CRP is an emerging CVD risk factor and independent predictor of cardiovascular events in individuals with and without CVD (30). Although changes in CRP correlate
with weight loss in clinical trials (31), individuals on a whole-grain enriched diet in our study lowered their CRP concentration in a manner that did not correlate with weight loss. The magnitude of reduction in CRP in the whole grain group was similar to that seen with statins (32-34). The finding of decreased CRP independent of weight loss is consistent with a study by Esposito et al. who compared a 2-year Mediterranean diet high in whole grains, fruits, vegetables, nuts and olive oil, with a prudent diet (50-60% carbohydrate, 15-20% protein, <30% fat) in 180 men and women with metabolic syndrome. In this study, CRP concentration decreased in the Mediterranean diet group independent of weight loss, whereas there was no change in CRP concentration in men and women who consumed a prudent diet. Although this study demonstrated a benefit of a Mediterranean diet on CRP, the specific impact of whole grain foods was not studied. Some (35, 36) but not all (37) clinical trials have reported that supplementation with dietary fiber does not affect CRP concentration, suggesting that the reduction in CRP in participants consuming whole grains may be due to other components intrinsic to whole grains.

At the end of the study period, there were no significant differences between groups in changes in biometric outcomes, triacylglycerol, total, LDL, and HDL cholesterol, and glucose and insulin measures. However, there were improvements in many of these parameters with weight loss, which is in agreement with other studies (38, 39). In the whole grain group, total and LDL cholesterol decreased compared with baseline, which is in line with the ATP III guidelines for management of metabolic syndrome and high blood cholesterol (17, 40). HDL-C was also decreased in participants in the whole grain group, an outcome often observed with weight loss. Based on
previous findings, it is likely that HDL-C will increase if the reduction in body weight is maintained (41).

To assess dietary intake throughout the study period, participants completed a 3-day recall at baseline and every 4 weeks. Energy intake was significantly decreased from baseline in the refined grain group, but the reduction in energy intake was not significant in the whole grain group. This could be due to participants in the whole group increasing their caloric intake to get in their recommended number of whole grain servings, or from eating refined grains in addition to the recommended number of whole grain servings. When this study began, a greater emphasis was placed on participants in the whole grain group consuming all of their whole grain servings, rather then on avoiding refined grains. The first 9 participants who completed the study in the whole grain group averaged a -7 kcal deficit from baseline and -1.0 kg weight loss. After the first cohort of participants completed the study, a greater emphasis was placed on participants avoiding refined grains and consuming only whole grain foods. The subsequent 15 individuals who completed the study in the whole grain group averaged -430 kcal from baseline and -5.3 kg weight loss. Thus, a lower calorie deficit in the first cohort of participants may account for the non-significant caloric reduction in the whole grain group.

Participants in the whole grain group increased their intake of dietary fiber to near 14g per 1000 kcal, which is the amount recommended in the Dietary Guidelines for Americans (15) and is associated with the lowest risk of coronary heart disease (42). Participants in the whole grain group significantly increased their intake of magnesium, which improves insulin action and glucose metabolism in type 2 diabetic patients (43-46), and decreased their intake of total and saturated fat. Participants in the refined grain
group but not the whole grain group significantly decreased their intake of added sugar and sodium. However, this appears to be due to a higher intake of these components at baseline since participants in the whole grain and refined grain groups had a similar intake of added sugar and sodium at weeks 4, 8, and 12. Overall, the increased consumption of whole grain foods in this study was associated with an improvement in dietary quality by increasing the intake of dietary fiber and magnesium and reducing the intake of saturated fat.

This study had a high completion and compliance rate, suggesting that both diets were well tolerated. Forty-seven out of 50 participants completed the study, and these 47 participants came to 99% of their study visits. In support of this, ratings of overall diet satisfaction, sense of having a healthy lifestyle, and family dynamics increased in participants in both treatment groups. Participants in the whole grain group, but not the refined grain group, rated that their meal planning and preparation was more difficult than at baseline, which indicates that convenience may be a limitation to incorporating whole grains into the diet. There were no differences within or between groups in ratings of cost, suggesting that cost is not a limiting factor to including whole grains in the diet.

A strength of this study is that it was conducted in a free-living population with metabolic syndrome, so that the results easily translate to people at risk for CVD who want to include whole grains into their diet with the goal of losing weight. A limitation of this, however, is that other behavioral changes in exercise and diet may account for the effects that I observed. This study also had a small sample size and duration, which gave little power to detecting differences between groups in secondary outcomes. However, given that a wide range of whole grain foods are now available to consumers, the results
of this study are timely as they demonstrate that a diet high in whole grains can improve CVD risk factors. Future studies examining larger cohorts for longer periods are necessary to determine the long-term health benefits of whole grains.
Table 3.1. Characteristics of the study participants at baseline.

<table>
<thead>
<tr>
<th></th>
<th>Whole grain group (n=25)</th>
<th>Refined grain group (n=25)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Biometric</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Age (y)</td>
<td>45.4 ± 8.0</td>
<td>46.6 ± 9.7</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>103.1 ± 13.5</td>
<td>106.2 ± 16.0</td>
</tr>
<tr>
<td>BMI (kg/m$^2$)</td>
<td>35.5 ± 4.1</td>
<td>36.1 ± 4.9</td>
</tr>
<tr>
<td>Systolic BP (mmHg)</td>
<td>123.0 ± 9.4</td>
<td>130.3 ± 13.3$^2$</td>
</tr>
<tr>
<td>Diastolic BP (mmHg)</td>
<td>82.0 ± 7.5</td>
<td>83.2 ± 8.3</td>
</tr>
<tr>
<td>Waist circumference (cm)</td>
<td>116.6 ± 11.8</td>
<td>118.2 ± 10.3</td>
</tr>
<tr>
<td>Body fat (%)$^3$</td>
<td>36.9 ± 7.5</td>
<td>37.3 ± 7.5</td>
</tr>
<tr>
<td><strong>Serum lipids</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total cholesterol (mg/dL)</td>
<td>189.8 ± 47.0</td>
<td>187.9 ± 24.7</td>
</tr>
<tr>
<td>LDL-C (mg/dL)</td>
<td>119.1 ± 39.3</td>
<td>114.6 ± 19.8</td>
</tr>
<tr>
<td>LDL PPD (Å)</td>
<td>262.1 ± 7.4</td>
<td>259.1 ± 8.0</td>
</tr>
<tr>
<td>LDL-I (%)</td>
<td>17.0 ± 5.0</td>
<td>15.0 ± 6.9</td>
</tr>
<tr>
<td>LDL-II (%)</td>
<td>43.2 ± 10.5</td>
<td>38.8 ± 10.8</td>
</tr>
<tr>
<td>LDL-III (%)</td>
<td>18.2 ± 11.2</td>
<td>26.0 ± 12.8$^2$</td>
</tr>
<tr>
<td>LDL-IV (%)</td>
<td>6.6 ± 2.5</td>
<td>7.1 ± 2.7</td>
</tr>
<tr>
<td>HDL-C (mg/dL)</td>
<td>41.4 ± 9.0</td>
<td>40.8 ± 7.7</td>
</tr>
<tr>
<td>Triacylglycerol (mg/dL)</td>
<td>146.1 ± 63.1</td>
<td>162.4 ± 65.3</td>
</tr>
<tr>
<td>Total/HDL cholesterol ratio</td>
<td>4.7 ± 1.4</td>
<td>4.8 ± 1.0</td>
</tr>
<tr>
<td>Apolipoprotein A-I (mg/dL)</td>
<td>119.0 ± 17.5</td>
<td>122.8 ± 19.4</td>
</tr>
<tr>
<td>Apolipoprotein B (mg/dL)</td>
<td>83.9 ± 19.9</td>
<td>85.1 ± 14.3</td>
</tr>
<tr>
<td><strong>Glucose parameters</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fasting glucose (mg/dL)</td>
<td>96.0 ± 7.5</td>
<td>95.6 ± 5.5</td>
</tr>
<tr>
<td>Fasting insulin (µU/mL)</td>
<td>15.0 ± 7.6</td>
<td>13.8 ± 6.5</td>
</tr>
<tr>
<td>2-h glucose (OGTT) (mg/dL)</td>
<td>137.4 ± 37.5</td>
<td>137.0 ± 38.2</td>
</tr>
<tr>
<td>2-h insulin (OGTT) (µU/mL)</td>
<td>97.2 ± 78.9</td>
<td>85.3 ± 53.9</td>
</tr>
<tr>
<td>AUC glucose (OGTT)</td>
<td>18172 ± 3287</td>
<td>18130 ± 2527</td>
</tr>
<tr>
<td>AUC insulin (OGTT)</td>
<td>11289 ± 7965</td>
<td>10873 ± 7250</td>
</tr>
<tr>
<td>ISI</td>
<td>2.8 ± 1.5</td>
<td>2.75 ± 1.2</td>
</tr>
<tr>
<td><strong>Inflammation and fibrinolysis</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CRP (mg/L)</td>
<td>6.0 ± 8.0</td>
<td>5.9 ± 6.0</td>
</tr>
<tr>
<td>PAI-1 (ng/mL)</td>
<td>15.3 ± 9.7</td>
<td>18.9 ± 11.8</td>
</tr>
</tbody>
</table>

$^1$ All values are mean ± SD. BP, blood pressure; PPD, peak particle diameter; OGTT, oral glucose tolerance test; AUC, area under the curve; ISI, insulin sensitivity index; CRP, C reactive protein; PAI-1, plasminogen activator inhibitor-1.

$^2$ Significantly different between groups, $P<0.05$.

$^3$ Measured by DXA scan.
Table 3.2. Daily grain intake at baseline and weeks 4, 8, and 12 from 3-day food records of the study participants.

<table>
<thead>
<tr>
<th></th>
<th>Group</th>
<th>Baseline</th>
<th>Week 4</th>
<th>Week 8</th>
<th>Week 12</th>
</tr>
</thead>
<tbody>
<tr>
<td># Whole Grain Servings</td>
<td>WG</td>
<td>1.44 ± 0.3</td>
<td>5.12 ± 0.3</td>
<td>5.00 ± 0.3</td>
<td>5.00 ± 0.3</td>
</tr>
<tr>
<td></td>
<td>CO</td>
<td>1.40 ± 0.3</td>
<td>0.14 ± 0.3</td>
<td>0.11 ± 0.3</td>
<td>0.02 ± 0.3</td>
</tr>
<tr>
<td># Some Whole Grain Servings</td>
<td>WG</td>
<td>0.57 ± 0.2</td>
<td>0.75 ± 0.2</td>
<td>0.56 ± 0.2</td>
<td>0.70 ± 0.2</td>
</tr>
<tr>
<td></td>
<td>CO</td>
<td>0.81 ± 0.2</td>
<td>0.55 ± 0.2</td>
<td>0.61 ± 0.2</td>
<td>0.65 ± 0.2</td>
</tr>
<tr>
<td># Refined Grain Servings</td>
<td>WG</td>
<td>4.78 ± 0.4</td>
<td>1.41 ± 0.5</td>
<td>1.50 ± 0.4</td>
<td>1.40 ± 0.5</td>
</tr>
<tr>
<td></td>
<td>CO</td>
<td>5.96 ± 0.4</td>
<td>4.90 ± 0.4</td>
<td>4.29 ± 0.5</td>
<td>4.82 ± 0.5</td>
</tr>
</tbody>
</table>

1 All values are least squares mean (95% CI).
2 WG, whole grain group; RG, refined grain group.
3 Significantly different from baseline, P<0.05.
4 Significantly different from refined grain group, P<0.05.
Table 3.3. Changes in values of assessed variables at baseline and at the end of the 12-week diet period in participants in the whole grain and refined grain groups.

<table>
<thead>
<tr>
<th>Measurement</th>
<th>Whole grain Δ from baseline (n = 24)</th>
<th>Refined grain Δ from baseline (n = 23)</th>
<th>Difference between groups</th>
<th>P²</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Biometric</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>-3.7 (-5.2, -2.2)</td>
<td>-5.2 (-6.7, -3.7)</td>
<td>-1.5 (-3.6, 0.6)</td>
<td>0.28</td>
</tr>
<tr>
<td>Systolic BP (mmHg)</td>
<td>-3.1 (-7.5, 1.3)</td>
<td>-6.2 (-10.7, -1.8)</td>
<td>-3.1 (-9.4, 3.2)</td>
<td>0.69</td>
</tr>
<tr>
<td>Diastolic BP (mmHg)</td>
<td>-2.6 (-5.8, 0.6)</td>
<td>-3.0 (-6.3, 0.3)</td>
<td>-0.4 (-5.0, 4.2)</td>
<td>1.00</td>
</tr>
<tr>
<td>Waist circumference (cm)</td>
<td>-2.6 (-4.7, -0.4)</td>
<td>-4.4 (-6.5, -2.2)</td>
<td>-1.8 (-4.8, 1.2)</td>
<td>0.44</td>
</tr>
<tr>
<td>Body fat (%)</td>
<td>-1.2 (-2.0, -0.5)</td>
<td>-1.0 (-1.8, -0.2)</td>
<td>0.2 (-0.9, 1.3)</td>
<td>1.00</td>
</tr>
<tr>
<td>Total cholesterol (mg/dL)</td>
<td>-10.8 (-19.9, -1.7)</td>
<td>-5.8 (-15.1, 3.5)</td>
<td>5.0 (-8.0, 18.0)</td>
<td>1.00</td>
</tr>
<tr>
<td><strong>Serum lipids</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>LDL-C (mg/dL)</td>
<td>-8.1 (-16.0, -0.1)</td>
<td>-3.5 (-11.6, 4.7)</td>
<td>4.6 (-6.8, 16.0)</td>
<td>0.97</td>
</tr>
<tr>
<td>LDL PPD (Å)</td>
<td>0.7 (-1.9, 3.3)</td>
<td>1.5 (-1.1, 4.2)</td>
<td>0.8 (-2.9, 4.5)</td>
<td>1.00</td>
</tr>
<tr>
<td>LDL-I (%)</td>
<td>-0.1 (-1.8, 1.5)</td>
<td>-1.1 (-2.7, 0.6)</td>
<td>-0.9 (-3.3, 1.4)</td>
<td>0.96</td>
</tr>
<tr>
<td>LDL-II (%)</td>
<td>1.4 (-2.3, 5.3)</td>
<td>3.7 (-0.3, 7.7)</td>
<td>2.2 (-3.4, 7.9)</td>
<td>0.99</td>
</tr>
<tr>
<td>LDL-III (%)</td>
<td>-2.2 (-6.1, 1.7)</td>
<td>-3.3 (-7.3, 0.7)</td>
<td>-1.1 (-6.8, 4.7)</td>
<td>1.00</td>
</tr>
<tr>
<td>LDL-IV (%)</td>
<td>0.3 (-0.9, 1.5)</td>
<td>0.9 (-0.3, 2.1)</td>
<td>0.6 (-1.1, 2.3)</td>
<td>1.00</td>
</tr>
<tr>
<td>HDL-C (mg/dL)</td>
<td>-1.7 (-3.2, -0.3)</td>
<td>-0.7 (-2.1, 0.8)</td>
<td>1.1 (-1.0, 3.1)</td>
<td>0.61</td>
</tr>
<tr>
<td>Triacylglycerol (mg/dL)</td>
<td>-6.8 (-29.7, 16.0)</td>
<td>-5.6 (-29.0, 17.7)</td>
<td>1.2 (-31.6, 34.0)</td>
<td>1.00</td>
</tr>
<tr>
<td>TC/HDL-C ratio</td>
<td>-0.1 (-0.4, 0.2)</td>
<td>-0.1 (-0.4, 0.2)</td>
<td>0.0 (-0.4, 0.5)</td>
<td>1.00</td>
</tr>
<tr>
<td>Apolipoprotein A-I (mg/dL)</td>
<td>-3.9 (-8.9, 1.1)</td>
<td>-5.5 (-10.6, -0.3)</td>
<td>-1.6 (-8.8, 5.6)</td>
<td>1.00</td>
</tr>
<tr>
<td>Apolipoprotein B (mg/dL)</td>
<td>-2.7 (-8.1, 2.8)</td>
<td>-2.8 (-8.3, 2.8)</td>
<td>-0.1 (-7.9, 7.7)</td>
<td>1.00</td>
</tr>
<tr>
<td><strong>Glucose parameters</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fasting glucose (mg/dL)</td>
<td>-1.3 (-4.0, 1.5)</td>
<td>-1.6 (-4.4, 1.2)</td>
<td>-0.3 (-4.2, 3.6)</td>
<td>1.00</td>
</tr>
<tr>
<td>Fasting insulin (µU/mL)</td>
<td>-0.6 (-3.1, 1.9)</td>
<td>-2.1 (-4.7, 0.4)</td>
<td>-1.6 (-5.1, 2.0)</td>
<td>0.85</td>
</tr>
<tr>
<td>2-h glucose (mg/dL)</td>
<td>-4.1 (-19.1, 10.9)</td>
<td>-3.0 (-18.4, 12.3)</td>
<td>1.1 (-20.4, 22.6)</td>
<td>1.00</td>
</tr>
<tr>
<td>2-h insulin (µU/mL)</td>
<td>-15.5 (-37.8, 6.8)</td>
<td>-13.4 (-36.2, 9.4)</td>
<td>2.1 (-29.8, 34.1)</td>
<td>1.00</td>
</tr>
<tr>
<td>Measurement</td>
<td>Whole grain Δ from baseline (n = 24)</td>
<td>Refined grain Δ from baseline (n = 23)</td>
<td>Difference between groups</td>
<td>P&lt;sup&gt;3&lt;/sup&gt;</td>
</tr>
<tr>
<td>-----------------------</td>
<td>--------------------------------------</td>
<td>---------------------------------------</td>
<td>---------------------------</td>
<td>--------------</td>
</tr>
<tr>
<td>AUC glucose</td>
<td>-662 (-1931, 607)</td>
<td>-408 (-1705, 888)</td>
<td>232 (-1561, 2068)</td>
<td>1.00</td>
</tr>
<tr>
<td>AUC insulin</td>
<td>-1520 (-3524, 484)</td>
<td>-1120 (-3167, 927)</td>
<td>400 (-2465, 3265)</td>
<td>1.00</td>
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<tr>
<td>ISI</td>
<td>0.6 (-0.2, 1.3)</td>
<td>0.5 (-0.3, 1.3)</td>
<td>-0.1 (-1.2, 1.0)</td>
<td>1.00</td>
</tr>
</tbody>
</table>

**Inflammation and fibrinolysis**

<p>| | | | | |</p>
<table>
<thead>
<tr>
<th></th>
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</thead>
<tbody>
<tr>
<td>CRP (mg/L)</td>
<td>-2.3 (-3.6, -1.0)&lt;sup&gt;2&lt;/sup&gt;</td>
<td>0.1 (-1.2, 1.4)</td>
<td>2.4 (0.6, 4.3)</td>
<td>0.007</td>
</tr>
<tr>
<td>PAI-1 (ng/mL)</td>
<td>-2.9 (-6.1, 0.3)</td>
<td>-6.1 (-9.4, -2.8)&lt;sup&gt;2&lt;/sup&gt;</td>
<td>-3.2 (-7.8, 1.5)</td>
<td>0.28</td>
</tr>
</tbody>
</table>

<sup>1</sup> All values are least squares mean (95% CI). BP, blood pressure; PPD; peak particle diameter; OGTT, oral glucose tolerance test; AUC, area under the curve; ISI, insulin sensitivity index; CRP, C-reactive protein; PAI-1, plasminogen activator inhibitor-1.

<sup>2</sup> Significantly different from baseline, P<0.05.

<sup>3</sup> P value for difference between groups.
Table 3.4. Daily energy and nutrient intake at baseline and at week 12 from 3-day food records of the study participants.  

<table>
<thead>
<tr>
<th></th>
<th>Group</th>
<th>Baseline</th>
<th>Week 4</th>
<th>Week 8</th>
<th>Week 12</th>
</tr>
</thead>
<tbody>
<tr>
<td>Energy (kcal)</td>
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<td></td>
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<tr>
<td>WG</td>
<td>1967</td>
<td>(1758, 2175)</td>
<td>1807 (1593, 2022)</td>
<td>1742 (1529, 1954)</td>
<td>1595 (1374, 1815)</td>
</tr>
<tr>
<td>RG</td>
<td>2265</td>
<td>(2057, 2474)</td>
<td>1616 (1408, 1824)</td>
<td>1569 (1351, 1788)</td>
<td>1578 (1361, 1795)</td>
</tr>
<tr>
<td>Carbohydrate (% energy)</td>
<td></td>
<td></td>
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</tr>
<tr>
<td>WG</td>
<td>47.8</td>
<td>(44.3, 51.3)</td>
<td>53.9 (50.3, 57.5)</td>
<td>54.0 (50.4, 57.5)</td>
<td>54.4 (50.7, 58.1)</td>
</tr>
<tr>
<td>RG</td>
<td>47.5</td>
<td>(44.0, 51.0)</td>
<td>49.6 (46.1, 53.1)</td>
<td>48.1 (44.5, 51.8)</td>
<td>50.2 (46.5, 53.8)</td>
</tr>
<tr>
<td>Protein (% energy)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>WG</td>
<td>16.9</td>
<td>(15.3, 18.4)</td>
<td>18.1 (16.5, 19.7)</td>
<td>18.4 (16.8, 20.0)</td>
<td>19.0 (17.4, 20.6)</td>
</tr>
<tr>
<td>RG</td>
<td>16.5</td>
<td>(14.9, 18.0)</td>
<td>18.7 (17.2, 20.3)</td>
<td>19.0 (17.4, 20.7)</td>
<td>19.9 (18.3, 21.6)</td>
</tr>
<tr>
<td>Total fat (% energy)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>WG</td>
<td>35.4</td>
<td>(32.5, 38.3)</td>
<td>28.9 (26.0, 31.9)</td>
<td>29.6 (26.7, 32.5)</td>
<td>28.0 (25.0, 31.0)</td>
</tr>
<tr>
<td>RG</td>
<td>36.2</td>
<td>(33.3, 39.0)</td>
<td>32.3 (29.4, 35.1)</td>
<td>33.5 (30.5, 36.5)</td>
<td>30.4 (27.4, 33.4)</td>
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<tr>
<td>Saturated fat (% energy)</td>
<td></td>
<td></td>
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<td></td>
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</tr>
<tr>
<td>WG</td>
<td>12.3</td>
<td>(11.1, 13.5)</td>
<td>9.6 (8.4, 10.8)</td>
<td>9.4 (8.3, 10.6)</td>
<td>8.6 (7.4, 9.9)</td>
</tr>
<tr>
<td>RG</td>
<td>12.3</td>
<td>(11.1, 13.4)</td>
<td>10.8 (9.6, 11.9)</td>
<td>11.0 (9.8, 12.3)</td>
<td>10.1 (8.8, 11.3)</td>
</tr>
<tr>
<td>MUFA (% energy)</td>
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<tr>
<td>WG</td>
<td>13.1</td>
<td>(11.8, 14.4)</td>
<td>11.0 (9.6, 12.3)</td>
<td>11.3 (10.0, 12.7)</td>
<td>10.6 (9.2, 12.0)</td>
</tr>
<tr>
<td>RG</td>
<td>14.1</td>
<td>(12.8, 15.4)</td>
<td>12.3 (11.0, 13.7)</td>
<td>12.9 (11.5, 14.3)</td>
<td>11.3 (9.9, 12.7)</td>
</tr>
<tr>
<td>PUFA (% energy)</td>
<td></td>
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<tr>
<td>WG</td>
<td>7.2</td>
<td>(6.4, 8.0)</td>
<td>6.0 (5.2, 6.8)</td>
<td>6.4 (5.5, 7.2)</td>
<td>6.4 (5.5, 7.2)</td>
</tr>
<tr>
<td>RG</td>
<td>7.0</td>
<td>(6.2, 7.8)</td>
<td>6.7 (5.9, 7.5)</td>
<td>7.0 (6.2, 7.9)</td>
<td>6.6 (5.8, 7.4)</td>
</tr>
<tr>
<td>Cholesterol (mg)</td>
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<tr>
<td>WG</td>
<td>261</td>
<td>(214, 309)</td>
<td>163 (114, 212)</td>
<td>182 (134, 230)</td>
<td>158 (107, 208)</td>
</tr>
<tr>
<td>RG</td>
<td>269</td>
<td>(222, 316)</td>
<td>224 (177, 271)</td>
<td>244 (194, 294)</td>
<td>245 (196, 294)</td>
</tr>
<tr>
<td></td>
<td>Group</td>
<td>Baseline</td>
<td>Week 4</td>
<td>Week 8</td>
<td>Week 12</td>
</tr>
<tr>
<td>----------------------</td>
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<td>-------------------</td>
<td>--------------------</td>
<td>--------------------</td>
<td>--------------------</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Total fiber (g/1000kcal)</td>
<td></td>
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<td></td>
</tr>
<tr>
<td></td>
<td>WG</td>
<td>8.6 (7.3, 9.8)</td>
<td>12.5 (11.2, 13.8)</td>
<td>13.3 (12.0, 14.6)</td>
<td>12.9 (11.6, 14.2)</td>
</tr>
<tr>
<td></td>
<td>RG</td>
<td>9.1 (7.8, 10.3)</td>
<td>10.0 (8.7, 11.3)</td>
<td>9.6 (8.3, 10.9)</td>
<td>9.8 (8.4, 11.1)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Soluble fiber (g/1000 kcal)</td>
<td></td>
<td></td>
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</tr>
<tr>
<td></td>
<td>WG</td>
<td>2.2 (1.9, 2.5)</td>
<td>3.1 (2.7, 3.4)</td>
<td>3.3 (3.0, 3.6)</td>
<td>3.3 (3.0, 3.6)</td>
</tr>
<tr>
<td></td>
<td>RG</td>
<td>2.3 (2.0, 2.7)</td>
<td>2.7 (2.4, 3.0)</td>
<td>2.5 (2.2, 2.8)</td>
<td>2.7 (2.4, 3.1)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Insoluble fiber (g/1000 kcal)</td>
<td></td>
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<tr>
<td></td>
<td>WG</td>
<td>6.3 (5.2, 7.3)</td>
<td>9.3 (8.3, 10.4)</td>
<td>9.9 (8.9, 10.9)</td>
<td>9.5 (8.4, 10.5)</td>
</tr>
<tr>
<td></td>
<td>RG</td>
<td>6.6 (5.6, 7.6)</td>
<td>7.1 (6.1, 8.1)</td>
<td>6.9 (5.9, 8.0)</td>
<td>6.9 (5.8, 7.9)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Magnesium (mg)</td>
<td></td>
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</tr>
<tr>
<td></td>
<td>WG</td>
<td>273 (240, 305)</td>
<td>333 (299, 366)</td>
<td>343 (310, 377)</td>
<td>300 (266, 334)</td>
</tr>
<tr>
<td></td>
<td>RG</td>
<td>320 (288, 353)</td>
<td>237 (204, 269)</td>
<td>243 (209, 277)</td>
<td>230 (196, 264)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Sodium (mg)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>WG</td>
<td>3238 (2845, 3632)</td>
<td>3273 (2868, 3677)</td>
<td>3096 (2695, 3497)</td>
<td>2916 (2501, 3330)</td>
</tr>
<tr>
<td></td>
<td>RG</td>
<td>3846 (3452, 4239)</td>
<td>3162 (2768, 3555)</td>
<td>2937 (2526, 3348)</td>
<td>3089 (2680, 3499)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Vitamin B6 (mg)</td>
<td></td>
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</tr>
<tr>
<td></td>
<td>WG</td>
<td>2.0 (1.7, 2.3)</td>
<td>2.2 (1.9, 2.5)</td>
<td>2.0 (1.7, 2.3)</td>
<td>1.9 (1.6, 2.2)</td>
</tr>
<tr>
<td></td>
<td>RG</td>
<td>2.0 (1.7, 2.3)</td>
<td>1.7 (1.4, 2.0)</td>
<td>1.8 (1.5, 2.1)</td>
<td>1.8 (1.4, 2.1)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Added sugar (g)</td>
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</tr>
<tr>
<td></td>
<td>WG</td>
<td>56.1 (43.5, 68.7)</td>
<td>43.9 (30.9, 57.0)</td>
<td>38.6 (25.8, 51.5)</td>
<td>39.9 (26.5, 53.2)</td>
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<tr>
<td></td>
<td>RG</td>
<td>78.2 (65.6, 90.8)</td>
<td>37.8 (25.3, 50.4)</td>
<td>39.3 (26.0, 52.6)</td>
<td>44.1 (30.9, 57.2)</td>
</tr>
</tbody>
</table>

All values are least squares mean (95% CI). MUFA, monounsaturated fatty acids; PUFA, polyunsaturated fatty acids.

1. WG, whole grain group; RG, refined grain group.
2. Data were log transformed for analysis.
3. Significantly different from baseline, P < 0.05.
4. Significantly different from refined grain group, P < 0.05.
Table 3.5. Diet satisfaction ratings at baseline, week 4, and week 12 in participants in the whole grain and refined grain groups.

<table>
<thead>
<tr>
<th></th>
<th>Whole grain group</th>
<th>Refined grain group</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Sense of a healthy lifestyle</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Baseline</td>
<td>2.5 (2.2 to 2.8)</td>
<td>2.5 (2.2, 2.8)</td>
</tr>
<tr>
<td>Week 4</td>
<td>3.6 (3.3, 3.9)²</td>
<td>3.7 (3.5, 4.0)²</td>
</tr>
<tr>
<td>Week 12</td>
<td>3.9 (3.6, 4.1)²</td>
<td>3.9 (3.6, 4.2)²</td>
</tr>
<tr>
<td><strong>Convenience</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Baseline</td>
<td>3.8 (3.6, 4.1)</td>
<td>3.9 (3.7, 4.1)</td>
</tr>
<tr>
<td>Week 4</td>
<td>3.4 (3.1, 3.6)²</td>
<td>3.6 (3.4, 3.8)</td>
</tr>
<tr>
<td>Week 12</td>
<td>3.5 (3.3, 3.8)</td>
<td>3.8 (3.5, 4.0)</td>
</tr>
<tr>
<td><strong>Cost</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Baseline</td>
<td>3.2 (2.9, 3.5)</td>
<td>3.5 (3.2, 3.7)</td>
</tr>
<tr>
<td>Week 4</td>
<td>3.3 (3.1, 3.6)</td>
<td>3.7 (3.5, 4.0)</td>
</tr>
<tr>
<td>Week 12</td>
<td>3.5 (3.2, 3.8)</td>
<td>3.6 (3.4, 3.9)</td>
</tr>
<tr>
<td><strong>Family dynamics</strong></td>
<td></td>
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<tr>
<td>Baseline</td>
<td>3.2 (3.0, 3.5)</td>
<td>3.2 (2.9, 3.4)</td>
</tr>
<tr>
<td>Week 4</td>
<td>4.1 (3.8, 4.3)²</td>
<td>4.1 (3.9, 4.3)²</td>
</tr>
<tr>
<td>Week 12</td>
<td>4.2 (4.0, 4.5)²</td>
<td>4.2 (4.0, 4.5)²</td>
</tr>
<tr>
<td><strong>Preoccupation with food</strong></td>
<td></td>
<td></td>
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<tr>
<td>Baseline</td>
<td>3.2 (2.9, 3.5)</td>
<td>2.8 (2.5, 3.1)</td>
</tr>
<tr>
<td>Week 4</td>
<td>3.5 (3.1, 3.8)</td>
<td>3.1 (2.8, 3.4)</td>
</tr>
<tr>
<td>Week 12</td>
<td>3.5 (3.1, 3.8)²</td>
<td>3.3 (3.0, 3.6)²</td>
</tr>
<tr>
<td><strong>Presence of negative feelings</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Baseline</td>
<td>3.7 (3.5, 4.0)</td>
<td>3.7 (3.5, 4.0)</td>
</tr>
<tr>
<td>Week 4</td>
<td>3.6 (3.4, 3.9)</td>
<td>3.6 (3.4, 3.8)</td>
</tr>
<tr>
<td>Week 12</td>
<td>3.7 (3.4, 3.9)</td>
<td>3.8 (3.6, 4.0)</td>
</tr>
<tr>
<td><strong>Ease of meal planning and preparation</strong></td>
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<td></td>
</tr>
<tr>
<td>Baseline</td>
<td>3.9 (3.6, 4.2)</td>
<td>3.7 (3.4, 4.0)</td>
</tr>
<tr>
<td>Week 4</td>
<td>3.3 (3.0, 3.6)²</td>
<td>3.4 (3.1, 3.6)</td>
</tr>
<tr>
<td>Week 12</td>
<td>3.4 (3.1, 3.7)²</td>
<td>3.7 (3.4, 4.0)</td>
</tr>
<tr>
<td><strong>Overall Score</strong></td>
<td></td>
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<tr>
<td>Baseline</td>
<td>3.3 (3.2, 3.5)</td>
<td>3.3 (3.2, 3.5)</td>
</tr>
<tr>
<td>Week 4</td>
<td>3.5 (3.3, 3.6)²</td>
<td>3.6 (3.5, 3.7)²</td>
</tr>
<tr>
<td>Week 12</td>
<td>3.7 (3.5, 3.8)²</td>
<td>3.8 (3.6, 3.9)²</td>
</tr>
</tbody>
</table>

¹ All values are least squares mean (95%CI).
² Significantly different from baseline, \( P<0.05 \).
Table 3.6. Change in fat mass, lean mass, and percent fat in the abdominal region in participants in the whole grain and refined grain groups.\(^1\)

<table>
<thead>
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<th></th>
<th>Whole Grain Group</th>
<th>Refined Grain Group</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Change in fat mass (g)</td>
<td>-429 (-681, -178)</td>
<td>-441 (-693, -190)</td>
<td>1.00</td>
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<tr>
<td>Change in lean mass (g)</td>
<td>-185.4 (-521, 150)</td>
<td>-384 (-720, -49)</td>
<td>0.91</td>
</tr>
<tr>
<td>Change in percent fat (%)</td>
<td>-2.2 (-3.3, -1.2)</td>
<td>-0.9 (-1.9, 0.2)</td>
<td>0.08</td>
</tr>
</tbody>
</table>

\(^1\) All values are least squares mean (95%CI).

Table 3.7. Change in fat mass, lean mass, and percent fat in the abdominal region in men and women.\(^1\)

<table>
<thead>
<tr>
<th></th>
<th>Female</th>
<th>Male</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Change in fat mass (g)</td>
<td>-291 (-549, -33)</td>
<td>-567 (-814, -321)</td>
<td>0.20</td>
</tr>
<tr>
<td>Change in lean mass (g)</td>
<td>-245 (-593, 104)</td>
<td>-322 (-655, 12)</td>
<td>1.00</td>
</tr>
<tr>
<td>Change in percent fat (%)</td>
<td>-1.0 (-2.1, 0.2)</td>
<td>-2.1 (-3.2, -1.0)</td>
<td>0.32</td>
</tr>
</tbody>
</table>

\(^1\) All values are least squares mean (95%CI).
Figure 3.1. Flow of participants through the study.

- Screened for metabolic syndrome and other inclusion criteria (n = 163)
  - Excluded (n = 113)
    - Did not meet inclusion criteria (n = 106)
    - Decided not to participate (n = 7)

Randomized 50 men and women with metabolic syndrome

- Allocated to whole grain group (n = 25)
  - Dropped out
    - Could not adhere to diet (n = 1)
  - Analyzed (n = 24)

- Allocated to refined grain group (n = 25)
  - Dropped out
    - Family reasons (n = 1)
    - Time conflict (n = 1)
  - Analyzed (n = 23)
Figure 3.2. Mean (+ SE) number of servings/day of whole grain foods consumed by participants in the whole grain (■) and refined grain (□) groups at baseline and weeks 4, 8, and 12. Consumption of whole grain foods increased at weeks 4, 8, and 12 in the whole grain group ($P<0.001$) and decreased in the refined grain group ($P<0.001$).

Figure 3.3. Sources of whole grain servings for participants in the whole grain group.
Figure 3.4. Mean (±SE) cumulative weight loss at each bi-weekly visit in the whole grain and refined grain groups. There were no significant differences in weight loss between groups at any time point.
**Figure 3.5.** Mean (± SE) CRP concentration in participants in the whole grain and refined grain groups at baseline (□) and week 12 (■). CRP decreased 38% in the whole grain group but was unchanged in the refined grain group ($P = 0.007$ for between group difference).

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**Figure 3.6** Percent reduction in CRP in participants in the whole grain and refined grain groups that had a reduction in CRP.
Figure 3.7. Mean (±SE) percentage change in lipids, lipoproteins, and glucose and insulin measures in subjects with high (≥3.5 mg/L, n=23) and low (<3.5 mg/L, n=24) CRP levels at baseline. The CRP groups are defined by a median split. There were no significant differences between CRP groups.
Figure 3.8. Mean ± SE circulating concentration of glucose and insulin during an oral glucose tolerance test in participants in the whole grain and refined grain groups at baseline (—) and week 12 (---). There were no significant differences in fasting or postchallenge glucose or insulin within or between groups at any time point.
References


34. Mora S, Ridker PM. Justification for the Use of Statins in Primary Prevention: an Intervention Trial Evaluating Rosuvastatin (JUPITER)--can C-reactive protein be used to target statin therapy in primary prevention? Am J Cardiol 2006;97:33A-41A.


CHAPTER 4
SEXUALLY DIMORPHIC CHANGES IN CARDIOVASCULAR DISEASE RISK FACTORS FOLLOWING A 12-WEEK HYPOCALORIC DIET

Introduction

After a follow-up period of 40 years, investigators from the Framingham Heart Study reported that being overweight or obese is associated with a large decrease in life expectancy in men and women (1). Forty-year-old female nonsmokers lost 3.3 years and 40-year-old male nonsmokers lost 3.1 years of life expectancy because of being overweight (BMI of 25 to 29.9 kg/m²). Furthermore, forty-year-old female nonsmokers lost 7.1 years and 40-year-old male nonsmokers lost 5.8 years because of obesity (BMI ≥ 30 kg/m²). Although being overweight or obese presents a similar risk to men and women in terms of life expectancy, there are some noteworthy differences in patterns of obesity and metabolic syndrome between the sexes - particularly between men and pre-menopausal women.

One common difference between men and pre-menopausal women is that pre-menopausal women tend to store fat around the hips, called peripheral adiposity, whereas men are more prone to store fat around the abdomen, called central or visceral obesity (2). Central obesity is associated with lower HDL-C and increased triglycerides, total and LDL cholesterol, and blood pressure (3). Central obesity also increases one’s risk for type 2 diabetes and cardiovascular disease (4). After menopause, women tend to deposit fat more centrally, and their lipoprotein values and body fat distribution changes to a
more male pattern. The transition to menopause in women is also associated with an increased risk of CVD and type 2 diabetes (5).

Regarding sex hormones, reduced testosterone and sex hormone binding globulin (SHBG) levels are commonly observed in men with metabolic syndrome, coronary artery disease, and type 2 diabetes (6-9). Low testosterone levels in men are also associated with CVD risk factors including hypertension, elevated total and LDL cholesterol, increased plasminogen activator inhibitor-1 (PAI-1), visceral obesity, and insulin resistance (10). In women, SHBG is also reduced as body weight increases (11). However, testosterone levels in women are inversely related to the waist-hip-ratio and other markers of central obesity (12). Elevated testosterone and reduced SHBG levels are also characteristic of polycystic ovary syndrome, which carries increased likelihood of developing metabolic syndrome (13).

Since there are sexually dimorphic differences in many CVD risk factors, it is pertinent to determine if men and women have different responses to the hypocaloric diets that were studied. I also evaluated changes in testosterone and SHBG levels since being overweight or obese is associated with irregular levels of these hormones. My hypotheses were [1] that men and post-menopausal women would have elevated levels of CVD risk factors compared with pre-menopausal women, and [2] that there would be greater improvements in these risk factors in men and post-menopausal women compared with pre-menopausal women.
Methods

This section evaluates differences in biometric, glycemic, lipid, inflammatory, and hormonal endpoints between men and women in the whole grain study discussed in Chapter 3. In women, pre- or post-menopausal status, menstrual cycle regularity, and diagnosis of polycystic ovary syndrome were determined by participants’ responses about their menstrual history that they answered on medical history forms completed at screening. A woman was considered pre-menopausal if she had regular menstrual cycles. The frequency of strenuous, moderate, and easy physical activity was determined by a physical activity questionnaire completed at each study visit. For this analysis, the whole grain and refined grain groups were combined since there were no significant effects of treatment, with the exception of CRP. Baseline differences between men, pre-, and post-menopausal women were determined by a one-way analysis of variance. The statistical analysis used to compare changes following weight loss is as described in the “Statistical Analysis” section in Chapter 3.

Results

The baseline characteristics of the study participants by gender and menopausal status are listed in Table 4.1 and Table 4.2, respectively. At baseline, men had a significantly lower body fat percentage and greater level of testosterone than pre- and post-menopausal women. Men also had significantly lower 2-hour glucose and Apo A-I levels compared with postmenopausal women. There were no other significant differences between men, pre-, and post-menopausal women baseline levels of lipids, glucose and insulin measures, or markers of inflammation and fibrinolysis.
Changes in biometric measurements, lipids and lipoproteins, glucose and insulin measures, markers of inflammation and fibrinolysis, and sex hormones are listed in Table 4.3. Both men and women had significant reductions in body weight and waist circumference. Men had significantly greater improvements in total and LDL cholesterol, the total:HDL cholesterol ratio, and apo-B compared with women. However, when baseline values and changes in body weight were taken into account, there were no significant differences in any of the outcomes measured between men and women.

Figure 4.1 compares the average percent change in the measured outcomes in men, pre-menopausal, and post-menopausal women. Notably, LDL-C, triglycerides, the total:HDL ratio, apo B, and the AUC for insulin decreased only in men. Furthermore, HDL-C was also only increased in men but not in either group of women. Both groups had similar percent reductions in body weight and waist circumference.

I then evaluated nutrient intake and exercise frequency in men and women to determine if these factors may play a role in the different responses between men and women. As shown in Figure 4.2, the macronutrient content of the diet was similar in men and women, as was the intake of dietary fiber. There were no significant differences in frequency of strenuous, moderate, and easy physical activity between men and women throughout the study period, as shown in Table 4.4.

One study participant had been previously diagnosed with PCOS and her results are shown in Table 4.5. Interestingly, this participant had no reduction in body weight, yet she had dramatic reductions (>35%) in the AUC for insulin, the insulin sensitivity index (ISI), and the free androgen index (FAI). She also had notable improvements
 (>10%) in systolic and diastolic blood pressure, LDL-C, triglycerides, testosterone, and SHBG.

**Discussion**

The main findings in this analysis are that men had greater improvements in several CVD risk factors including total and LDL cholesterol, the total:HDL cholesterol ratio, and apo-B compared with pre- and post-menopausal women. When baseline values and changes in body weight were taken into account, there were no significant differences in any of the outcomes measured between men and women. There were also no significant differences in the change in any outcome measured between pre-and post-menopausal women. Based on data collected from the 3-day diet recalls and physical activity questionnaires, it does not appear that the different response between genders is due to differences in nutrient intake or exercise frequency.

Several investigators have reported greater reductions in body weight and improvements in CVD risk in men following lifestyle interventions compared with women. But for the most part these gender differences were no longer significant when baseline values and weight loss are taken into account. For example, Wing et al. compared changes in CVD risk factors in 159 moderately overweight men and women (13.6-31.8 kg above ideal body weight) age 25-45 who were participating in an 18-month behavior weight loss program (14). Subjects were prescribed a calorie goal of 1000 or 1500 kcal/d depending on their body weight and given exercise goals that started at 250 cal/week and gradually increased to 1000 kcal/week. Subjects recorded their intake and exercise daily for the first 20 weeks and then one week per month for the remainder of
the 18 months. Men had greater decreases in triglycerides, systolic blood pressure, and waist circumference at 6, 12, and 18 months and greater increases in HDL-C at 6 and 12 months. However, these gender differences were removed by adjusting for the baseline level of the risk factor and change in BMI.

In contrast to my hypothesis, there were no differences between pre- and post-menopausal women in baseline values or the response of any outcome. This could be because pre-menopausal women with metabolic syndrome are more likely to have abdominal obesity and higher levels of other CVD risk factors compared with premenopausal women without metabolic syndrome. The lack of significant improvements in most of the risk factors measured in women may be due to a short study period or a small reduction in body weight. Numerous clinical trials have shown that there are significant improvements in lipids and lipoproteins, glucose tolerance, and markers of inflammation in pre- and post-menopausal women following 6-month lifestyle studies or shorter term studies with a greater weight loss (7-10%) (15-17).

Since it appears that the greater reductions in CVD risk factors in men is due to a higher initial value and greater weight loss, the question remains: why do men lose more weight than women? One reason proposed is that men have more muscle mass than women, which means that they have a higher metabolic rate (18). On average, the 24-hour energy expenditure of a man at rest is 5 to 10 percent higher than that of a woman of the same weight and height (19). As a result, men burn more calories even when they're resting, so the result is more rapid weight loss in men when they reduce their calorie intake. Since the women in this study had a significantly greater percentage of body fat than men, it is likely that the women had a lower metabolic rate per kg of body weight.
A second reason that men lose more weight than women when following the same weight loss programs could be that men can lose weight while eating more calories, since men tend to be bigger and have higher energy needs. For example, a 30 year old male who is 6’0, 280 lbs (BMI = 38) can achieve a 500 calorie deficit with 2800 calories, whereas a female who is 5’0, 195lbs (BMI = 38) can only consume 1600 calories to maintain a 500 calorie deficit. In this study, the energy needs for men calculated using the Mifflin equation were about 450 calories higher than the energy needs for women.

A third reason that men may lose more weight than women is that men are more likely than women to lose weight when they exercise regularly (20). This is a consistent finding in clinical trials that have evaluated the magnitude of weight loss from an exercise regimen while keeping dietary intake constant. Interestingly, Krotkiewski and Bjorntorp found that in a 3-month conditioning study of obese men and women, having gynoid obesity (based on waist to hip ratio) in women was associated with significant weight gain, while android obesity in women was associated with no change in body weight or a slight weight loss (21). These findings suggest that obese women with an abdominal distribution of adipose tissue may be more likely to respond to physical training like men (i.e. with greater weight loss). In the whole grain study, exercise frequency in men and women throughout the study period was similar. However, it is possible that a difference in the response to exercise could account for gender differences observed.

I also evaluated nutrient intake in men and women to determine if diet composition may account for the different responses. I found no difference in nutrient intake in men and women based on their three-day diet recalls. Findings from previous
studies suggest that it is unlikely that men and women would respond differently to a particular diet composition. For example, in a review of the gender differences in plasma lipid responses to a high fat and low fat diet, Lapointe et al reported that the response of female and male subjects is similar and consistent (22). Both LDL and HDL concentrations were reduced in men and women as the total and saturated fat content of the diet declined. When the magnitude of decrease reached statistical significance, it was significant for both genders.

It is intriguing that the study participant with PCOS had such dramatic metabolic improvements without weight loss. Although antecdotally women with PCOS are thought to have greater difficulty losing weight than healthy pre-menopausal women, there were no differences in weight loss between subjects with and without PCOS following hypocaloric diets for 2-7 months (23-25). It is possible that the metabolic improvements in this participant were due in part to changes from exercise, since some studies have shown that exercise training improves insulin sensitivity without weight loss (26, 27). However, this participant did not report an increase in exercise frequency compared with baseline. Studies have also reported improvements in total cholesterol, triglycerides, and LDL-C when body weight is maintained following a low carbohydrate diet compared with a high carbohydrate diet (28, 29). However, this participant did not have any dramatic changes in the macronutrient composition of her diet compared with baseline according to her three day diet recalls. Her intake of dietary fiber increased from (19.6g at baseline to 28.4g throughout the study period), which may account for the effects that were observed. A high fiber diet has been shown to reduce 24-hour glucose
and insulin levels as well as LDL-C and triglycerides when body weight is maintained (30).

In summary, the results I presented demonstrate that men have greater improvements in some CVD risk factors during weight loss compared with women. These differences can largely be accounted for by larger baseline values and greater weight loss in men. It is apparent that gender differences certainly increase the variability of response to a hypocaloric diet. This presents a difficult decision in study design, especially for a small study. Limiting the study to one gender would reduce the variability and increase the likelihood of detecting differences, but it would reduce the generalizability of the study.
Table 4.1. Characteristics of the study participants at baseline by gender.

<table>
<thead>
<tr>
<th>Outcome</th>
<th>Female n = 25</th>
<th>Male n = 25</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Biometric</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>104.5 ± 16.9</td>
<td>108.8 ± 11.1</td>
<td>0.04</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>37.0 ± 5.3</td>
<td>34.6 ± 3.1</td>
<td>0.06</td>
</tr>
<tr>
<td>Systolic BP (mmHg)</td>
<td>123.4 ± 13.8</td>
<td>129.8 ± 9.1</td>
<td>0.06</td>
</tr>
<tr>
<td>Diastolic BP (mmHg)</td>
<td>80.9 ± 8.2</td>
<td>84.3 ± 7.2</td>
<td>0.12</td>
</tr>
<tr>
<td>Waist Circumference (cm)</td>
<td>119.5 ± 13.1</td>
<td>115.3 ± 8.0</td>
<td>0.18</td>
</tr>
<tr>
<td>Body Fat %</td>
<td>43.0 ± 4.5</td>
<td>31.4 ± 4.7</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td><strong>Serum Lipids</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cholesterol (mg/dL)</td>
<td>190.6 ± 42.8</td>
<td>187.1 ± 31.4</td>
<td>0.74</td>
</tr>
<tr>
<td>LDL-C (mg/dL)</td>
<td>115.5 ± 34.1</td>
<td>118.3 ± 27.9</td>
<td>0.75</td>
</tr>
<tr>
<td>LDL-PPD (Å)</td>
<td>262.3 ± 7.3</td>
<td>258.9 ± 8.0</td>
<td>0.12</td>
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<tr>
<td>LDL-I (%)</td>
<td>16.9 ± 5.8</td>
<td>15.1 ± 6.3</td>
<td>0.31</td>
</tr>
<tr>
<td>LDL-II (%)</td>
<td>41.8 ± 10.6</td>
<td>40.2 ± 11.2</td>
<td>0.61</td>
</tr>
<tr>
<td>LDL-III (%)</td>
<td>19.5 ± 11.7</td>
<td>24.6 ± 13.1</td>
<td>0.15</td>
</tr>
<tr>
<td>LDL-IV (%)</td>
<td>6.4 ± 2.6</td>
<td>7.3 ± 2.6</td>
<td>0.20</td>
</tr>
<tr>
<td>HDL-C (mg/dL)</td>
<td>43.5 ± 7.6</td>
<td>38.7 ± 8.4</td>
<td>0.04</td>
</tr>
<tr>
<td>Triglycerides (mg/dL)</td>
<td>158.0 ± 77.4</td>
<td>150.5 ± 48.6</td>
<td>0.68</td>
</tr>
<tr>
<td>TC:HDLC</td>
<td>4.4 ± 1.0</td>
<td>5.0 ± 1.3</td>
<td>0.09</td>
</tr>
<tr>
<td>Apo A-I (mg/dL)</td>
<td>126.3 ± 19.2</td>
<td>115.5 ± 16.1</td>
<td>0.04</td>
</tr>
<tr>
<td>Apo B (mg/dL)</td>
<td>82.8 ± 19.0</td>
<td>86.2 ± 15.3</td>
<td>0.49</td>
</tr>
<tr>
<td><strong>Glucose Parameters</strong></td>
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<td></td>
<td></td>
</tr>
<tr>
<td>Fasting glucose (mg/dL)</td>
<td>95.1 ± 7.0</td>
<td>96.5 ± 6.0</td>
<td>0.45</td>
</tr>
<tr>
<td>Fasting insulin (µU/mL)</td>
<td>14.8 ± 7.6</td>
<td>14.0 ± 6.5</td>
<td>0.69</td>
</tr>
<tr>
<td>2-h glucose (mg/dL)</td>
<td>149.4 ± 40.0</td>
<td>125.0 ± 31.0</td>
<td>0.02</td>
</tr>
<tr>
<td>2-h insulin (µU/mL)</td>
<td>100.7 ± 72.6</td>
<td>81.7 ± 61.3</td>
<td>0.32</td>
</tr>
<tr>
<td>AUC glucose (OGTT)</td>
<td>18406 ± 3451</td>
<td>17895 ± 2267</td>
<td>0.54</td>
</tr>
<tr>
<td>AUC insulin (OGTT)</td>
<td>10921 ± 7816</td>
<td>11242 ± 7412</td>
<td>0.88</td>
</tr>
<tr>
<td>ISI</td>
<td>2.7 ± 1.2</td>
<td>2.9 ± 1.5</td>
<td>0.65</td>
</tr>
<tr>
<td><strong>Inflammation and Fibrinolysis</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>PAI-1 (ng/mL)</td>
<td>17.1 ± 11.5</td>
<td>17.0 ± 10.5</td>
<td>0.96</td>
</tr>
<tr>
<td>CRP (mg/L)</td>
<td>6.5 ± 4.8</td>
<td>5.4 ± 8.7</td>
<td>0.59</td>
</tr>
<tr>
<td>IL-1 (pg/mL)</td>
<td>26.9 ± 42.7</td>
<td>42.5 ± 71.2</td>
<td>0.35</td>
</tr>
<tr>
<td>IL-6 (pg/mL)</td>
<td>88.7 ± 139.9</td>
<td>173.4 ± 288.9</td>
<td>0.19</td>
</tr>
<tr>
<td>TNF-α (pg/mL)</td>
<td>29.6 ± 25.9</td>
<td>63.0 ± 76.1</td>
<td>0.05</td>
</tr>
<tr>
<td><strong>Sex Hormones</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Testosterone (ng/dL)</td>
<td>30.2 ± 17.2</td>
<td>379.3 ± 110.9</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>SHBG (nmol/L)</td>
<td>40.2 ± 16.4</td>
<td>34.2 ± 18.0</td>
<td>0.22</td>
</tr>
<tr>
<td>FAI</td>
<td>3.1 ± 2.2</td>
<td>47.3 ± 23.5</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

1 All values are mean ± SD. BP, blood pressure; PPD, peak particle diameter; OGTT, oral glucose tolerance test; AUC, area under the curve; ISI, insulin sensitivity index; CRP, C reactive protein; PAI-1, plasminogen activator inhibitor-1; SHBG, sex hormone binding globulin; FAI, free androgen index.

2 Measured by DXA scan.
Table 4.2. Characteristics of the study participants at baseline by gender and menopausal status.

<table>
<thead>
<tr>
<th>Outcome</th>
<th>Male n = 25</th>
<th>Female – Pre n = 14</th>
<th>Female – Post n = 8</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Biometric</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>108.8 ± 11.1</td>
<td>101.8 ± 20.2</td>
<td>95.2 ± 9.6</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>34.6 ± 3.1</td>
<td>37.0 ± 2.4</td>
<td>36.3 ± 4.0</td>
</tr>
<tr>
<td>Systolic BP (mmHg)</td>
<td>129.8 ± 9.1</td>
<td>121.0 ± 15.6</td>
<td>122.6 ± 9.3</td>
</tr>
<tr>
<td>Diastolic BP (mmHg)</td>
<td>84.3 ± 7.2</td>
<td>80.1 ± 7.6</td>
<td>79.3 ± 8.4</td>
</tr>
<tr>
<td>Waist Circumference (cm)</td>
<td>115.3 ± 8.0</td>
<td>119.6 ± 15.3</td>
<td>117.3 ± 8.1</td>
</tr>
<tr>
<td>Body Fat (%)</td>
<td>31.4 ± 4.7</td>
<td>42.0 ± 4.5</td>
<td>44.7 ± 4.6</td>
</tr>
<tr>
<td><strong>Serum Lipids</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cholesterol (mg/dL)</td>
<td>187.1 ± 31.4</td>
<td>176.7 ± 43.8</td>
<td>209.0 ± 39.6</td>
</tr>
<tr>
<td>LDL-C (mg/dL)</td>
<td>118.3 ± 27.9</td>
<td>103.3 ± 37.1</td>
<td>131.1 ± 25.7</td>
</tr>
<tr>
<td>LDL PPD (Å)</td>
<td>258.9 ± 8.0</td>
<td>262.3 ± 7.7</td>
<td>263.3 ± 7.2</td>
</tr>
<tr>
<td>LDL-I (%)</td>
<td>15.1 ± 6.3</td>
<td>16.8 ± 5.4</td>
<td>18.5 ± 6.8</td>
</tr>
<tr>
<td>LDL-II (%)</td>
<td>40.2 ± 11.2</td>
<td>42.8 ± 11.9</td>
<td>40.4 ± 8.5</td>
</tr>
<tr>
<td>LDL-III (%)</td>
<td>24.6 ± 13.1</td>
<td>17.8 ± 10.2</td>
<td>19.4 ± 12.8</td>
</tr>
<tr>
<td>LDL-IV (%)</td>
<td>7.3 ± 2.6</td>
<td>6.4 ± 2.9</td>
<td>6.7 ± 3.8</td>
</tr>
<tr>
<td>HDL-C (mg/dL)</td>
<td>38.7 ± 8.4</td>
<td>43.0 ± 8.7</td>
<td>45.9 ± 3.8</td>
</tr>
<tr>
<td>Triglycerides (mg/dL)</td>
<td>150.5 ± 48.6</td>
<td>151.7 ± 75.4</td>
<td>159.1 ± 87.0</td>
</tr>
<tr>
<td>TC:HDL</td>
<td>5.0 ± 1.3</td>
<td>4.2 ± 1.1</td>
<td>4.5 ± 0.7</td>
</tr>
<tr>
<td>Apo A-I (mg/dL)</td>
<td>115.5 ± 16.1</td>
<td>123.9 ± 22.2</td>
<td>134.0 ± 15.0³</td>
</tr>
<tr>
<td>Apo B (mg/dL)</td>
<td>86.2 ± 15.3</td>
<td>76.3 ± 18.4</td>
<td>92.9 ± 19.4</td>
</tr>
<tr>
<td><strong>Glucose Parameters</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fasting glucose (mg/dL)</td>
<td>96.5 ± 6.0</td>
<td>96.6 ± 8.3</td>
<td>93.0 ± 4.8</td>
</tr>
<tr>
<td>Fasting insulin (µU/mL)</td>
<td>14.0 ± 6.5</td>
<td>14.4 ± 7.2</td>
<td>13.1 ± 8.1</td>
</tr>
<tr>
<td>2-h glucose (mg/dL)</td>
<td>125.0 ± 31.0</td>
<td>142.5 ± 40.1</td>
<td>162.1 ± 47.0³</td>
</tr>
<tr>
<td>2-h insulin (µU/mL)</td>
<td>81.7 ± 61.3</td>
<td>81.7 ± 70.6</td>
<td>122.5 ± 79.8</td>
</tr>
<tr>
<td>AUC glucose (OGTT)</td>
<td>17,895 ± 2,267</td>
<td>17,884 ± 3,752</td>
<td>19,037 ± 3,583</td>
</tr>
<tr>
<td>AUC insulin (OGTT)</td>
<td>11,242 ± 7,412</td>
<td>9,864 ± 8,838</td>
<td>10,791 ± 5,889</td>
</tr>
<tr>
<td>ISI</td>
<td>2.9 ± 1.5</td>
<td>2.9 ± 1.0</td>
<td>2.9 ± 1.4</td>
</tr>
<tr>
<td><strong>Inflammation &amp; Fibrinolysis</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>PAI-1 (ng/mL)</td>
<td>17.0 ± 10.5</td>
<td>16.3 ± 11.5</td>
<td>17.3 ± 12.9</td>
</tr>
<tr>
<td>CRP (mg/L)</td>
<td>5.4 ± 8.7</td>
<td>5.8 ± 5.6</td>
<td>7.2 ± 3.8</td>
</tr>
<tr>
<td>IL-1 (pg/mL)</td>
<td>42.5 ± 71.2</td>
<td>17.8 ± 22.6</td>
<td>18.7 ± 17.3</td>
</tr>
<tr>
<td>IL-6 (pg/mL)</td>
<td>173.4 ± 288.9</td>
<td>39.1 ± 55.0</td>
<td>126.5 ± 187.4</td>
</tr>
<tr>
<td>TNF-α (pg/mL)</td>
<td>63.0 ± 76.1</td>
<td>22.8 ± 22.6</td>
<td>33.8 ± 26.1</td>
</tr>
<tr>
<td><strong>Sex Hormones</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Testosterone (ng/dL)</td>
<td>379.3 ± 110.9</td>
<td>36.6 ± 19.9³</td>
<td>20.8 ± 6.6³</td>
</tr>
<tr>
<td>SHBG (nmol/L)</td>
<td>34.2 ± 18.0</td>
<td>43.9 ± 11.8</td>
<td>35.8 ± 18.7</td>
</tr>
<tr>
<td>FAI</td>
<td>47.3 ± 23.5</td>
<td>3.3 ± 2.4³</td>
<td>2.5 ± 1.3³</td>
</tr>
</tbody>
</table>

1 All values are mean ± SD.
2 Measured by DXA scan.
3 Significantly different from males, P < 0.05.
Table 4.3. Changes in values of assessed variables at baseline and at the end of the 12-week diet period in men and women.

<table>
<thead>
<tr>
<th>Outcome</th>
<th>Female</th>
<th>Male</th>
<th>P value with no adjustment</th>
<th>P value adjusted for baseline value</th>
<th>P value adjusted for baseline value &amp; weight change</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Biometric</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>-3.3 (-5.6, -1.1)</td>
<td>-5.6 (-7.7, -3.4)</td>
<td>0.25</td>
<td>0.13</td>
<td>---</td>
</tr>
<tr>
<td>Systolic BP (mmHg)</td>
<td>-3.0 (-7.3, 1.4)</td>
<td>-5.8 (-10.0, -1.6)</td>
<td>0.75</td>
<td>1.00</td>
<td>1.00</td>
</tr>
<tr>
<td>Diastolic BP (mmHg)</td>
<td>-0.9 (-4.1, 2.3)</td>
<td>-3.7 (-6.8, -0.5)</td>
<td>0.38</td>
<td>0.59</td>
<td>1.00</td>
</tr>
<tr>
<td>Waist Circumference (cm)</td>
<td>-3.6 (-6.3, -0.8)</td>
<td>-3.6 (-6.3, -0.9)</td>
<td>1.00</td>
<td>1.00</td>
<td>0.39</td>
</tr>
<tr>
<td>Body Fat (%)</td>
<td>-0.7 (-1.5, 0.0)</td>
<td>-1.5 (-2.2, -0.7)</td>
<td>0.26</td>
<td>0.057</td>
<td>1.00</td>
</tr>
<tr>
<td><strong>Serum Lipids</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cholesterol (mg/dL)</td>
<td>-1.5 (-10.1, 7.0)</td>
<td>-14.8 (-23.2, -6.3)</td>
<td>0.03</td>
<td>0.03</td>
<td>0.15</td>
</tr>
<tr>
<td>LDL-C (mg/dL)</td>
<td>-0.3 (-7.9, 7.3)</td>
<td>-11.0 (-18.5, -3.5)</td>
<td>0.049</td>
<td>0.053</td>
<td>0.30</td>
</tr>
<tr>
<td>LDL PPD (Å)</td>
<td>-0.4 (-3.3, 2.5)</td>
<td>2.6 (-0.3, 5.4)</td>
<td>0.24</td>
<td>0.77</td>
<td>1.00</td>
</tr>
<tr>
<td>LDL-I (%)</td>
<td>-0.9 (-3.4, 1.6)</td>
<td>-0.3 (-2.8, 2.2)</td>
<td>1.00</td>
<td>1.00</td>
<td>1.00</td>
</tr>
<tr>
<td>LDL-II (%)</td>
<td>1.0 (-3.3, 5.3)</td>
<td>4.1 (-0.1, 8.2)</td>
<td>0.63</td>
<td>0.88</td>
<td>1.00</td>
</tr>
<tr>
<td>LDL-III (%)</td>
<td>-1.2 (-3.9, 3.5)</td>
<td>-4.4 (-9.0, 0.2)</td>
<td>0.72</td>
<td>1.00</td>
<td>1.00</td>
</tr>
<tr>
<td>LDL-IV (%)</td>
<td>0.7 (-0.5, 1.9)</td>
<td>0.5 (-0.7, 1.6)</td>
<td>1.00</td>
<td>1.00</td>
<td>1.00</td>
</tr>
<tr>
<td>HDL-C (mg/dL)</td>
<td>-2.1 (-3.9, -0.4)</td>
<td>-0.3 (-2.0, 1.4)</td>
<td>0.19</td>
<td>0.85</td>
<td>0.58</td>
</tr>
<tr>
<td>Triglycerides (mg/dL)</td>
<td>4.6 (-21.1, 30.3)</td>
<td>-16.8 (-41.9, 8.4)</td>
<td>0.44</td>
<td>0.18</td>
<td>0.77</td>
</tr>
<tr>
<td>TC:HDL</td>
<td>0.2 (-0.1, 0.5)</td>
<td>-0.4 (-0.7, -0.1)</td>
<td>0.002</td>
<td>0.007</td>
<td>0.09</td>
</tr>
<tr>
<td>Apo A-I (mg/dL)</td>
<td>-3.3 (-8.6, 2.0)</td>
<td>-6.0 (-11.2, -0.8)</td>
<td>1.00</td>
<td>0.36</td>
<td>1.00</td>
</tr>
<tr>
<td>Apo B (mg/dL)</td>
<td>2.0 (-3.0, 7.0)</td>
<td>-7.3 (-12.1, -2.4)</td>
<td>0.006</td>
<td>0.007</td>
<td>0.054</td>
</tr>
<tr>
<td><strong>Glucose Parameters</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fasting glucose (mg/dL)</td>
<td>-0.6 (-3.6, 2.4)</td>
<td>-2.2 (-5.1, 0.7)</td>
<td>1.00</td>
<td>1.00</td>
<td>1.00</td>
</tr>
<tr>
<td>Fasting insulin (µU/mL)</td>
<td>-0.4 (-3.1, 2.4)</td>
<td>-2.3 (-4.9, 0.4)</td>
<td>0.70</td>
<td>0.51</td>
<td>1.00</td>
</tr>
<tr>
<td>2-h glucose (mg/dL)</td>
<td>-5.4 (-22.0, 11.2)</td>
<td>-1.8 (-18.0, 14.4)</td>
<td>1.00</td>
<td>1.00</td>
<td>1.00</td>
</tr>
<tr>
<td>2-h insulin (µU/mL)</td>
<td>-10.6 (-38.1, 16.9)</td>
<td>-18.1 (-45.0, 8.8)</td>
<td>1.00</td>
<td>0.71</td>
<td>1.00</td>
</tr>
<tr>
<td>AUC glucose (OGTT)</td>
<td>-187 (-1519, 1144)</td>
<td>-869 (-2172, 434)</td>
<td>1.00</td>
<td>0.79</td>
<td>1.00</td>
</tr>
<tr>
<td>Outcome</td>
<td>Female</td>
<td>Male</td>
<td>$P$ value with no adjustment</td>
<td>$P$ value adjusted for baseline value</td>
<td>$P$ value adjusted for baseline value &amp; weight change</td>
</tr>
<tr>
<td>------------------------------</td>
<td>-------------------------</td>
<td>-----------------------------</td>
<td>------------------------------</td>
<td>--------------------------------------</td>
<td>------------------------------------------------------</td>
</tr>
<tr>
<td>AUC insulin (OGTT)</td>
<td>-376 (-2617, 1866)</td>
<td>-2225 (-4418, -32) $^2$</td>
<td>0.45</td>
<td>0.43</td>
<td>1.00</td>
</tr>
<tr>
<td>ISI</td>
<td>0.1 (-0.7, 0.8)</td>
<td>0.9 (0.2, 1.6) $^2$</td>
<td>0.13</td>
<td>0.13</td>
<td>0.63</td>
</tr>
<tr>
<td><strong>Inflammation and Fibrinolysis</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>PAI-1 (ng/mL)</td>
<td>-3.9 (-8.1, 0.3)</td>
<td>-5.1 (-9.1, -1.1) $^2$</td>
<td>1.00</td>
<td>1.00</td>
<td>1.00</td>
</tr>
<tr>
<td>CRP (mg/L)</td>
<td>-1.0 (-3.2, 1.2)</td>
<td>-1.2 (-3.3, 0.9)</td>
<td>1.00</td>
<td>1.00</td>
<td>0.82</td>
</tr>
<tr>
<td>IL-1 (pg/mL)</td>
<td>0.0 (-7.1, 7.2)</td>
<td>-3.9 (-10.9, 3.1)</td>
<td>1.00</td>
<td>1.00</td>
<td>1.00</td>
</tr>
<tr>
<td>IL-6 (pg/mL)</td>
<td>-6.2 (-20.7, 8.2)</td>
<td>-11.6 (-25.7, 2.5)</td>
<td>1.00</td>
<td>1.00</td>
<td>1.00</td>
</tr>
<tr>
<td>TNF-α (pg/mL)</td>
<td>-0.2 (-3.2, 2.8)</td>
<td>-2.7 (-5.7, 0.3)</td>
<td>0.46</td>
<td>1.00</td>
<td>1.00</td>
</tr>
<tr>
<td><strong>Sex Hormones</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Testosterone (ng/dL)</td>
<td>-0.9 (-25.1, 23.3)</td>
<td>42.1 (18.4, 65.8) $^2$</td>
<td>0.009</td>
<td>n/a</td>
<td>n/a</td>
</tr>
<tr>
<td>SHBG (nmol/L)</td>
<td>0.8 (-3.0, 4.5)</td>
<td>2.0 (-1.6, 5.7)</td>
<td>1.00</td>
<td>n/a</td>
<td>n/a</td>
</tr>
<tr>
<td>FAI</td>
<td>-0.1 (-4.3, 4.1)</td>
<td>0.0 (-4.1, 4.1)</td>
<td>1.00</td>
<td>n/a</td>
<td>n/a</td>
</tr>
</tbody>
</table>

$^1$All values are least squares mean (95% CI).

$^2$Significantly different from baseline, $P<0.05$. 
Table 4.4. Times per week that male and female subjects participated in strenuous, moderate, and easy physical activity$^1$.

**Strenuous Intensity**

<table>
<thead>
<tr>
<th></th>
<th>Baseline</th>
<th>Week 2</th>
<th>Week 4</th>
<th>Week 6</th>
<th>Week 8</th>
<th>Week 10</th>
<th>Week 12</th>
</tr>
</thead>
<tbody>
<tr>
<td>Female</td>
<td>0.5 ± 1.2</td>
<td>1.1 ± 1.6</td>
<td>0.8 ± 1.4</td>
<td>0.6 ± 1.4</td>
<td>0.9 ± 1.6</td>
<td>0.8 ± 1.9</td>
<td>1.0 ± 1.8</td>
</tr>
<tr>
<td>Male</td>
<td>0.9 ± 1.9</td>
<td>1.5 ± 2.8</td>
<td>1.7 ± 2.4$^2$</td>
<td>1.2 ± 2.3</td>
<td>2.0 ± 2.7$^2$</td>
<td>1.3 ± 1.9</td>
<td>1.6 ± 2.3$^2$</td>
</tr>
</tbody>
</table>

**Moderate Intensity**

<table>
<thead>
<tr>
<th></th>
<th>Baseline</th>
<th>Week 2</th>
<th>Week 4</th>
<th>Week 6</th>
<th>Week 8</th>
<th>Week 10</th>
<th>Week 12</th>
</tr>
</thead>
<tbody>
<tr>
<td>Female</td>
<td>1.9 ± 3.2</td>
<td>2.3 ± 2.6</td>
<td>2.9 ± 2.4$^2$</td>
<td>3.2 ± 4.3$^2$</td>
<td>3.6 ± 2.9$^2$</td>
<td>2.6 ± 2.3</td>
<td>3.2 ± 2.8$^2$</td>
</tr>
<tr>
<td>Male</td>
<td>1.8 ± 2.1</td>
<td>2.6 ± 2.2</td>
<td>2.5 ± 1.9</td>
<td>2.5 ± 1.9</td>
<td>2.3 ± 1.8</td>
<td>2.1 ± 2.0</td>
<td>3.0 ± 2.0$^2$</td>
</tr>
</tbody>
</table>

**Easy Intensity**

<table>
<thead>
<tr>
<th></th>
<th>Baseline</th>
<th>Week 2</th>
<th>Week 4</th>
<th>Week 6</th>
<th>Week 8</th>
<th>Week 10</th>
<th>Week 12</th>
</tr>
</thead>
<tbody>
<tr>
<td>Female</td>
<td>2.2 ± 1.5</td>
<td>2.1 ± 2.5</td>
<td>2.4 ± 4.3</td>
<td>3.0 ± 4.4</td>
<td>2.6 ± 4.5</td>
<td>2.5 ± 1.9</td>
<td>3.0 ± 6.2</td>
</tr>
<tr>
<td>Male</td>
<td>2.5 ± 2.0</td>
<td>1.9 ± 2.4</td>
<td>2.3 ± 2.1</td>
<td>2.2 ± 2.0</td>
<td>1.7 ± 1.9</td>
<td>3.7 ± 8.2</td>
<td>2.0 ± 2.1</td>
</tr>
</tbody>
</table>

$^1$ All values are mean ± SE.

$^2$ Significantly different from baseline, $P < 0.05$
**Table 4.5.** Percent change in biometric, lipid, glycemic, and hormonal endpoints in the study participant with polycystic ovary syndrome.

<table>
<thead>
<tr>
<th>Outcome</th>
<th>Percent Change</th>
</tr>
</thead>
<tbody>
<tr>
<td>Weight</td>
<td>0.41</td>
</tr>
<tr>
<td>Waist Circumference</td>
<td>-1.26</td>
</tr>
<tr>
<td>Body Fat Percentage</td>
<td>-2.1</td>
</tr>
<tr>
<td>Systolic Blood Pressure</td>
<td>-12.4</td>
</tr>
<tr>
<td>Diastolic Blood Pressure</td>
<td>-14.1</td>
</tr>
<tr>
<td>AUC Glucose</td>
<td>-8.9</td>
</tr>
<tr>
<td>AUC Insulin</td>
<td>-51.1</td>
</tr>
<tr>
<td>ISI</td>
<td>43.8</td>
</tr>
<tr>
<td>Testosterone</td>
<td>-23.1</td>
</tr>
<tr>
<td>SHBG</td>
<td>21.1</td>
</tr>
<tr>
<td>FAI</td>
<td>-36.5</td>
</tr>
<tr>
<td>LDL-C</td>
<td>-16.9</td>
</tr>
<tr>
<td>HDL-C</td>
<td>-16.0</td>
</tr>
<tr>
<td>Triglycerides</td>
<td>21.1</td>
</tr>
<tr>
<td>Total:HDL-C</td>
<td>4.8</td>
</tr>
</tbody>
</table>
Figure 4.1. Average percent change in biometric measurements, lipids and lipoproteins, glucose and insulin measures, and sex hormones by reproductive status.
Figure 4.2. Nutrient intake at week 12 from 3-day food records of the study participants. Values are mean ± SE.
References


CHAPTER 5

PROLONGED REDUCTION IN POSTPRANDIAL TESTOSTERONE LEVELS AFTER A HIGH-FAT, WESTERN MEAL COMPARED WITH A LOW-FAT, HIGH-FIBER MEAL IN WOMEN WITH POLYCYSTIC OVARY SYNDROME

Introduction

Polycystic ovary syndrome (PCOS) is one of the most common causes of infertility and anovulation in women. The most consistent biochemical feature of PCOS is hyperandrogenemia (1), which is characterized by an elevated total or free testosterone concentration. The hyperandrogenemia associated with PCOS can lead to peripheral hyperandrogenism as hirsutism, acne, and alopecia, and is also implicated in the pathological development of PCOS (2, 3). Reducing circulating androgens is often used as a surrogate outcome in clinical trials of women with PCOS (4). A reduction in circulating androgens is associated with increased menstrual cyclicity and fertility in women with PCOS (1, 5-7), and consequently is a treatment goal.

In women with PCOS, diet is an effective means of reducing androgens when accompanied by a weight loss of at least five percent of body weight (7-10). In light of this, there is current interest in whether a particular diet composition is more effective in lowering testosterone levels (8, 9, 11). Knowing the acute effects of meals with varying macronutrient content may lend insight into which diet composition is most beneficial for women with PCOS in the long term. Studies in men and also women with PCOS have demonstrated that testosterone levels are reduced as soon as two hours after a meal (12-
However, the short-term effect of varying diet composition on testosterone levels has not been studied in women.

The aim of the present study was to determine if a high fat, Western meal or a low-fat, high-fiber meal produces a greater postprandial reduction in testosterone levels in women with PCOS. Since people are generally in a postprandial state for the majority of the day, a reduction in postprandial testosterone levels in women with PCOS may lead to long-term improvements if the diet composition is maintained over a longer period of time.

Methods

Participants

I studied 15 women with PCOS between the ages of 19-40. Women were considered to have PCOS if they [1] had a history of chronic anovulation, determined by intermenstrual periods of \( \geq 45 \) days or \( \leq 8 \) menstrual cycles/y, and [2] had hyperandrogenism, determined by total testosterone \( >50 \) ng/dL or free androgen index \( >1.5 \) (6, 16, 17). Hyperprolactinemia was excluded by the measurement of prolactin, nonclassical adrenal 21-hydroxylase deficiency was excluded by the measurement of 17-hydroxyprogesterone, androgen-secreting tumors were excluded by the measurement of testosterone, and thyroid disorders were excluded by the measurement of thyroid-stimulating hormone (TSH).

All participants were in good health, were non-smokers, and were not pregnant or lactating. For at least 3 months before the study, all participants were not taking any medication known to affect sex hormones or carbohydrate or lipid metabolism.
Participants were excluded if they were anemic, had type 1 or type 2 diabetes, or drank more than two alcoholic drinks/d. The study was carried out in the General Clinical Research Centers at the Pennsylvania State University campuses located at the Milton S. Hershey Medical Center in Hershey, PA and at the University Park campus in University Park, PA. The study opened to accrual in March of 2005 and completed enrollment in October of 2006. The Institutional Review Board at the Pennsylvania State University College of Medicine approved the study, and each participant provided written informed consent.

**Study design**

Participants were screened prior to their first study visit to ensure that they were in the follicular phase of their menstrual cycle. To confirm that they were in the follicular phase, participants provided a blood sample for measurement of progesterone within five days of their first study visit. A progesterone level \( \leq 2 \text{ ng/dL} \) was used to confirm that the participant was in the follicular phase of their menstrual cycle. After the progesterone test, participants used a Clearblue Easy daily ovulation test daily until completion of the study to ensure that they did not ovulate during the study period. If the kit did detect ovulation, I asked that the participant reschedule their study visit in the week after they menstruated.

A random number table was used to randomize participants to one of two meal sequences using a 2x2 crossover design: [1] the low-fat, high-fiber meal at visit 1 and the high-fat, Western meal at visit 2, or [2] the high-fat, Western meal at visit 1 and the low-fat, high-fiber meal at visit 2. At least 7 days separated each visit to diminish the
likelihood of carryover effects. The nutrient composition of the meals is listed in Table 5.1. The high-fat, Western meal contained a Jimmy Dean sausage, egg, and cheese croissant with whole milk. The low-fat, high-fiber meal contained Fiber One cereal with 1% milk, Dannon yogurt + fiber, and a fruit salad. The high-fat, Western meal and low-fat, high-fiber meals were isocaloric and were 62% and 6% fat, 24% and 81% carbohydrate, and had 1g and 26.8g of fiber, respectively.

For three days prior to both study visits, participants followed a standard 2,000 calorie meal plan of approximately 30% fat, 55% carbohydrate, and 15% protein, and recorded all food and drinks consumed in a diet log. Participants were instructed to fast from 1900 h the night before each study visit and to avoid strenuous exercise and alcoholic beverages for at least 24 hours prior to each study visit.

On the morning of the two study visits, participants arrived at the General Clinical Research Center at 0700 h. A venicatheter was inserted into an antecubital or hand vein for collection of blood samples and the catheter was kept open with saline. A baseline blood sample was taken after the IV was inserted for measurement of progesterone, glucose, insulin, testosterone, and sex hormone binding globulin (SHBG).

Participants were then served the test meal and asked to consume it within 15 minutes. After each meal, a blood sample was taken at 30 minutes and then every hour for six hours for measurement of testosterone, SHBG, glucose, and insulin. During this time participants remained comfortably seated or reclined and were allowed to read, listen to music, and watch TV. Participants were allowed to consume water and were allowed to go to the bathroom as needed. After the last blood draw, the catheter was removed and participants were given a complementary meal.
Clinical and biochemical measurements

Each participant had a dual energy x-ray absorptiometry (DXA) scan to obtain body composition data including percent body fat and lean body measurements. The DXA scans were performed using a Hologic QDR-4500W in fan-beam mode (Hologic Corp, Waltham MA). Proper operation of the x-ray subsystem was verified daily using a spine phantom and tissue composition calibration was performed at least once/wk on a tissue equivalent phantom.

Serum and plasma were separated by centrifugation for 15 minutes at 3,200 rpm and 4°C, then aliquoted into cryovials and stored at −80°C until analysis. Plasma glucose concentration was determined by the glucose oxidase technique using the Yellow Springs Instruments Model 2300 Stat Plus Glucose Analyzer. Insulin was measured using radioimmunoassay kits from Linco (St. Charles, MO) (18). Serum levels of testosterone, estradiol, and progesterone were determined by radioimmunoassay using commercial reagents (Diagnostic Products Corporation, Los Angeles, CA). Intra-assay CVs were: testosterone 5-7%; estradiol 3.5 – 5.8%; progesterone 3-7%. Inter-assay CVs were: testosterone 7-11%; estradiol 4-9%; progesterone 4-8%. Analytical sensitivity for the steroid assays were; testosterone 15 ng/dl; estradiol 10 pg/ml; progesterone 0.5 ng/ml. SHBG was measured by Immunoradiometric Assay (IRMA) (Diagnostic Systems Laboratories, Webster, TX). Intra-assay CVs for SHBG were 4-6% while inter-assay CVs were 6-9%.
Statistical Analysis

Power Calculation

I proposed a sample size of 16 subjects for this 2×2 crossover design. The justification for the sample size of 16 subjects follows:

The sample size formula for a two-sided alternative hypothesis is given by the expression

\[ N = \left( z_{1-\alpha/2} + z_{1-\beta} \right)^2 \frac{\sigma^2}{\Delta^2} \]

where \( N \) is the total sample size, equally allocated over the two sequences, \( \alpha \) represents the probability of a Type I error (significance level), \( \beta \) represents the probability of a Type II error (1 - \( \beta \) represents statistical power), \( z_{1-\alpha/2} \) and \( z_{1-\beta} \) represent appropriate percentiles from the standard normal distribution, \( \sigma^2 = 1.0 \omega_{AA} + 1.0 \omega_{BB} - 2.0 \omega_{AB} + \sigma_A^2 + \sigma_B^2 \) represents the standard deviation, and \( \Delta = \mu_A - \mu_B \) represents the effect size. The Type I error, \( \alpha \), was set equal to 0.05 (5% significance level), and \( \beta \) is set equal to 0.20 (80% statistical power). The standard normal percentiles corresponding to these choices are 1.96 and 0.84, respectively.

I assumed that \( \omega_{AA} = \omega_{BB} = \omega_{AB} \) and \( \sigma_A^2 = \sigma_B^2 \); thus, \( \sigma^2 = 2\sigma_A^2 \). A sample size of 16 subjects yielded 80% statistical power for a two-sided, 0.05 significance level test to detect a standardized effect size of \( \Delta/\sigma = 1.0 \), i.e., an effect size of 1.0 standard deviation. I anticipated a very low withdrawal rate (< 10%), so that the statistical power is would be affected only slightly by dropouts (if there is a 10% withdrawal rate, then there is 80% statistical power to detect a standardized effect size of 1.1). For the primary outcome variable of circulating testosterone level change from baseline, an estimate of the
standard deviation is $\sigma = 20$ ng/dL. Thus, the effect size for this trial was also 20 ng/dL and is considered clinically meaningful.

**Statistical Analysis**

A mixed-effects linear model was used to compare testosterone, SHBG, glucose, insulin, and free androgen index (FAI) levels at each time point with baseline values, as well as to compare responses to the different diets at identical time points (19). All variables were analyzed using SAS PROC MIXED (SAS v. 9.1, Cary, NC, USA). Models included treatment, time, and visit number as fixed effects and participant as a random effect. $P$-values and confidence intervals were adjusted for multiple comparisons testing using Tukey’s multiple comparison procedure.

For each subject, the area under the curve (AUC) for each outcome was calculated using the trapezoidal rule. The FAI was calculated as the ratio of total testosterone divided by SHBG, multiplied by 100 (20). A mixed-effects linear model, as described above, was then fit to the AUC data. To reduce bias, I incorporated all available data on randomized patients in the statistical analysis. This is known as the “intent-to-treat” analysis. A $P$ value < 0.05 was used to determine statistical significance.

**Results**

Fifteen women were enrolled in the study and all women completed both study visits. Fifty two women were screened in order to enroll these 15 participants. The study period ended before the final participant could be enrolled. The participants were age 19 to 39 years and had a BMI ranging from 19.9 to 53.5 kg/m$^2$. Seven participants were
normal weight (BMI 18.5-24.9), three were overweight (BMI 25-29.9), and five were obese (BMI $\geq 30$). Descriptive statistics of the study participants are listed in Table 5.2. Fasting glucose and SHBG were significantly different between the two randomization groups at baseline ($P=0.002$ and $P=0.03$, respectively). However, there was no effect of the order of treatment on the outcomes measured. Of the 15 participants, 12 were Caucasian, 1 was Hispanic, 1 was Asian, and 1 was African American. Two participants were determined to be ovulatory at both study visits based on their progesterone level, most likely due to problems using the daily ovulation kit. The two ovulatory participants were included in our intent to treat analysis. Excluding these participants from the analysis did not significantly affect the results.

Testosterone and SHBG levels at baseline and after the low-fat, high-fiber meal and the high-fat, Western meal are shown in Figure 5.1. Testosterone levels decreased within two hours after both meals by 27% ($P<0.001$). However, testosterone levels remained below premeal values for four hours after the low-fat, high-fiber meal ($P<0.004$) and for six hours after the high-fat, Western meal ($P<0.004$). Testosterone levels began to return to baseline 60 minutes after consuming the low-fat, high-fiber meal, whereas testosterone levels continued to drop 60 minutes after the high-fat, Western meal and did not begin to rise until 120 minutes after the meal. Testosterone levels were significantly lower at 60 minutes after the low-fat, high-fiber meal compared with the high-fat, Western meal ($P=0.008$).

SHBG levels increased compared with baseline at 60, 300, and 360 minutes after the low-fat, high-fiber meal ($P<0.05$). There were no significant changes in SHBG levels after the high-fat, Western meal and no significant differences in SHBG levels
between groups. The change in the free androgen index was similar to the testosterone response (Figure 5.2). There were no differences in the area under the curve for testosterone, SHBG, or the free androgen index after the two test meals (Table 5.3).

Glucose and insulin levels at baseline and after the low-fat, high-fiber meal and high-fat, Western meal are shown in Figure 5.3. Glucose levels increased 30 minutes after both meals ($P < 0.001$), as well as 60 minutes after the low-fat, high-fiber meal ($P < 0.001$). Glucose levels were significantly higher at 30 and 60 minutes after the low-fat, high-fiber meal compared with the high-fat, Western meal ($P < 0.003$). Compared with premeal values, insulin levels were increased 30 and 60 minutes after the high-fat, Western meal and the low-fat, high-fiber meal ($P < 0.001$), as well as 120 minutes after the low-fat, high-fiber meal ($P < 0.001$). Insulin levels were almost two fold higher 30, 60, and 120 minutes after the low-fat, high-fiber meal compared with the high fat meal ($P < 0.03$). There were no significant differences in the area under the curve for glucose or insulin following the two test meals (Table 3).

**Discussion**

To our knowledge, this is the first study evaluating the short-term effects of meal composition on testosterone levels in women with PCOS. I observed a 27% reduction in testosterone levels within two hours after eating a high-fat, Western meal and a low-fat, high-fiber meal in women with PCOS. Testosterone levels were reduced for two hours longer after the high-fat, Western meal compared with the low-fat, high-fiber meal. However there was no difference in the area under the curve.
The postprandial reduction in testosterone levels that I observed is consistent with previous findings in men and in women with PCOS. In several studies in men, testosterone levels decreased 20-25% within two hours after ingestion of a meal (12, 15). Likewise, Panidis et al. reported that serum testosterone levels decreased significantly three hours after oral administration of 75g dextrose in insulin resistant and insulin sensitive women with PCOS, as well as in lean and overweight women without PCOS (21). In another study, Parra et al. measured changes in serum androgens after a 725-calorie breakfast (55% CHO, 31% fat) in six women with PCOS (13). In five of the six women, free testosterone levels decreased an average of 62% at 90 minutes. The greater postprandial reduction in testosterone levels in this study could possibly be due to a higher calorie and macronutrient content of the meal.

A few studies in men have investigated the role of diet composition on postprandial testosterone levels, but the results are inconclusive. Meikle et al. reported that total and free testosterone levels were approximately 30% lower for four hours after consuming an 800-calorie high-fat milkshake (57% fat, 34% carbohydrate). However, they observed no significant change in testosterone levels following a mixed carbohydrate and protein milkshake (26% protein, 73% carbohydrate) (14). Habito and Ball compared meals with different sources of protein (soy vs. meat), different amounts of saturated fat (lean meat vs. meat with animal fat) and different sources of fat (animal fat vs. vegetable oil) in 15 healthy men (15). Mean testosterone levels decreased significantly 2 hours after all of the meals except the high animal fat meal, suggesting that the type of fat influenced postprandial androgen levels. Although Habito and Ball did not observe a change in testosterone levels after a high saturated fat meal, Volek et al.
observed a 22% and 23% decrease and total and free testosterone levels following a fat-
rich meal (86% fat) containing 52g saturated fat, 59g monounsaturated fat and 12g
polyunsaturated fat in 11 healthy men (12). Taken together, there is not a consistent
effect of diet composition in men in previous studies.

The results of the present study suggest that in women with PCOS, there is a
reduction in testosterone levels after meals of varying composition. The postprandial
testosterone response in our study is strikingly similar to the postprandial cortisol and
DHEA response observed in a recent study by Kasim-Karakas et al. (11). This study
compared DHEA and cortisol levels after a high-protein drink (75 g 98% pure, intact
whey protein isolate) compared with a 75g OGTT in women with PCOS. Their rationale
for measuring DHEA and cortisol was that cortisol can cause insulin resistance and
DHEA is a substrate for peripheral testosterone synthesis. Serum cortisol and DHEA
levels were significantly reduced from baseline during the 5 hour period after the high-
protein drink, whereas following the OGTT, serum cortisol and DHEA concentrations
were below baseline until the third hour and were significantly higher than after the high-
protein meal at the fourth and fifth hour. Postprandial glucose and insulin levels were
significantly higher than baseline for two hours after the OGTT but not after the high-
protein drink. When this study is considered with our study, it appears that a high-
carbohydrate meal promotes the fastest return to baseline levels of testosterone, DHEA,
and cortisol compared with a high-fat or high-protein meal, in addition to increasing
postprandial glucose and insulin levels.

Although hyperinsulinemia is implicated in the pathogenesis of PCOS, it is
unlikely that insulin is directly responsible for the postprandial changes in testosterone
levels that I observed. Several investigators have reported no relationship between the change in insulin and testosterone levels after a meal (12, 15) or after an oral glucose load (22, 23). In addition, no relationship was observed between androgen and insulin levels following IV infusion of glucose or insulin (23-25). In support of this, Parra et al. conducted an elegant study in which women with PCOS were fed a 725 calorie breakfast with simultaneous infusion of epinephrine and propranolol to cause an acute blockade on pancreatic insulin secretion. After 90 minutes, the infusion was stopped and blood samples were collected for the remaining 90 minutes. The investigators observed that testosterone levels decreased after the breakfast despite the suppression of pancreatic insulin secretion. There was also no acute increase in androgens after cessation of the epinephrine and propranol infusion. These data suggest that insulin itself is not responsible for the postprandial decline in testosterone levels.

The prolonged reduction in testosterone levels after a high fat meal could be due to slower postprandial blood flow to the mesenteric artery, resulting in a longer period of nutrient absorption, or to greater portal blood flow, resulting in increased testosterone metabolism and clearance. Previous studies have reported that there is a faster peak in mean velocity and volume flow in the superior mesenteric artery following a high carbohydrate meal compared with a high fat meal (26, 27). In addition, Høst et al. observed that Doppler estimated portal blood flow increased 107% one hour after a fat-rich meal (80-83% fat), compared with a 62% increase after an isocaloric, isovolumetric carbohydrate rich meal (81% carbohydrate) in healthy non-obese males (28). The differing responses in mesenteric and portal blood flow after high carbohydrate and high fat meals could be due to differences in gastric emptying, release of gastrointestinal
hormones, and sympathetic nervous system activation (26, 29). The specific mechanisms are unknown (28).

A limitation of this study is that since I did not control for a single nutrient, it is not possible to determine what specific dietary component(s) causes the prolonged reduction in testosterone levels after the high-fat, Western meal. In designing this study, my aim was to test two meals that had very different nutrient compositions to increase the likelihood of detecting any differences in postprandial testosterone levels. Further studies are needed to determine the specific nutrients that affect testosterone levels and the mechanisms involved.

Since testosterone levels are reduced as long as six hours after eating, eating small, frequent meals throughout the day could be a means of reducing testosterone levels in women with PCOS. In theory, keeping the body in the postprandial state could suppress testosterone levels and prevent a return to baseline levels. Spreading out food intake over 6 or more small meals compared with three or fewer large meals may also have additional benefits in women with PCOS. In clinical trials, eating small, frequent meals reduced day-long insulin levels and reduced cholesterol and triglyceride levels compared with an isocaloric diet with three or fewer meals (30-32). Some observational studies have also reported a lower body weight and body fat percentage in people who consume frequent meals compared with fewer meals (33-35). However these findings have not been confirmed in clinical studies. Further studies are needed to test the effect of meal frequency on testosterone levels.

Despite limitations, our results do support the dietary recommendations advocated by several clinicians to choose diets that minimize postprandial glucose and insulin levels.
in PCOS. Additionally, this study suggests that altering meal composition can have reproductive benefits in women with PCOS without weight loss, which is an interesting concept that warrants further investigation.
Table 5.1. Energy and nutrient composition of the two test meals.

<table>
<thead>
<tr>
<th></th>
<th>High-Fat, Western Meal</th>
<th>Low-Fat, High-Fiber Meal</th>
</tr>
</thead>
<tbody>
<tr>
<td>Calories</td>
<td>600</td>
<td>600</td>
</tr>
<tr>
<td>Carbohydrate (g)</td>
<td>35 (24%)</td>
<td>133 (81%)</td>
</tr>
<tr>
<td>Protein (g)</td>
<td>20 (13%)</td>
<td>20 (13%)</td>
</tr>
<tr>
<td>Total fat (g)</td>
<td>41 (62%)</td>
<td>4 (6%)</td>
</tr>
<tr>
<td>Saturated fat (g)</td>
<td>14 (22%)</td>
<td>2 (2%)</td>
</tr>
<tr>
<td>Total fiber (g)</td>
<td>1</td>
<td>26.8</td>
</tr>
<tr>
<td>Cholesterol (mg)</td>
<td>158</td>
<td>12.2</td>
</tr>
</tbody>
</table>
Table 5.2. Baseline characteristics of the study subjects (n = 15)\(^1\).

<table>
<thead>
<tr>
<th></th>
<th>High-Fat, Western → Low-Fat, High-Fiber (n=7)</th>
<th>Low-Fat, High-Fiber → High-Fat, Western (n=8)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>26.4 ± 5.4</td>
<td>27.4 ± 7.7</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>69.5 ± 17.7</td>
<td>90.9 ± 40.0</td>
</tr>
<tr>
<td>BMI (kg/m(^2))</td>
<td>25.6 ± 6.0</td>
<td>33.0 ± 13.1</td>
</tr>
<tr>
<td>Systolic blood pressure (mmHg)</td>
<td>108.1 ± 7.0</td>
<td>108.3 ± 18.1</td>
</tr>
<tr>
<td>Diastolic blood pressure (mmHg)</td>
<td>65.6 ± 4.6</td>
<td>65.9 ± 10.2</td>
</tr>
<tr>
<td>Waist circumference (cm)</td>
<td>83.2 ± 12.7</td>
<td>103.9 ± 30.8</td>
</tr>
<tr>
<td>Body Fat Percentage</td>
<td>34.4 ± 4.1</td>
<td>33.0 ± 10.6</td>
</tr>
<tr>
<td>Testosterone (ng/dL)</td>
<td>62.6 ± 19.7</td>
<td>56.1 ± 18.8</td>
</tr>
<tr>
<td>SHBG (nmol/L)</td>
<td>61.1 ± 42.1</td>
<td>32.6 ± 14.7(^2)</td>
</tr>
<tr>
<td>Free Androgen Index</td>
<td>5.4 ± 3.4</td>
<td>7.5 ± 4.7</td>
</tr>
<tr>
<td>Fasting glucose (mg/dL)</td>
<td>81.1 ± 5.0</td>
<td>90.6 ± 9.8(^2)</td>
</tr>
<tr>
<td>Fasting insulin (µU/mL)</td>
<td>9.1 ± 5.5</td>
<td>16.1 ± 13.3</td>
</tr>
<tr>
<td>Cholesterol (mg/dL)</td>
<td>160.6 ± 18.0</td>
<td>161.8 ± 25.6</td>
</tr>
<tr>
<td>Triglycerides (mg/dL)</td>
<td>112.4 ± 81.4</td>
<td>105.1 ± 68.9</td>
</tr>
</tbody>
</table>

\(^1\)Data represent mean ± SD. BMI, body mass index; SHBG, sex hormone binding globulin.

\(^2\)Significantly different between groups, \(P<0.05\).
Table 5.3. Area under the curve values after the high fat and high carbohydrate meals$^1$.

<table>
<thead>
<tr>
<th></th>
<th>High-Fat, Western Meal</th>
<th>Low-Fat, High-Fiber Meal</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Testosterone (ng/dL * min)</td>
<td>17,541 ± 4,873</td>
<td>17,214 ± 4,747</td>
<td>0.86</td>
</tr>
<tr>
<td>SHBG (nmol/L * min)</td>
<td>15,884 ± 12,479</td>
<td>18,171 ± 14,910</td>
<td>0.30</td>
</tr>
<tr>
<td>Free Androgen Index (units * min)</td>
<td>2,021 ± 1406</td>
<td>1,815 ± 1,157</td>
<td>0.27</td>
</tr>
<tr>
<td>Glucose (mg/dL * min)</td>
<td>31,852 ± 2,528</td>
<td>32,256 ± 2,778</td>
<td>0.61</td>
</tr>
<tr>
<td>Insulin (µU/mL * min)</td>
<td>10,282 ± 6,935</td>
<td>16,549 ± 12,986</td>
<td>0.24</td>
</tr>
</tbody>
</table>

$^1$Data represent mean ± SD. SHBG, Sex hormone binding globulin.
**Figure 5.1.** Least squares mean (± SE) change in testosterone and SHBG levels after a high-fat, Western meal (---) and a low-fat, high-fiber meal (—) in women with PCOS.

![Graph showing changes in testosterone and SHBG levels over time](image1)

* p<0.05 for within group comparison to baseline; ‡ p<0.05 for between group comparison

**Figure 5.2.** Least squares mean (± SE) change in the free androgen index (FAI) after a high-fat, Western meal (---) and a low-fat, high-fiber meal (—) in women with PCOS.

![Graph showing changes in FAI over time](image2)

* p<0.05 for within group comparison to baseline
‡ p<0.05 for between group comparison
**Figure 5.3.** Least squares mean (± SE) change in glucose and insulin levels after a high fat (---) and high carbohydrate (—) meal in women with PCOS

* p<0.05 for within group comparison to baseline; † p<0.05 for between group comparison
References


CHAPTER 6

SUMMARY, STRENGTHS AND LIMITATIONS, AND FUTURE DIRECTIONS

Insulin resistance is implicated in the pathogenesis of many chronic conditions including metabolic syndrome and polycystic ovary syndrome (1). As a result, high-fiber diets are frequently recommended because fiber lowers postprandial glucose and insulin levels, leading to greater insulin sensitivity (2). Whole grain foods are advocated because they are a rich source of dietary fiber and also contain other protective nutrients and phytochemicals and are associated with reduced risk of CVD (3, 4).

My thesis research addressed two important questions regarding whole grains and high-fiber foods that have not been tested in clinical trials. The first question is, “Does including whole grains into a hypocaloric diet enhance weight loss and reduce cardiovascular disease risk?” Since cardiovascular disease is the leading cause of death in the United States and being overweight or obese increases one’s risk (5), determining which foods and dietary patterns lead to greater weight loss and reduced risk of CVD can have a beneficial public health impact.

The second question that my thesis research addressed is, “Does a meal high in fiber reduce postprandial testosterone levels in women with polycystic ovary syndrome more than low fiber foods?” Elevated testosterone levels are a clinical feature of polycystic ovary syndrome, and treatments that reduce testosterone levels improve fertility, as well as reduce acne, hirsutism, and hair growth (6). If there is a greater reduction in testosterone levels after a particular meal, it would indicate that there could be long term improvements in testosterone levels if the diet composition was maintained over a longer period of time.
In the first study, 50 men and women with metabolic syndrome received dietary advice to eat either avoid whole grain foods or to have all of their grain servings each day from whole grains for 12 weeks. Body weight, the primary endpoint, was significantly reduced in both groups at the end of the study period. However, there was no significant difference in weight loss between the two diet groups. Since participants in both groups were making changes to reduce body weight and CVD risk, it is not possible to conclude that whole grains affected weight loss. However, my findings do demonstrate that weight loss can be achieved when whole grains are incorporated into the diet, and that there may be other benefits to the cardiovascular risk profile beyond those achieved by weight loss alone.

The most interesting finding in this study was that CRP levels decreased an average of 38% in the whole grain group, but were unchanged in the refined grain group despite similar weight loss. This was unexpected since previous studies have reported that CRP decreases in relation to the amount of weight that is lost. However, I did not observe any significant changes in cytokines that affect inflammation and CRP production including IL-1, IL-6, and TNF-α. Since many of the samples were below the level of detection for the cytokine assays I used, retesting these samples using an assay with a greater sensitivity would be needed to confirm this finding.

Participants in both groups had significant improvements in other CVD risk factors including a decrease in total and LDL cholesterol in the whole grain group, and a decrease in PAI-1 and systolic blood pressure in the refined grain group. However, the changes between groups were not significantly different from each other. Since these changes were correlated with weight loss, it reinforces previous, consistent findings that
weight loss improves CVD risk factors. Overall, men had greater improvements in several outcomes compared with women including total cholesterol and the total:HDL cholesterol ratio, however, these differences were largely due to differences in baseline values and changes in body weight.

In the second study, I evaluated postprandial changes in testosterone, SHBG, glucose, and insulin levels following a high-fat, Western meal and a low-fat, high-fiber meal in 15 women with PCOS. Testosterone levels of the study participants decreased within two hours after both meals by 27%, confirming that there is a significant postprandial reduction in testosterone levels in women with PCOS.

The participants’ testosterone and FAI levels remained below premeal values for four hours after the low-fat, high-fiber meal compared with six hours after the high-fat, Western meal. In designing this study, my goal was to see if a “healthy” low-fat, high-fiber meal reduces testosterone levels compared with an “unhealthy” meal high in saturated fat and low in fiber. However, I saw a greater reduction in testosterone levels after the “unhealthy” meal. Although it is possible that having more fiber in the diet diminishes the reduction in testosterone, it is more likely that this effect is due to the meal having more carbohydrate and/or less fat since fiber is not absorbed.

Both the whole grain and meal studies have several strengths and limitations. A strength of the whole grain study is that it was conducted in a free living population. This increases the study’s external validity, or the ability of the results to generalize to the larger population. A limitation of this is that other changes in the diet or activity could account for the changes I observed, even though participants were given the same instructions on all aspects of their diet except for instructions on what type of grains they
should eat. A controlled feeding study that manipulates only the source of grains would be needed to be sure that the effects observed are truly due to whole grains.

A second aspect of the whole grain study that is both a strength and limitation is that participants in only the whole grain group were given a target number of grain servings. Having a whole grain serving recommendation ensured that participants in the whole grain group were eating a high quantity of whole grain foods. This increased the likelihood of detecting biochemical changes due to whole grains. A limitation of only giving grain recommendations to the whole grain group is that these participants may have been eating more food in order to get in their total number of whole grain servings. This increase in calories may have limited weight loss in the whole grain group.

A final strength of the whole grain study is the 94% completion rate, which is much higher than was expected for a lifestyle study. There are many factors that I attribute to the high completion rate. One is that all of the study participants had individualized diet counseling that included informational lessons. Even if participants were not losing weight, they still felt that they were learning. Second, the dietitians working on the study were experienced in counseling for weight loss and had families themselves, so they were very understanding of the “life on the run”. The staff at the GCRC also made the study participants feel at home and like they were being part of important research. Even the oral glucose tolerance test was not a bother for most of the participants because they were so well taken care of. The short study period also made the study manageable for most people.

In the case of the meal study, a limitation is that several nutrients were manipulated in each of the test meals, so the effects observed cannot be attributed to a
single dietary component. However, the purpose of the study was to determine if diet composition can affect testosterone levels. By having dramatically different meal compositions, I was able to detect differences in testosterone levels that I may not have been able to detect otherwise. Other strengths of the meal study are that it had a long postprandial period and a 100% completion rate. In addition, having a crossover design reduced the variance and increased the statistical power to detect differences.

Although these two studies provide new information on the short-term (6 hours) and longer-term (12 weeks) effects of whole grain and high fiber foods, many questions remain unanswered that can be addressed in future studies.

The observation that C-reactive protein was reduced following consumption of whole grain foods is a new finding that needs to be confirmed in a more controlled setting. One way to do this would be to conduct a controlled feeding study comparing whole grains with refined grains. A good example of controlled feeding study that did this was published by Pereira et al. in 2002 (7). This study was a randomized, crossover controlled feeding trial with two 6-week feeding periods and included 11 overweight or obese hyperinsulinemic adults. One menu contained refined grains, which contained no bran or germ and little fiber, and the other contained whole grains. The whole-grain diet was created by substituting an equal volume of whole-grain food items for the refined-grain products. The food was otherwise identical in the 2 treatment periods. In this study, insulin sensitivity was improved when subjects were on the whole grain diet compared with the refined grain diet.

Future studies may also consider changes in gene expression in adipose tissue in addition to blood levels of inflammatory markers. This would require an adipose tissue
biopsy from subcutaneous abdominal tissue at the beginning and end of the study period, which is a more invasive procedure than a blood draw. Changes in gene expression could be profiled using microarrays, which contain probes for several thousand genes. The results of microarrays are typically confirmed using real-time quantitative polymerase chain reaction (PCR). This genetic screening technique could help elucidate what molecular pathways are affected by whole grains.

Future studies are also needed to determine if whole grain foods affect energy intake and weight loss. The biggest challenge I foresee in studying this is in designing a study that is not confounded by other differences in diet. One option would be a controlled feeding study where participants are randomized to a whole grain or refined grain diet group and all of their meals are provided. The quantity of food that participants would be given would be 25% more than the amount needed to meet their energy needs to allow self-selection for quantity of food (8). Energy and nutrient intakes of the study participants could be calculated by weighing the amount of uneaten foods and subtracting this from the total amount of food given. The drawback of this study design is that it is cost and labor intensive. However, a crossover study design could be used, which would reduce the number of subjects that would be needed.

A second study design that would be less cost and labor intensive, but also less controlled, would be a study similar to the one that I conducted, but where participants in the whole grain and refined grain groups were both be given a target number grain servings. All participants would also be given dietary counseling and instruction on appropriate food choices, however a recommended number of servings for these foods
would not be given. The draw back of this study design is that other differences in dietary intake could still affect calorie intake and weight loss.

A final future direction would be to determine the long-term effects of whole grain foods on weight loss. My study will address this question by having a 1-year follow-up. At this visit, participants will complete a 3-day diet recall and answer a question regarding how many whole grain servings they eat in the average week. However, since participants were not given specific dietary instructions at the end of the 12-week study period, this is more of an observational analysis than a randomized study. Determining the long term effects of whole grains versus refined grains with a randomized, clinical trial could be difficult because many people are unwilling to avoid whole grain foods for an extended period of time.

Regarding future directions for the meal study, since testosterone levels are reduced ~25% in the two hour period after a high-fat and a low-fat meal, as indicated by my study and previous studies in men and women with PCOS, an interesting opportunity for future research is to test whether increasing meal frequency reduces testosterone levels. Some investigators have reported that eating more frequent meals reduces day-long insulin and triglyceride levels, suggesting that eating frequent meals may be beneficial to women with PCOS. This could be carried out as a one-day, crossover study comparing day long testosterone levels after eating identical foods spread out either over 1-2 meals or over 6 meals.

A second direction for future research would be to determine which nutrients affect testosterone levels and what mechanisms are involved. This could involve comparing postprandial responses to meals that are primarily carbohydrate, protein, and
fat. Liquid meals could be used to facilitate this (9, 10). Future studies should also evaluate changes in FSH and LH pulsatility since these hormones regulate testosterone production.

A final direction for future research is to determine the long-term effects of varying diet composition in women with PCOS. The study that I have conducted indicates that diet composition affects testosterone levels in the short term. To date, two studies have compared a 55% carbohydrate with a 40% carbohydrate diet during 4-12 weeks of weight loss and found similar improvements in both groups (11, 12). Since I have shown that there is a prolonged reduction in testosterone levels after a very low-carbohydrate meal, and several studies have shown greater weight loss and improvements in CVD risk factors after a very low carbohydrate diet compared with a low fat diet (13-16), it is pertinent to test whether there are metabolic and reproductive benefits of a very low carbohydrate diet in women with PCOS. A low carbohydrate diet has been advocated for treatment of PCOS because it reduces fasting insulin concentrations and insulin sensitivity, which would be expected to improve the endocrine profile (17).

Given the strengths of my thesis research and the limitations noted, the studies I conducted show that whole grains and high fiber foods have metabolic and reproductive effects in metabolic syndrome and polycystic ovary syndrome. Specifically, I found that a diet high in whole grain foods reduced CRP, an important predictor of cardiovascular events, compared with a high in refined grain foods. Additionally, a high-fiber, low-fat meal reduced postprandial testosterone levels in women with PCOS and had a faster return to baseline levels compared with a high-fat, Western meal. However, further research is needed before recommendations can be made. The evidence available to date
indicates that weight loss is the most effective strategy in treating metabolic syndrome and polycystic ovary syndrome, so the diet composition that can best achieve weight loss is most appropriate for treating these insulin resistant conditions.
References


8. Gerhard GT, Ahmann A, Meeuws K, McMurry MP, Duell PB, Connor WE. Effects of a low-fat diet compared with those of a high-monounsaturated fat diet


APPENDIX A

WHOLE GRAIN STUDY
RECRUITMENT MATERIALS
Weight Loss and Heart Disease Prevention Study

- Are you between 20 and 65 years of age?
- Do you have some weight you’d like to lose?
- Do you want to change your eating habits in order to lose weight?

If you answered YES to these questions, you may be eligible to participate in a nutrition research study.

The purpose of this study is to determine how whole grains affect weight loss and risk of heart disease

Requirements for Participation
- Men and women ages 20-65
- Non-smoker
- Not pregnant

Volunteers will receive compensation for their participation

Interested Persons Please Contact:
Heather Katcher at 1-866-PSU-DIET (1-866-778-3438) or e-mail huk107@psu.edu

This study is under the direction of Penny Kris-Etherton, Ph.D., R.D., Department of Nutritional Sciences, Penn State University and Richard Legro, M.D., Department of Obstetrics and Gynecology, Penn State Milton S. Hershey Medical Center

This research study has been approved by the Institutional Review (IRB) Board under federal regulations, at Penn State Hershey Medical Center.
Weight Loss and Heart Disease Prevention Study

- Are you between 20 and 65 years of age?
- Do you have at least 20 pounds you’d like to lose?
- Do you want to change your eating habits in order to lose weight?

If you answered YES to these questions, you may be eligible to participate in a nutrition research study.

The purpose of this study is to determine how whole grains affect weight loss and risk of heart disease.

Requirements for Participation

- Men and women ages 20-65
- Non-Smoker
- Not Pregnant
- Not taking medications for cholesterol

Volunteers will receive compensation for their participation.

Interested Persons Please Contact:
1-866-PSU-DIET (1-866-778-3438) and leave your name and phone number or e-mail: huk107@psu.edu

This study is under the direction of Penny Kris-Etherton, Ph.D., R.D., Department of Nutritional Sciences, Penn State University and Richard Legro, M.D., Department of Obstetrics and Gynecology, Milton S. Hershey Medical Center.

This research study has been approved by the Institutional Review (IRB) Board under federal regulations, at Penn State Hershey Medical Center.
Weight Loss and Cardiovascular Disease Prevention via a Whole Grain Diet in Men and Women with Metabolic Syndrome

Telephone Interview Form

Date ____________
Interviewer: _____________________

1. Subjects call us in response to an advertisement
2. We give a synopsis of the study:

This research is being done to see if whole grains and a weight loss diet affect heart disease risk factors. This study involves consuming a reduced-calorie diet with or without whole grains for three-months. If you meet the criteria for the study and would like to participate, you will meet with a nutritionist to develop a balanced meal plan for the study and will be asked to eat either zero or about six servings of whole grain foods each day. You will continue to meet with the nutritionist every other week to achieve weight loss and a balanced diet. At each bi-weekly visit we will measure your weight, waist circumference, body composition and blood pressure and these visits will be approximately 45 minutes. At the beginning and end of the study, you will have a three-hour visit where we will measure your body composition, take a blood sample, and perform a blood test to determine how your body responds to a sugary drink. For these two visits, you would need to fast for 10 hours prior to arriving for your visit and would have an IV inserted. Are you still interested in the study?"

YES _____ (continue with interview)
NO _____ (thank them for their time and interest)

“I will be asking you some questions about your health information and medical history to determine if you are eligible for this research. By answering these questions, you are consenting to allow us to use this information to pre-screen you for the study. With your permission we will retain this information in the locked research office, and if you are interested in participating in additional studies in our department, we will contact you for future studies. If you do not want us to keep your information, we will destroy it following the study.” Do you want us to keep your information at the end of this study so that we can contact you for future studies?

YES _____ NO _____

1. Please give us your:

Name ______________________________________ Date of Birth ____________
Home address

____________________________________________

Daytime Phone# ___________________ Evening Phone # ___________________
E-mail address__________________________
2. What is your age? __________
   Your Height (ft and in)__________
   Your Weight (lbs) __________

   **Interviewer:**
   Age between 20 and 65 y □Yes □No
   BMI >30 □Yes □No

3. For women only:
   (a) Are you currently pregnant or wishing to become pregnant during the next 3 months?
      □ Yes □ No
   (b) Are you currently lactating or been lactating any time during the last 3 weeks?
      □ Yes □ No
   (c) Are you currently taking an oral contraceptive? □ Yes □ No
      If yes, please specify the type of medication used and duration of use:
      _______________________________________________________
      _______________________________________________________

5. Have you lost any weight during the past 6 months? □ Yes □ No
   If yes, how much:________________________________________

6. Are you currently on a weight-loss diet or program? □ Yes □ No
   If yes, please specify: ____________________________________
   How long have you been on this weight loss diet? _________
   How much weight have you lost? _________

7. Will you be in the area for the entire 12 weeks of the study? □ Yes □ No

8. Do you have any of the following medical conditions:
   a. heart disease □ Yes □ No
   b. stroke □ Yes □ No
   c. TIA (mini stroke) □ Yes □ No
   d. diabetes □ Yes □ No
   e. high blood pressure □ Yes □ No
   f. renal or kidney disease □ Yes □ No
   g. rheumatoid arthritis □ Yes □ No
   h. gastrointestinal disease (such as Crohn’s disease, irritable bowel syndrome, ulcer or history of bowel surgery, lactose intolerance). □ Yes □ No
   i. blood clotting disorder □ Yes □ No
   j. liver disease or cirrhosis □ Yes □ No
   k. any condition that requires the use of steroids □ Yes □ No
   l. gout (requiring treatment) □ Yes □ No
   m. anemia (or sickle cell anemia) □ Yes □ No
   n. lung disease (such as bronchitis, emphysema, asthma) □ Yes □ No
   o. cancer within the last 10 years □ Yes □ No
   p. thyroid disease □ Yes □ No
q. Problems with immune system (hepatitis, AIDS) □ Yes □ No
r. Peripheral vascular disease or circulation problems such as Reynaud’s □ Yes □ No
s. any other medical condition not specified in this list □ Yes □ No
specify ___________________________________

Explain any “yes” answers: _______________________________________________

10. Do you take any medication prescribed by a doctor? (This includes medications for any diseases, any type of pain medicine, and any drugs for treatment of depression or other mental health problems.) □ Yes □ No
If yes, please specify the type of medication used, duration of use and reason:
________________________________________________________________________

11. Do you take any blood pressure or cholesterol-lowering medication? □ Yes □ No
Example: Captopril, Hydrochlorothiazide, Atenol or Zocor, Ezetimibe, Questran, Colestid, Orlistat

12. Are you taking any OTC cholesterol-lowering substances? □ Yes □ No
Example: psyllium, fish capsules, soy lecithin, phytoestrogen: if so, what?

13. Do you take any medication not prescribed by a doctor? Or any type of nutritional supplement, herb or vitamin? □ Yes □ No
If yes, please specify the type of medication used, duration of use and reason: ______________________________________________________
_______________________________________________________________________

If yes, are you willing to discontinue use during the study? □ Yes □ No

15. Are you allergic to latex? □ Yes □ No

16. Are you lactose intolerant or allergic to dairy products? □ Yes □ No

17. Do you have any food restrictions related to religious practices? Or are there any foods you refuse to eat? If yes, please specify (specifically ask about vegetarian) □ Yes □ No
18. Approximately how many servings of whole grains do you eat each day?  Whole grain foods include whole wheat bread, oatmeal, popcorn, brown rice, wild rice, whole grain cereals (cheerios, grape nuts, wheat chex, frosted mini-wheats), whole grain crackers like triscuits and corn chips. _________________

☐ ≤3  ☐ >3

18. Depending on the group that you are assigned to, are you willing to eat six or zero servings of whole grains each day?  ☐ Yes  ☐ No

19. Do you exercise intensely more than 10 hours a week or play sports regularly?  ☐ Yes  ☐ No
   If yes, please specify: _________________________________

20. Do you currently smoke?  ☐ Yes  ☐ No
   If no, have you ever smoked before?  ☐ Yes  ☐ No
   Explain: _________________________________

21. Do you consume alcohol?  ☐ Yes  ☐ No
   If yes, how much/how often? _______________________________

If female and premenopausal, complete the following questions

22. Approximately when was your last three menstrual periods   LMP_______ PMP_______
    PMP________

23. Do you have intermenstrual periods of ≥45 days or ≤8 menses per year.  ☐ Yes  ☐ No

If subject is eligible, schedule visit to General Clinical Research Center to sign informed consent and for screening if necessary.

☐ Yes, subject eligible
   ☐ Screening will be performed on: _______________________________
   ☐ Dr. ________________’s office will be contacted for medical records to determine eligibility at _______________________________

☐ No, subject is not eligible – Reason: _______________________________

____________________________________________________________________
APPENDIX B

WHOLE GRAIN STUDY
INFORMED CONSENT
Title of Project: Weight loss and cardiovascular disease prevention via a whole grain diet in men and women with metabolic syndrome

Principal Investigator: Penny Kris-Etherton, Ph.D., R.D.


Participant’s Printed Name: _____________________________

This is a research study. Research studies include only people who voluntarily choose to take part. This consent form gives you information about this research, which will be discussed with you. This consent form may contain words or procedures that you do not understand. You are urged to ask questions about anything that is unclear to you. Discuss it with your family and friends and take your time to make your decision. You will receive a copy of the signed and dated consent form to keep.

1. **Purpose of the Research:**

You will have the opportunity to take part in this research because you want to lose weight. You were selected because you have a condition known as metabolic syndrome, which means that you have at least three of the following five risk factors for developing heart disease, diabetes and stroke: high blood pressure, high glucose levels, elevated triglycerides, abdominal obesity, and low HDL cholesterol.

This research is being done to see if whole grains and a weight loss diet affect cardiovascular risk factors. These risk factors include abdominal obesity, insulin resistance, blood cholesterol levels and blood markers of inflammation.
This study is being conducted at the Hershey Medical Center and University Park Campus. Approximately 200 men and women will be evaluated for participation in the study at both campuses.

2. Procedures to be Followed:
   Informed Consent
   If you are interested in participating in this research study, you will first be asked to sign this consent form. If you are eligible to participate in this study, you will be informed of all events involved in this study. The study coordinator will discuss every step with you that you see listed below including any negative events that could potentially occur. Please note that at any time, you should feel free to ask questions.

   Initial visit/Screening visit
   You will be asked to not eat or drink anything for at least twelve hours before this visit. To determine if you are eligible for this study, we will take a blood sample (about 1 tablespoon) to determine your cholesterol, triglyceride and glucose levels and measure your height, weight, waist circumference and blood pressure. If you are a woman and are able to bear children, we will ask you to provide a urine sample to screen for pregnancy. If you are pregnant, you will not be allowed to participate in this research study. We will notify you if any of your blood values are abnormal.

   At this visit we will also ask you to complete two questionnaires. The first is an eating attitudes questionnaire to assess your likelihood of having an eating disorder. If your responses on this questionnaire indicate that you may have an eating disorder, you will not be able to participate in this study and will be referred to a counseling service at Penn State and/or to your own physician for further discussion about this issue. The second questionnaire is a physical activity questionnaire, which is to ensure that you will be physically able to meet the activity requirement of the study (30 minutes, three times a week). If your answers indicate that you may have difficulty with the physical activity component of the study, we will ask you to get written permission from your doctor stating that it is OK for you to participate in this study.

   If you have medical records from your doctor from the past year that show that you have metabolic syndrome, the screening visit will not be necessary. If your doctor is at Hershey Medical Center, we will access your medical records to confirm that you have three of the five indications for metabolic syndrome. If your doctor is outside of Hershey Medical Center, we will ask your doctor for your medical records to confirm that you have metabolic syndrome. If your doctor has diagnosed you with metabolic syndrome, you will be asked to complete the eating attitudes questionnaire and physical activity questionnaire at the baseline visit and you will also be screened for pregnancy at the baseline visit if you are a woman able to bear children.
Before you begin the study, you will meet briefly with the dietitian, either at your screening visit or at another date, who will ask you to keep track of your diet for three days before you begin the study. She will explain to you how to record what you ate on those days. The dietitian will also give you a hunger questionnaire to complete that evening after your evening meal.

**Randomization**
When you have met all of the criteria above for participation, you will be assigned to one of two diet groups. You will have an equal chance of being assigned to either of these groups. One group will be instructed to consume between four and eight servings of whole grain foods each day and the other group will be instructed to consume zero servings of whole grain foods each day. The number of whole grain foods that you will be asked to have per day is based on your calorie needs. Whole grain foods include foods such as whole-grain breakfast cereals, whole wheat bread, brown rice, whole-wheat pasta and oatmeal. Aside from the instruction on the amount of whole grains to eat, both groups will receive the same dietary counseling. The diet that you will follow during the study should meet your nutrient needs, and we therefore ask that you do not take a multivitamin or any supplements for the three-month study period.

**Baseline Visit**
Prior to the baseline visit, you will be instructed to complete a three-day diet record, which will be used as a basis for dietary instruction. You will also be asked to fast for twelve hours before this visit. At this visit you will have an oral glucose tolerance test and DXA scan and will meet with the dietitian. At this visit we will also measure your weight, blood pressure, percent body fat and waist circumference and ask you to complete a questionnaire on how satisfied you are with your current diet.

**Oral Glucose Tolerance Test:** This blood test will be done to see how your body responds to a sugary drink. The oral glucose tolerance test is a routine test done to determine if someone has diabetes. This test will be done between 8am and 10am in the morning. You will have a small flexible tube inserted into your forearm vein by a nurse. This tube, called a catheter, will allow for blood samples to be taken without puncturing your skin with a needle each time. Once the catheter is in place, samples can be taken while you read or watch TV. Before the test begins, a baseline blood sample will be drawn for measurement of various blood markers used to determine cardiovascular risk as well as sex hormones and hormones secreted by your gut and adipose tissue. You will then be asked to drink a sugary beverage and a blood sample will be taken every 30 minutes for two hours. Approximately six tablespoons of blood will be drawn at this visit.

**DXA Scan:** At this visit and at the end of the study we will perform a dual energy x-ray absorptiometry (DXA) scan to determine your percent body fat
and bone mineral density. The DXA scan uses a low dose x-ray of two different energies to distinguish between bone and soft tissue, giving a very accurate measurement of bone density. DXA is a painless, non-invasive test. You will be asked to lie still and quiet on a padded table, but you will be able to breathe normally. The study lasts only a few minutes. The x-ray dose you will be exposed to is less than half of the exposure from a routine chest x-ray.

**Percent Body Fat:** Your percent body fat will be measured using a scale that measures how fast a small electric signal passes through your body.

**Dietary Counseling:** At this visit you will meet with the dietitian to discuss your diet for the three-month study period.

If you are randomized to the whole grain group, you will be given a list of whole grain foods and asked to consume at least six servings of foods from the list each day. If you require a large amount of calories each day, you may be asked to have seven or eight servings of whole grains per day. We will ask that you gradually increase the number of whole grain foods you eat each day. We will recommend that you have three servings of whole grain foods for the first two weeks of the study, and then increase to six or more servings of whole grain foods per day for the remainder of the study. We will provide you with two to three whole-grain foods such as whole-grain breakfast cereals, brown rice, whole-wheat pasta or oatmeal to give you the opportunity to sample foods you may not have tried before. However, your whole grain intake should include other whole grain foods in addition to the ones you receive from the study.

If you are randomized to the group instructed not to eat any whole grains, you will be given a list of whole grain foods and asked not to consume any foods on the list for the duration of the study. You will be also be provided with two to three healthy cereals, crackers, pastas and other snacks.

Regardless of the diet group that you are in, the dietitian will work with you to develop a meal plan to achieve weight loss. You will be instructed to consume at least five servings of fruit and vegetables and three servings of low-fat dairy products per day, and two servings of lean meat, fish and poultry per day. The dietitian also will discuss with you ways to reduce your intake of saturated and trans fat. You will also be asked to exercise three times a week for thirty minutes per session throughout the three-month study period.

**Diet and Activity Record:** For the three-month study period, you will be required to keep track of your daily food and whole grain intake in a diet diary and record when and how long you exercise for. You will be advised to keep your food log current since the best time to record is right after eating the meal or snack.
**Bi-weekly Visits**

Every other week you will visit the study site and review your diet with the dietitian, who will offer nutritional guidance, suggestions for improvement, and discuss a nutritional topic of interest. At each visit we will record your weight, blood pressure, percent body fat and waist circumference. You will also be given the choice of two to three new foods to take with you.

**Weight loss:** Your goal is to lose one to two pounds per week throughout the study period. The dietitian will review your diet and calorie intake with you at each visit to ensure that you are eating at least 500 calories per day less than the number of calories needed to maintain your body weight. If you are eating too many calories each day or not losing at least one pound a week, the dietitian will discuss with you additional ways to reduce your caloric intake.

**3-Day Diet Record:** Every four weeks, you will be asked to keep a detailed, three-day diet record to estimate your total energy and nutrient intake. You will be asked to be as specific as possible by providing information on how the food was cooked, portion size, brand name, and anything added to the food such as margarine, salad dressing or jam.

**Appetite and Diet Satisfaction Questionnaire:** At your visit on week 5 and week 11, you will be asked to fill out a questionnaire on how satisfied you are with your diet. You will also be given a questionnaire to rate your appetite, which you will be asked to complete in the evening after your evening meal.

**Bi-weekly Contact**

On the weeks that you do not have a scheduled visit, you will be contacted by the dietitian or study coordinator to review your progress and discuss any concerns or questions that you might have. You will be asked to monitor and report your weight on a weekly basis to provide additional feedback on your progress in the study.

At the end of three months, a repeat of your baseline evaluation will be done, including DXA scan, oral glucose tolerance test, blood work, height, weight, percent body fat, waist circumference and blood pressure. If you are a woman and able to bear children you will have a pregnancy test before the DXA scan is performed. Approximately four tablespoons of blood will be drawn.

**Final Visit**

Upon completion of the study, you will be advised to continue your dietary regimen and exercise routine. To determine the long term effects of the study, at nine months after your completion of the study we will ask you to complete a three-day diet record and go to the GCRC at Hershey Medical Center or University Park for a final visit. Your weight, waist circumference,
percent body fat and blood pressure will be measured. A fasting blood sample (approx. 4 tablespoons) will also be taken. Once this final visit is completed, your participation in this research will be complete.

3. Discomforts and Risks:
   DXA Scan: The DXA bone density procedures results in a small amount of x-ray radiation exposure. For each procedure, the dose to the whole body is approximately 0.5 mrad (mrad is a measure of the radiation dose). When averaged over the entire body, this amount of radiation poses no more risk than the natural background radiation (continuous radiation exposure from cosmic rays, radioactive materials present in the earth and building materials and radioactive materials normally present within the human body) that is received each day from living in Pennsylvania. For further comparison purposes, this is less radiation than is received from a routine chest x-ray or from cosmic rays during a coast-to-coast flight.
   Blood Draws: The discomfort associated with removing blood by venipuncture (by needle from a vein) is a slight pinch or pin prick when the sterile needle enters the skin. The risks include mild discomfort and/or a black and blue mark at the site of puncture. Less common risks include a small blood clot, infection or bleeding at the puncture site, and on rare occasions fainting during the procedure.
   High-Fiber Diet: A rapid increase in fiber intake may result in gas, cramping, bloating or diarrhea. If you are in the whole grain group, you will be instructed to increase your fiber intake gradually to minimize such effects. Additionally, men and women in both groups will be advised to consume at least 8 cups of water per day, which will also minimize any gastrointestinal side effects.
   Foods containing fiber are known to reduce the amount minerals available to your body, however the amount of reduction is not of concern because your diet will be nutritionally adequate and the reduction in minerals will have little effect on nutritional status.
   Eating Attitudes Questionnaire: Some questions in the EAT-26 may make you feel uncomfortable to answer.

4. Possible Benefits:
   a. Possible benefits to you:
   The possible benefit you may experience from participating in this research study includes weight loss. There is no guarantee that you will benefit from being in this research.
   b. Possible benefits to society:
   Information gained from this study may help to define the appropriate dietary management of metabolic syndrome and will form the basis for future research into the long-term effects of whole grains on cardiovascular health and the specific effects of individual components of whole grains.
5. **Other Options that Could be Used Instead of this Research:**
You can elect not to participate in this study. If you decline to participate in this study, it will not affect any care or treatment you would normally receive from your regular doctor.

6. **Time Duration of the Procedures and Study:**
If you agree to take part in this study, your involvement will last three months. You will be asked to return to the clinic eight times (every other week during the study plus an additional visit at nine months). The baseline and final visits will be approximately 3 hours and the bi-weekly visits will be about 45-60 minutes long.

7. **Statement of Confidentiality:**
   a. **Privacy and Confidentiality Measures**
   Your research records and samples of your blood that are reviewed, stored, and analyzed at either The Milton S. Hershey Medical Center (HMC) and Penn State College of Medicine (PSU) or at the University Park campus will be labeled with a code number and your initials. The lists that match your name with the code number will be kept in locked files in Dr. Kris-Etherton’s and Dr. Legro’s offices. Your research information will be kept in a password-protected computer file and locked filing cabinet. Your samples will be stored in a freezer and will be locked in Dr. Legro’s lab at the Hershey Medical Center. If you give permission, your blood samples will be stored for use in future research. If you do not give permission to store your blood samples, they will be destroyed once the study is complete.

   In the event of any publication or presentation resulting from the research, no personally identifiable information will be shared.

7b. **The Use of Private Health Information:**

   Health information about you will be collected if you choose to be part of this research study. Health information is protected by law as explained in the Privacy Notice. If you have not received this notice, please request a copy from the researcher. At The Milton S. Hershey Medical Center (HMC) and Penn State College of Medicine (PSU) and at the University Park Campus your information will only be used or shared as explained and authorized in this consent form or when required by law. It is possible that some of the other people/groups who receive your health information may not be required by Federal privacy laws to protect your information and may share it without your permission.

   To participate in this research you must allow the research team to use your health information. If you do not want us to use your protected health information, you may not participate in this research.
Your permission for the use, retention, and sharing of your identifiable health information will continue indefinitely. If you consent to the collection of samples of your blood for future research, the period for the use of your samples is unknown. If you do not consent to the collection of samples of your blood for future research, they will be destroyed at the end of the research.

If you choose to participate, you are free to withdraw your permission for the use and sharing of your health information and your samples at any time. You must do this in writing as indicated in the Privacy Notice. Write to Dr. Kris-Etherton and let her know that you are withdrawing from the research study. Her mailing address is:

Penny Kris-Etherton, Ph.D., R.D.
Department of Nutritional Sciences
The Pennsylvania State University
S-126 Henderson Building
University Park, PA  16802

If you withdraw your permission:
- We will no longer use or share medical information about you or your samples for the reasons covered by your written authorization, except when the law allows us to do so.
- We are unable to take back anything we have already done or any information we have already shared with your permission.
- We may continue using and sharing the information obtained prior to your withdrawal if it is necessary for the soundness of the overall research.
- We will need to keep our records of the care that we provided to you as long as the law requires.

The research team may use the following sources of health information:
- Personal health history and information from your medical records
- Information from your medical records
- Measurements of your height, weight, blood pressure, waist circumference and body composition
- Blood sample results and DXA scan results
- Information from your diet diaries

Representatives of the following people/groups within HMC/PSU and University Park are allowed to use your health information and to share it with other specific groups in connection with this research study.
- The principal investigator, Penny Kris-Etherton, Ph.D., R.D.
- The HMC/PSU and University Park Institutional Review Board
- The HMC/PSU Human Subjects Protection Office and University Park Office for Research Protections
• The research team at the Hershey Medical Center and College of Medicine and at University Park

The people or groups listed in the above paragraph may share your health information with the following people/groups outside HMC/PSU for their use in connection with this research study. These groups, while monitoring the research study, may also review and/or copy your original PSU/HMC records.

• The Office of Human Research Protections in the U. S. Department of Health and Human Services
• The General Mills Bell Institute of Health and Nutrition

8. **Costs for Participation:**
   **Costs:** The DXA scan, blood tests, and dietary counseling will be provided at no cost to you. Expenses for procedures done for study purposes during your participation in the study will be covered by the study and will not be billed to you or your insurance company.

   **Treatment and Compensation for Injury:** Every effort to prevent injury as a result of your participation will be taken. It is possible, however, that you could develop complications or injuries as a result of participating in this research study. In the event of injury resulting from this research, medical treatment is available but will be provided at the usual charge. It is the policy of this institution to provide neither financial compensation nor free medical treatment for research-related injury.

   Costs for the treatment of research-related injuries will be charged to your insurance carrier or to you. Some insurance companies may not cover costs associated with research studies. If for any reason these costs are not covered by your insurance, they will be your responsibility.

   You are not waiving any legal rights you may have by signing this form.

9. **Compensation for Participation:**
   You will be paid a total of $100 for your participation in the study. You will be paid $10 for each visit and an additional $20 upon completion of your final visit during the three-month study period. You will be paid $10 for completion of your follow-up visit 9 months after finishing the study. We will ask you for your social security number for tax purposes.

   If you are an employee of Penn State University, the compensation you receive for participation will be treated as taxable income and therefore taxes will be taken from the total amount. If you are not employed by Penn State University, total payments within one calendar year that exceed $600 will require the University to annually report these payments to the IRS. This may require you to claim the compensation that you receive for participation in this study as taxable income.
10. **Research Funding:**
The institution and investigators are receiving funding from a grant from the General Mills Bell Institute of Health and Nutrition to support the activities that are required to conduct this research.

11. **Voluntary Participation:**
Taking part in this research study is voluntary. If you choose to take part in this research, your major responsibilities will include compliance with visits and protocol instructions. You do not have to participate in this research. If you choose to take part, you have the right to stop at any time. If you decide not to participate or if you decide to stop taking part in the research at a later date, there will be no penalty or loss of benefits to which you are entitled. In other words, your decision to decline to participate in this research or to stop taking part in the research will not affect your medical care.

Your research doctor or the sponsor may take you out of the research study without your permission. Possible reasons for this are: you become pregnant, you did not follow the instructions of the study, or you experience serious side effects. Also, the sponsor of the research may end the research study early. If your participation in the research ends early, you may be asked to visit the research doctor for a final visit.

If you will be participating in another clinical trial while in this research, you should discuss the procedures and/or treatments with your physician or the investigators. This precaution is intended to protect you from possible side effects from interactions of research drugs, treatments or testing.

During the course of the research you will be informed of any new findings that may affect your willingness to continue participating in this research.

12. **Contact Information for Questions or Concerns:**
You have the right to ask any questions you may have about this research. If you have questions or concerns or believe you may have developed an injury that is related to this research, contact Dr. Kris-Etherton 814-863-2923 or Dr. Legro at (717) 531-8478.

If you have questions or concerns regarding your rights as a research participant or about your privacy and the use of your personal health information, you may contact the research protection advocate in the HMC Human Subjects Protection Office at (717) 531-5687 or the University Park Office for Research Protections at (814) 865-1775.

For more information about participation in a research study and about the Institutional Review Board (IRB), a group of people who review the research to protect your rights, please visit the HMC IRB’s Web site at [http://www.hmc.psu.edu/irb](http://www.hmc.psu.edu/irb). Included on this web site, under the heading
“Links”, you can access the federal regulations for the protection of human research participants. If you do not have access to the internet, copies of these regulations are available by calling the HSPO at (717) 531-5687.

**Signature and Consent/Permission to be in the Research**

Before making the decision regarding enrollment in this research you should have:
- Discussed this study with an investigator,
- Reviewed the information in this form, and
- Had the opportunity to ask any questions you may have.

Your signature below means that you have received this information, have asked the questions you currently have about the research and those questions have been answered. You will receive a copy of the signed and dated form to keep for future reference.

**Participant**: By signing this consent form, you indicate that you are voluntarily choosing to take part in this research.

_________________________ __________ ______     __________________
Signature of Participant   Date  Time        Printed Name

**Person Explaining the Research**: Your signature below means that you have explained the research to the participant/participant representative and have answered any questions he/she has about the research.

__________________________          _________   ______  ______
Signature of person who explained this research*   Date          Time        Printed Name
(*Only approved investigators for this research may explain the research and obtain informed consent.)

In addition the main part of the research study, there is an optional part of the research. You can participate in the main part of the research without agreeing to take part in this optional part.

**Optional Tissue Storage for Future Use**
As part of this study, we are obtaining blood from you. If you agree, the researchers would like to store leftover samples of your blood so that your blood can be studied in the future after this study is over. These future studies may provide additional information that will be helpful in understanding metabolic syndrome, but it is unlikely that these studies will have a direct benefit to you. The results of these tests will not have an effect on your care. Neither your doctor nor you will receive results of these future research tests, nor will the results be put in your health record. Sometimes tissue is used for genetic research about diseases that are passed on in families. Even if your samples are used for this kind of research, the results will not be put in
your health records. It is possible that your blood might be used to develop products or tests that could be patented and licensed. There are no plans to provide financial compensation to you should this occur. If you have any questions, you should contact Dr. Kris-Etherton at (814) 863-2923.

Your leftover samples will be labeled with a code number and stored in Dr. Legro’s locked laboratory at the Hershey Medical Center. If you consent to the collection of samples of your blood for future research, the period for the use of the samples is unknown. If you agree to allow your blood to be kept for future research, you will be free to change your mind at any time. You should contact Dr. Kris-Etherton at (814) 863-2923 and let her know you wish to withdraw your permission for your blood to be used for future research. Any unused blood will be destroyed and not used for future research studies.

You should initial below to indicate your preferences regarding the optional storage of your leftover blood for future research studies.

a. Your samples may be stored and used for future research studies to learn about, prevent, treat or cure metabolic syndrome

______ Yes  ______ No

b. Your samples may be stored and used for research about other health problems.

______ Yes  ______ No

c. Your samples may be shared with other investigator/groups without any identifying information.

______ Yes  ______ No

Participant: By signing below, you indicate that you are voluntarily choosing to take part in this optional part of the research.

__________________________    _______    _______    ________________
Signature of Participant  Date  Time  Printed Name

Person Explaining the Research: Your signature below means that you have explained the optional part of the research to the participant/participant representative and have answered any questions he/she has about the research.

__________________________    _______    _______    ________________
Signature of person who explained this research  Date  Time  Printed Name
APPENDIX C

WHOLE GRAIN STUDY
STUDY MATERIALS AND EDUCATIONAL HANDOUTS
# Recommended Whole Grain Servings at Each Calorie Level

<table>
<thead>
<tr>
<th>Calorie Level</th>
<th># Servings Whole Grains</th>
</tr>
</thead>
<tbody>
<tr>
<td>1200</td>
<td>4</td>
</tr>
<tr>
<td>1300</td>
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<td>1400</td>
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<td>2600</td>
<td>8</td>
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</tbody>
</table>
# Daily Diet Monitoring Form

**ID#:** ________________

**Date:** ________________  **Physical Activity Today?** Yes [ ] No [ ] **How Many Minutes?** ______  **Activity type** ________________

<table>
<thead>
<tr>
<th>Meal</th>
<th>Foods and Beverages (provide amounts and detailed description)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
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</tbody>
</table>

<table>
<thead>
<tr>
<th>Whole Grains (___)</th>
<th>Fruits &amp; Vegetables</th>
<th>Low Fat Dairy</th>
<th>Lean Meat, Fish &amp; Poultry</th>
</tr>
</thead>
<tbody>
<tr>
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</tbody>
</table>
**ID#: _______________**

**Daily Diet Monitoring Form**

Date: _______________  Physical Activity Today? Yes ☐  No ☐  How Many Minutes? _______  Activity type __________

<table>
<thead>
<tr>
<th>Meal</th>
<th>Foods and Beverages (provide amounts and detailed description)</th>
</tr>
</thead>
<tbody>
<tr>
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</tbody>
</table>

<table>
<thead>
<tr>
<th>Fruits &amp; Vegetables</th>
<th>Low Fat Dairy</th>
<th>Lean Meat, Fish &amp; Poultry</th>
</tr>
</thead>
<tbody>
<tr>
<td>☐ ☐ ☐ ☐ ☐</td>
<td>☐ ☐ ☐</td>
<td>☐ ☐ ☐</td>
</tr>
</tbody>
</table>
Weekly Weight Log

<table>
<thead>
<tr>
<th>ID#___________</th>
<th>Date</th>
<th>Weight (pounds)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Week 1</td>
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<td>Week 2</td>
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<td>Week 3</td>
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<td>Week 4</td>
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<td>Week 5</td>
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<td>Week 6</td>
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<td>Week 7</td>
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<td>Week 8</td>
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<td>Week 9</td>
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<td>Week 10</td>
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<tr>
<td>Week 11</td>
<td></td>
<td></td>
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<tr>
<td>Week 12</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
### D-SAT Questionnaire

For each of the statements listed below, circle the number that best represents your response as it applies to the way you currently eat and your current level of physical activity. Please read each statement carefully before responding.

**For example:** For the following question, "I think I exercise a lot," you would base your answer on your current level of physical activity. If you feel that you currently exercise a lot, you would circle 5 to indicate that you strongly agree.

<table>
<thead>
<tr>
<th>Statement</th>
<th>Disagree strongly</th>
<th>Disagree somewhat</th>
<th>Neither disagree nor agree</th>
<th>Agree somewhat</th>
<th>Agree strongly</th>
</tr>
</thead>
<tbody>
<tr>
<td>I think I exercise a lot.</td>
<td>1</td>
<td>2</td>
<td>3</td>
<td>4</td>
<td>5</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th></th>
<th>Disagree strongly</th>
<th>Disagree somewhat</th>
<th>Neither disagree nor agree</th>
<th>Agree somewhat</th>
<th>Agree strongly</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. I have a lot of energy.</td>
<td>1</td>
<td>2</td>
<td>3</td>
<td>4</td>
<td>5</td>
</tr>
<tr>
<td>2. I feel good about myself.</td>
<td>1</td>
<td>2</td>
<td>3</td>
<td>4</td>
<td>5</td>
</tr>
<tr>
<td>3. I think I eat a healthy diet.</td>
<td>1</td>
<td>2</td>
<td>3</td>
<td>4</td>
<td>5</td>
</tr>
<tr>
<td>4. I believe that I am reducing my risk for disease by the way that I eat.</td>
<td>1</td>
<td>2</td>
<td>3</td>
<td>4</td>
<td>5</td>
</tr>
<tr>
<td>5. I believe that I am reducing my risk for disease by the way that I exercise.</td>
<td>1</td>
<td>2</td>
<td>3</td>
<td>4</td>
<td>5</td>
</tr>
<tr>
<td>6. I think I have a healthy lifestyle.</td>
<td>1</td>
<td>2</td>
<td>3</td>
<td>4</td>
<td>5</td>
</tr>
<tr>
<td>7. I am satisfied with my current diet.</td>
<td>1</td>
<td>2</td>
<td>3</td>
<td>4</td>
<td>5</td>
</tr>
<tr>
<td>8. The way I currently eat makes me feel guilty.</td>
<td>1</td>
<td>2</td>
<td>3</td>
<td>4</td>
<td>5</td>
</tr>
<tr>
<td>9. The way I currently eat prevents me from eating in restaurants frequently.</td>
<td>1</td>
<td>2</td>
<td>3</td>
<td>4</td>
<td>5</td>
</tr>
<tr>
<td>10. When dining out, I can easily choose foods from the menu that fit into my current diet.</td>
<td>1</td>
<td>2</td>
<td>3</td>
<td>4</td>
<td>5</td>
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<td>---</td>
</tr>
<tr>
<td>Disagree strongly</td>
<td>Disagree somewhat</td>
<td>Neither disagree nor agree</td>
<td>Agree somewhat</td>
<td>Agree strongly</td>
<td></td>
</tr>
<tr>
<td>11. Finding appropriate food choices at restaurants is difficult.</td>
<td>1</td>
<td>2</td>
<td>3</td>
<td>4</td>
<td>5</td>
</tr>
<tr>
<td>12. I have to prepare most of my foods from “scratch”.</td>
<td>1</td>
<td>2</td>
<td>3</td>
<td>4</td>
<td>5</td>
</tr>
<tr>
<td>13. I find eating satisfying.</td>
<td>1</td>
<td>2</td>
<td>3</td>
<td>4</td>
<td>5</td>
</tr>
<tr>
<td>14. I have difficulty finding the foods I want when eating out.</td>
<td>1</td>
<td>2</td>
<td>3</td>
<td>4</td>
<td>5</td>
</tr>
<tr>
<td>15. I find it easy to shop for the kinds of foods I eat at my grocery store.</td>
<td>1</td>
<td>2</td>
<td>3</td>
<td>4</td>
<td>5</td>
</tr>
<tr>
<td>16. I limit my choice of restaurants.</td>
<td>1</td>
<td>2</td>
<td>3</td>
<td>4</td>
<td>5</td>
</tr>
<tr>
<td>17. I have plenty of different types of foods to choose from with my current diet.</td>
<td>1</td>
<td>2</td>
<td>3</td>
<td>4</td>
<td>5</td>
</tr>
<tr>
<td>18. I feel I spend a large amount of my budget on the foods I eat.</td>
<td>1</td>
<td>2</td>
<td>3</td>
<td>4</td>
<td>5</td>
</tr>
<tr>
<td>19. I think preparing food/meals for the way I eat now is economical</td>
<td>1</td>
<td>2</td>
<td>3</td>
<td>4</td>
<td>5</td>
</tr>
<tr>
<td>20. I think preparing food/meals for the way I eat now costs a lot of money</td>
<td>1</td>
<td>2</td>
<td>3</td>
<td>4</td>
<td>5</td>
</tr>
<tr>
<td>21. I spend a lot of money on food.</td>
<td>1</td>
<td>2</td>
<td>3</td>
<td>4</td>
<td>5</td>
</tr>
<tr>
<td>22. It’s hard for me to afford the kind of foods I eat</td>
<td>1</td>
<td>2</td>
<td>3</td>
<td>4</td>
<td>5</td>
</tr>
<tr>
<td>23. I feel the way I eat now bothers my family.</td>
<td>1</td>
<td>2</td>
<td>3</td>
<td>4</td>
<td>5</td>
</tr>
<tr>
<td>Question</td>
<td>1</td>
<td>2</td>
<td>3</td>
<td>4</td>
<td>5</td>
</tr>
<tr>
<td>-------------------------------------------------------------------------</td>
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</tr>
<tr>
<td>24. My family encourages me to keep eating the way I am eating now.</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>25. My family supports my efforts to eat a healthy diet.</td>
<td>1</td>
<td>2</td>
<td>3</td>
<td>4</td>
<td>5</td>
</tr>
<tr>
<td>26. My family thinks my current diet is a healthy diet.</td>
<td>1</td>
<td>2</td>
<td>3</td>
<td>4</td>
<td>5</td>
</tr>
<tr>
<td>27. My family discourages me from eating the way I am eating now.</td>
<td>1</td>
<td>2</td>
<td>3</td>
<td>4</td>
<td>5</td>
</tr>
<tr>
<td>28. The way I currently eat causes stress within my family.</td>
<td>1</td>
<td>2</td>
<td>3</td>
<td>4</td>
<td>5</td>
</tr>
<tr>
<td>29. Thoughts of food are always on my mind.</td>
<td>1</td>
<td>2</td>
<td>3</td>
<td>4</td>
<td>5</td>
</tr>
<tr>
<td>30. I think about food between almost every meal.</td>
<td>1</td>
<td>2</td>
<td>3</td>
<td>4</td>
<td>5</td>
</tr>
<tr>
<td>31. I have cravings for some of my favorite foods.</td>
<td>1</td>
<td>2</td>
<td>3</td>
<td>4</td>
<td>5</td>
</tr>
<tr>
<td>32. I always feel like I want to snack between meals.</td>
<td>1</td>
<td>2</td>
<td>3</td>
<td>4</td>
<td>5</td>
</tr>
<tr>
<td>33. I often feel hungry.</td>
<td>1</td>
<td>2</td>
<td>3</td>
<td>4</td>
<td>5</td>
</tr>
<tr>
<td>34. I feel that my diet controls my life.</td>
<td>1</td>
<td>2</td>
<td>3</td>
<td>4</td>
<td>5</td>
</tr>
<tr>
<td>35. I feel deprived based on what I order when eating in a restaurant.</td>
<td>1</td>
<td>2</td>
<td>3</td>
<td>4</td>
<td>5</td>
</tr>
<tr>
<td>36. I feel self-conscious trying to eat my current diet at social events.</td>
<td>1</td>
<td>2</td>
<td>3</td>
<td>4</td>
<td>5</td>
</tr>
<tr>
<td>37. I feel embarrassed if I order specially prepared foods in a restaurant.</td>
<td>1</td>
<td>2</td>
<td>3</td>
<td>4</td>
<td>5</td>
</tr>
<tr>
<td>Question</td>
<td>1</td>
<td>2</td>
<td>3</td>
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<td>-------------------------------------------------------------------------</td>
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<tr>
<td>38. My family eats the same foods that I currently eat.</td>
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<tr>
<td>39. I feel deprived when I choose to avoid some of my favorite foods.</td>
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<td></td>
</tr>
<tr>
<td>Disagree strongly</td>
<td>Disagree somewhat</td>
<td>Neither disagree nor agree</td>
<td>Agree somewhat</td>
<td>Agree strongly</td>
<td></td>
</tr>
<tr>
<td>40. I have to prepare separate meals for my family and myself.</td>
<td></td>
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<tr>
<td>41. I spend a lot of time planning my meals.</td>
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<tr>
<td>42. I spend a lot of time shopping for food.</td>
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<tr>
<td>43. I think preparing food/meals for the way I eat now is time consuming.</td>
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<tr>
<td>44. I think preparing food/meals for the way I eat now requires a lot of effort.</td>
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<tr>
<td>45. I spend a lot of time looking for new food/meal ideas that fit into my current diet.</td>
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</tbody>
</table>

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Physical Activity Questionnaire

Subject ID_______________   Visit___________    Date __________

In the past week how many times on the average did you do the following kinds of exercise for more than 15 minutes during your free time?

Times Per Week

_____ A. Strenuous exercise (heart beats rapidly)

(For example: running, jogging, hockey, football, soccer, squash, basketball, cross country skiing, judo, roller blading, vigorous swimming, vigorous long distance bicycling)

_____ B. Moderate exercise (not exhausting)

(For example: fast walking, baseball, tennis, easy bicycling, volleyball, badminton, easy swimming, alpine skiing, dancing)

_____ C. Mild exercise (minimal effort)

(For example: easy walking, golf, yoga, bowling, horseshoes, archery, fishing from river boat)
**What are whole grains?**
Whole grains include all three parts of a grain: the bran, germ and endosperm.

The bran and germ are removed when grains are milled to produce “regular” white flour. The health benefits of whole grains come from the “whole grain package” and not just individual components. The fiber, vitamins, minerals, and phytochemicals found in whole grains work together to help protect against diseases like heart disease, certain cancers, and diabetes.

**How do I know if a food is made from whole grains?**
One way to identify whole grains food is to look for the whole grain statement on the food package, called a *health claim*. It should state:

“*Diets rich in whole grain foods and other plant foods and low in total fat, saturated fat, and cholesterol may reduce the risk of heart disease and certain cancers.*”

However, not all whole grain foods will display this statement. Therefore, another way to identify whole grain foods is to look at the ingredients list on the package. Look for foods with the whole grain ingredient listed first – for example, whole wheat flour, whole oats, whole grain corn, or brown rice. The phrase “whole grain” or the word “whole” before the grain’s name tells you that a food is made from the entire grain.

**A food cannot be identified as a whole grain food by its fiber content. Some foods can be high in fiber, but are not a “whole grain” food.**
The following whole grains should be consumed in the serving sizes indicated. Please consume ____ servings of any combination from this list each day. On the back page, more specific suggestions can be found.

<table>
<thead>
<tr>
<th>Whole Grain Foods</th>
<th>Serving Size</th>
</tr>
</thead>
<tbody>
<tr>
<td>Whole Wheat Bread</td>
<td>1 slice</td>
</tr>
<tr>
<td>Whole Wheat Pasta</td>
<td>½ cup, cooked</td>
</tr>
<tr>
<td>Brown Rice</td>
<td>½ cup, cooked</td>
</tr>
<tr>
<td>Oatmeal</td>
<td>½ cup, cooked</td>
</tr>
<tr>
<td>Whole Grain Cereals</td>
<td>~1 cup</td>
</tr>
<tr>
<td>Buckwheat Groats or Kasha</td>
<td>½ cup, cooked</td>
</tr>
<tr>
<td>Bulgur</td>
<td>½ cup, cooked</td>
</tr>
<tr>
<td>Wild Rice</td>
<td>½ cup, cooked</td>
</tr>
<tr>
<td>Whole Grain Crackers</td>
<td>5-7</td>
</tr>
<tr>
<td>Whole Wheat Tortillas, Pitas</td>
<td>1</td>
</tr>
<tr>
<td>Popcorn</td>
<td>2 cups</td>
</tr>
<tr>
<td>Wheat berries (found at health food stores)</td>
<td>½ cup, cooked</td>
</tr>
<tr>
<td>Millet</td>
<td>½ cup, cooked</td>
</tr>
</tbody>
</table>

The following whole grains can be found in health food stores and some supermarkets. These are usually flours (to be used in baking), or flakes, which can be cooked and used as hot cereals or added to other foods.

- Triticale (hybrid of wheat and rye)
- Barley (flakes or flour is less refined than "pearled" barley)
- Amaranth
- Rye
- Quinoa
- Cornmeal (yellow or white) *(If cornmeal is labeled as "degermed" or "degerminated" it indicates the germ and bran have been removed and, therefore, the product is not a whole grain food.)*
- Kamut
- Spelt
Whole Wheat Bread

Truly whole wheat bread will be labeled as **100% whole wheat**. Whole wheat flour should be listed as the first ingredient. The following terms do not necessarily mean whole wheat: 100% wheat, multigrain, stone-ground, pumpernickel, cracked wheat, oat bran, wheat flour, 7-, 9-, or 12-grain bread. Examples of 100% Whole Wheat Bread: Roman Meal, Wonder 100% Whole Wheat bread, Brownberry 100% Whole Wheat bread, Schmidt’s Old Tyme 100% Whole Wheat bread.

Whole Wheat Pasta

Truly whole wheat pasta will be labeled as such, and is available in various shapes and varieties. Some good ones are DeBoles Whole Wheat Angel Hair/Spaghetti, Hodgson Mill Whole Wheat Egg Noodles, and San Giorgio Healthy Harvest Pasta.

Oats

In all forms, oats are a whole grain. Oats may be called Oat Groats, Scotch Oats (steel-cut oats), or Oatmeal (Old-fashioned or Quick cooking). Be careful about “flavored” instant oatmeal. While still a whole grain, these contain lots of extra sodium and sugar. Some whole grain ready-to-eat oat cereals include Cheerios (original flavor) and General Mills Oatmeal Crisp.

Cereals

Look for a whole grain as the first ingredient. It can be whole wheat, whole rye, whole corn, whole grain oats, whole barley, millet. Avoid cereals that list sugar as the first ingredient. Good examples of true whole grain cereals are Wheatena, Shredded Wheat, Total, Grape-Nuts, Raisin Bran, and Wheaties. Cereals that are mostly refined and **not** whole grain include Basic 4, Cornflakes, Special K, Product 19, Smart Start, Cream of Wheat, Rice Krispies, and many others.

Whole Grain Crackers

Again, look for a whole grain as the first ingredient. Many crackers use the terms like multi-grain and cracked wheat, but may be highly refined. Some good examples of whole grain crackers are: Ry Krisp, Triscuit (all varieties), and Wasa Fibre Rye.
Please avoid the following whole grains while you are participating in this study:

<table>
<thead>
<tr>
<th>Whole Wheat Bread</th>
<th>Brown Rice</th>
<th>Kasha</th>
</tr>
</thead>
<tbody>
<tr>
<td>Whole Wheat Pasta</td>
<td>Wild Rice Bulgur</td>
<td>Millet</td>
</tr>
<tr>
<td>Whole Wheat Tortillas</td>
<td>Barley</td>
<td>Buckwheat/Oat Groats</td>
</tr>
<tr>
<td>Whole Wheat Pitas</td>
<td>Popcorn</td>
<td>Oatmeal</td>
</tr>
<tr>
<td>Whole Grain Cereals</td>
<td>Corn</td>
<td></td>
</tr>
<tr>
<td>Whole Grain Crackers</td>
<td>Cornmeal</td>
<td></td>
</tr>
</tbody>
</table>

**General Guidelines:**

Please check the ingredient list on the food package to be sure that a food is not a whole grain. Try to avoid foods during the study that have a whole grain included anywhere on the food label, especially as the first, second, or third ingredient. Examples of a whole grain ingredient are whole wheat, rye, corn, oats, whole barley, millet, or corn. Please avoid these foods. Also, a whole grain may also have the following health claim on the package:

“Diets rich in whole grain foods and other plant foods and low in total fat, saturated fat, and cholesterol may reduce the risk of heart disease and certain cancers.”

**Specific Guidelines:**

**Bread:** Truly whole wheat bread will be labeled as **100% whole wheat**. Whole wheat flour would be listed as the first ingredient. The following terms do not necessarily mean whole wheat: 100% wheat, multigrain, stone-ground, pumpernickel, cracked wheat, oat bran, wheat flour, 7-, 9-, or 12-grain bread. You may enjoy these fiber-rich breads throughout the study if the whole-grain health claim is not on the label and a whole grain is not an ingredient.

**Cereals:** A whole grain should not be listed as the first ingredient, and check for the whole-grain health claim. Some examples of cereals you should **not** consume during this study are: Oatmeal, Cheerios, Shredded Wheat, Total, Grape nuts, Raisin Bran, and Wheaties. Healthy cereals you can enjoy during the study are: Cornflakes, Special K, Cream of Wheat, Cream of Rice, grits, Rice Krispies, and many others.

**Crackers:** Many crackers use terms like multi-grain or cracked wheat, but may not be a whole grain cracker. Please avoid the following crackers: Ry Krisp, Triscuit, Wasa Fibre Rye or any other cracker that displays the whole grain health claim on the package or contains a whole grain as an ingredient.
This hand-out is intended as a starting point for a weight-loss plan that we will be helping you with for the duration of the study. Our goal for weight loss will be 1-2 lbs/week. Many topics will be covered in greater detail later in the study.

You can start with these simple guidelines:

* Eat a **healthy diet** that includes fruits, vegetables (5 servings/day), low-fat dairy products (3 servings/day), and lean meat/fish (2 servings/day) -- and be sure to **control portion sizes**.

* Get physically active at least three times a week, for thirty minutes or more.

* Complete your Daily Diet Monitoring form everyday. Studies have shown that self-monitoring of your diet can be very helpful when losing weight.

A healthy lifestyle is made up of lots of small choices. Below, we offer a few ideas -- ways that you can improve your eating habits by choosing to do things just slightly differently.

<table>
<thead>
<tr>
<th>If you normally...</th>
<th>then try this instead!</th>
</tr>
</thead>
<tbody>
<tr>
<td>use whole or 2% milk</td>
<td>use fat free milk</td>
</tr>
<tr>
<td>drink soda</td>
<td>drink diet soda, 100% juice, or water</td>
</tr>
<tr>
<td>eat dessert</td>
<td>eat fruit for dessert</td>
</tr>
<tr>
<td>have second helpings</td>
<td>put the food away as soon as you serve</td>
</tr>
<tr>
<td>eat big portions</td>
<td>use smaller plates, start with a small portion, and eat SLOW</td>
</tr>
<tr>
<td>eat out</td>
<td>Avoid buffets when hungry, choose restaurants with “light” offerings, consider eating out less often</td>
</tr>
<tr>
<td>use butter</td>
<td>try olive oil, canola oil, and low-fat spreads</td>
</tr>
<tr>
<td>eat fast food on the road</td>
<td>pack fruits and vegetables for car rides</td>
</tr>
<tr>
<td>eat red meat</td>
<td>Prepare lean cuts of beef or pork, skinless chicken or turkey, or fish</td>
</tr>
<tr>
<td>snack on chips and junk food</td>
<td>keep fruits and veggies in the fridge instead</td>
</tr>
<tr>
<td>skip breakfast</td>
<td>try eating a healthy breakfast each day</td>
</tr>
<tr>
<td>fry your food</td>
<td>grill or roast your food instead</td>
</tr>
</tbody>
</table>
ID:________________
Calorie Level:__________________
Servings Recommended (per day):

**Dairy Food: 3 servings/day**
**Examples and Serving Sizes**

<table>
<thead>
<tr>
<th>One Serving Size =</th>
<th>Milk – 1 cup</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Non-fat dry milk – 1/3 cup dry</td>
</tr>
<tr>
<td></td>
<td>Soy milk – 1 cup</td>
</tr>
<tr>
<td></td>
<td>Plain nonfat yogurt – ¾ cup</td>
</tr>
<tr>
<td></td>
<td>Low-fat or fat-free fruit yogurt – ½ cup</td>
</tr>
<tr>
<td></td>
<td>Cheese – 1 ½ ounce</td>
</tr>
<tr>
<td></td>
<td>Low-fat Cottage Cheese – ½ cup</td>
</tr>
<tr>
<td></td>
<td>Frozen Yogurt – ½ cup</td>
</tr>
</tbody>
</table>

**Fruit & Vegetables: 5 servings/day**
**Examples and Serving Sizes**

| One Serving Size = | Chopped raw vegetables – ½ cup |
|--------------------| Cooked vegetables – ½ cup |
|                    | Leafy Vegetables (ex: lettuce) – 1 cup |
|                    | Avocado – ½ medium |
|                    | Fresh whole fruit – 1 baseball size piece |
|                    | Fresh diced fruit – ½ cup |
|                    | Canned fruit – ½ cup |
|                    | Dried fruit – ¼ cup |
|                    | Pure fruit juice – 3/4 cup |
|                    | Vegetable juice – 3/4 cup |

**Lean Protein: 2 servings/day**
**Examples and Serving Sizes**

| One Serving Size = | Cooked meat, poultry, or fish - 3 oz. * |
|--------------------| Cooked beans - ½ cup |
|                    | Nuts – 1/3 cup |
|                    | Egg – 1 |
|                    | Egg Substitute – ¼ cup |
|                    | Tofu – 4 oz. or ½ cup |
|                    | Peanut butter – 2 TBS |

* 3 oz of cooked meat is the size of the deck of cards. Four ounces of raw meat makes approximately 3 oz cooked meat. Meat in ounces listed on restaurant menus is listed as raw weight.
Fruits and Vegetables

"For optimum health, scientists say, eat a rainbow of colors. Your plate should look like a box of Crayolas." - Janice M. Horowitz, TIME, January 12, 2002

Colorful fruits and vegetables provide the wide range of vitamins, minerals, fiber, antioxidants and phytochemicals your body uses to maintain good health and energy levels, protect against the effects of aging, and reduce the risk of cancer and heart disease.

What are antioxidants and phytochemicals?

**Antioxidants** – Antioxidants help protect the body from harmful substances called “free radicals”. Free radicals are naturally produced by the body and can cause damage to DNA and cell membranes. Free radical damage may contribute to health problems such as heart disease, aging, hardening of the arteries, cancer, arthritis and cataracts. Damage caused by free radicals can often be reversed or prevented by a regular supply of antioxidants from fruits and vegetables.

**Phytochemicals** – A phytochemical is a natural plant substance that works with nutrients and dietary fiber to protect against disease. Research suggests that phytochemicals working together with other nutrients found in fruits, vegetables, and nuts may help slow the aging process and reduce the risk of many diseases, including cancer, heart disease, stroke, high blood pressure, cataracts, and urinary tract infections.

Many of the phytochemicals and other compounds that make fruits and vegetables good for us also give them their color. That’s why it’s essential to sample the complete color spectrum every day to get the full preventive benefits of fruits and vegetables. The more reds, oranges, greens, yellows and blues you see on the plate, the more health-promoting properties you are also getting from your fruit and vegetable choices. The specific benefits for each color are listed below.

What counts as a serving for fruits and vegetables?

- One medium size fruit
- ½ cups raw, cooked, frozen or canned fruit (in 100% juice) or vegetables
- ¼ cup (6 oz.) 100% fruit or vegetable juice
- 1 cup raw, leafy vegetables
- ¼ cup dried fruit
BLUE and PURPLE

Include **BLUE/PURPLE** in your low-fat diet to help maintain:
- A lower risk of some cancers
- Urinary tract health
- Memory function
- Healthy aging

Get **BLUE/PURPLE** every day with foods such as:

<table>
<thead>
<tr>
<th>Blackberries</th>
<th>Blueberries</th>
</tr>
</thead>
<tbody>
<tr>
<td>Black currants</td>
<td>Dried plums</td>
</tr>
<tr>
<td>Elderberries</td>
<td>Purple figs</td>
</tr>
<tr>
<td>Purple grapes</td>
<td>Plums</td>
</tr>
<tr>
<td>Raisins</td>
<td>Purple asparagus</td>
</tr>
<tr>
<td>Purple carrots</td>
<td>Eggplant</td>
</tr>
<tr>
<td>Purple Belgian endive</td>
<td>Purple peppers</td>
</tr>
<tr>
<td>Purple potatoes</td>
<td>Purple cabbage</td>
</tr>
</tbody>
</table>

GREEN

Add **GREEN** in your diet to maintain:
- A lower risk of some cancers
- Vision health
- Strong bones and teeth

Go **GREEN** every day with fruits and vegetables like these:

<table>
<thead>
<tr>
<th>Avocados</th>
<th>Green apples</th>
</tr>
</thead>
<tbody>
<tr>
<td>Green grapes</td>
<td>Honeydew melon</td>
</tr>
<tr>
<td>Kiwifruit</td>
<td>Limes</td>
</tr>
<tr>
<td>Green pears</td>
<td>Artichokes</td>
</tr>
<tr>
<td>Arugula</td>
<td>Asparagus</td>
</tr>
<tr>
<td>Broccoli</td>
<td>Broccoli rabe</td>
</tr>
<tr>
<td>Brussels sprouts</td>
<td>Chinese cabbage</td>
</tr>
<tr>
<td>Green beans</td>
<td>Green cabbage</td>
</tr>
<tr>
<td>Cucumbers</td>
<td>Endive</td>
</tr>
<tr>
<td>Leafy greens</td>
<td>Leeks</td>
</tr>
<tr>
<td>Lettuce</td>
<td>Green onion</td>
</tr>
<tr>
<td>Okra</td>
<td>Peas</td>
</tr>
<tr>
<td>Green pepper</td>
<td>Spinach</td>
</tr>
<tr>
<td>Zucchini</td>
<td>Celery</td>
</tr>
</tbody>
</table>
### WHITE

Working **WHITE** into your diet helps maintain:
- Heart health
- Cholesterol levels that are already health
- A lower risk of some cancers

Get the health benefits of **WHITE** by choosing foods such as:

<table>
<thead>
<tr>
<th>Bananas</th>
<th>Brown pears</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dates</td>
<td>White nectarines</td>
</tr>
<tr>
<td>White peaches</td>
<td>Cauliflower</td>
</tr>
<tr>
<td>Garlic</td>
<td>Ginger</td>
</tr>
<tr>
<td>Jicama</td>
<td>Mushrooms</td>
</tr>
<tr>
<td>Onions</td>
<td>Parsnips</td>
</tr>
<tr>
<td>White potatoes</td>
<td>Shallots</td>
</tr>
<tr>
<td>Turnips</td>
<td></td>
</tr>
</tbody>
</table>

### YELLOW and ORANGE

Make **YELLOW/ORANGE** part of your diet to help maintain:
- A health heart
- Vision health
- A healthy immune system
- A lower risk of some cancers

Include **YELLOW** and **ORANGE** fruits and vegetables like these:

<table>
<thead>
<tr>
<th>Yellow apples</th>
<th>Apricots</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cantaloupe</td>
<td>Yellow figs</td>
</tr>
<tr>
<td>Grapefruit</td>
<td>Golden kiwifruit</td>
</tr>
<tr>
<td>Lemon</td>
<td>Mangoes</td>
</tr>
<tr>
<td>Nectarines</td>
<td>Oranges</td>
</tr>
<tr>
<td>Papayas</td>
<td>Peaches</td>
</tr>
<tr>
<td>Yellow pears</td>
<td>Persimmons</td>
</tr>
<tr>
<td>Pineapples</td>
<td>Tangerines</td>
</tr>
<tr>
<td>Yellow watermelon</td>
<td>Yellow beets</td>
</tr>
<tr>
<td>Butternut squash</td>
<td>Carrots</td>
</tr>
<tr>
<td>Yellow peppers</td>
<td>Yellow potatoes</td>
</tr>
<tr>
<td>Pumpkin</td>
<td>Rutabagas</td>
</tr>
<tr>
<td>Yellow summer squash</td>
<td>Sweet corn</td>
</tr>
<tr>
<td>Sweet potatoes</td>
<td>Yellow tomatoes</td>
</tr>
<tr>
<td>Yellow winter squash</td>
<td></td>
</tr>
</tbody>
</table>
Be sure to include RED in your diet to help maintain:
- A health heart
- Memory function
- A lower risk of some cancers
- Urinary tract health

Get your REDS every day by eating fruits and vegetables such as:

<table>
<thead>
<tr>
<th>Red apples</th>
<th>Blood oranges</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cherries</td>
<td>Cranberries</td>
</tr>
<tr>
<td>Red grapes</td>
<td>Pink/Red grapefruit</td>
</tr>
<tr>
<td>Red pears</td>
<td>Pomegranates</td>
</tr>
<tr>
<td>Raspberries</td>
<td>Strawberries</td>
</tr>
<tr>
<td>Watermelon</td>
<td>Beets</td>
</tr>
<tr>
<td>Red cabbage</td>
<td>Red peppers</td>
</tr>
<tr>
<td>Radishes</td>
<td>Radicchio</td>
</tr>
<tr>
<td>Red onions</td>
<td>Red potatoes</td>
</tr>
<tr>
<td>Rhubarb</td>
<td>Tomatoes</td>
</tr>
</tbody>
</table>

Besides providing vitamins, minerals, antioxidants and phytochemicals, fruits and vegetables also provide fiber and water which help to make you feel fuller for a longer period of time.

Adapted from the Produce for Better Health Foundation’s 5-a-day program.
Dietary Fat

Polyunsaturated and monounsaturated fats – Both of these fats may help lower your blood cholesterol level when you use them in place of saturated fats in your diet. They're found primarily in oils from plants. But a moderate intake of all types of fat is best. Use polyunsaturated or monounsaturated oils — and margarines and spreads made from them — in limited amounts.

Polyunsaturated fats — These include safflower, sesame and sunflower seeds, corn and soybeans, many nuts and seeds, and their oils.

Monounsaturated fats — Found in canola, olive and peanut oils, and avocados.

Omega 3 fatty acids - Omega-3 fatty acids benefit the heart of healthy people, and those at high risk of — or who have — cardiovascular disease.

- **Eat fish (particularly fatty fish) at least two times a week.** Fish doesn't have the high saturated fat that fatty meat products do. Fatty fish like mackerel, lake trout, herring, sardines, albacore tuna and salmon are high in two kinds of omega-3 fatty acids.
- **Try tofu and other forms of soybeans, canola, walnuts, flaxseed, and their oils.** These contain alpha-linolenic acid (LNA), which can become omega-3 fatty acid in the body.

Saturated fats - Saturated fat is the main dietary cause of high blood cholesterol. The American Heart Association recommends that you limit your saturated fat intake to 7–10 percent of total calories each day. Saturated fat is found mostly in foods from animals and some plants.

- **From animals** — These include beef, beef fat, veal, lamb, pork, lard, poultry fat, butter, cream, milk, cheeses and other dairy products made from whole milk. These foods also contain dietary cholesterol.

- **From plants** — These include coconut oil, palm oil and palm kernel oil (often called tropical oils), and cocoa butter.

Trans-fatty acids - Trans-fatty acids (or “trans fat”) are fats found in foods such as shortening, some margarines, crackers, candies, baked goods, cookies, snack foods, fried foods, and many processed foods. Trans fat is formed during a process called “hydrogenation” which manufacturers use to keep food fresh and to make margarines a solid form. Liquid and tub margarines have less trans fat than stick margarines. In clinical studies, trans fats tend to raise total blood cholesterol levels, which may increase the risk of heart disease. Recently the FDA passed a regulation requiring trans fat to be listed on the nutrition label, and
food manufacturers have until 2006 to list the amount of trans fat in food to food labels.

<table>
<thead>
<tr>
<th></th>
<th><strong>Instead of</strong></th>
<th><strong>Try</strong></th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Breakfast foods</strong></td>
<td>Donuts and pastries</td>
<td>Cereals &amp; fresh fruit</td>
</tr>
<tr>
<td></td>
<td>Croissants and bagels with butter or full-fat cream cheese</td>
<td>Small bagel with low-fat cheese and tomato</td>
</tr>
<tr>
<td><strong>Dairy Products</strong></td>
<td>Whole or 2% milk</td>
<td>Skim or low-fat (1%) milk</td>
</tr>
<tr>
<td></td>
<td>Full-fat cheeses</td>
<td>Part-skim or fat-free cheese</td>
</tr>
<tr>
<td></td>
<td>Half ‘n’ Half</td>
<td>Fat-free coffee creamer</td>
</tr>
<tr>
<td>**Meat, Poultry, fish, beans,</td>
<td>High-fat meats</td>
<td>Lean meats such as sirloin, lean pork, and</td>
</tr>
<tr>
<td>eggs and nuts**</td>
<td>Fried poultry or fish</td>
<td>turkey breast</td>
</tr>
<tr>
<td></td>
<td>High-fat bologna or hot dogs</td>
<td>Roasted or grilled meat, poultry or fish</td>
</tr>
<tr>
<td></td>
<td>Tuna canned in oil</td>
<td>Lean roast beef or ham, chicken or</td>
</tr>
<tr>
<td></td>
<td></td>
<td>turkey breast, low-fat bologna and hot</td>
</tr>
<tr>
<td></td>
<td></td>
<td>dogs</td>
</tr>
<tr>
<td><strong>Vegetables</strong></td>
<td>French fries or baked potatoes with butter and sour cream</td>
<td>Baked potatoes with low-fat sour</td>
</tr>
<tr>
<td></td>
<td></td>
<td>cream or salsa</td>
</tr>
<tr>
<td></td>
<td>Tossed salad with full-fat dressing</td>
<td>Tossed salad with low-fat or fat-free</td>
</tr>
<tr>
<td></td>
<td></td>
<td>dressing</td>
</tr>
<tr>
<td></td>
<td>Cooked vegetables with butter/high-fat sauces</td>
<td>Steamed vegetables with little or no</td>
</tr>
<tr>
<td></td>
<td></td>
<td>butter or high-fat sauce</td>
</tr>
<tr>
<td><strong>Rice and Pasta</strong></td>
<td>Pasta with butter, alfredo or cheese sauce</td>
<td>Pasta with tomato sauce and vegetables</td>
</tr>
<tr>
<td></td>
<td>Rice with butter or high-fat sauce</td>
<td>Rice with low-fat sauce, vegetables or</td>
</tr>
<tr>
<td></td>
<td></td>
<td>broth</td>
</tr>
<tr>
<td><strong>Desserts</strong></td>
<td>Cakes or cookies</td>
<td>Reduced-fat cakes or cookies (compare</td>
</tr>
<tr>
<td></td>
<td></td>
<td>labels!)</td>
</tr>
<tr>
<td></td>
<td>Full-fat ice cream</td>
<td>Low-fat frozen yogurt or soft-serve ice</td>
</tr>
<tr>
<td></td>
<td></td>
<td>cream</td>
</tr>
<tr>
<td><strong>Snacks</strong></td>
<td>Regular potato chips or tortilla chips</td>
<td>Baked chips, reduced-fat crackers,</td>
</tr>
<tr>
<td></td>
<td></td>
<td>pretzels</td>
</tr>
<tr>
<td></td>
<td>Chocolate bars</td>
<td>Fresh fruit, fudgesicles or low-fat ice</td>
</tr>
<tr>
<td></td>
<td></td>
<td>cream</td>
</tr>
<tr>
<td><strong>Soups</strong></td>
<td>Cream Soups</td>
<td>Vegetable or broth-based soups</td>
</tr>
</tbody>
</table>
Physical Activity

Why is Physical Activity Important?

Increasing physical activity is an important part of your weight-management program. Adding exercise increases the number of calories you burn so that you can speed up your weight-loss efforts. You also build muscle, which keeps your metabolism in high gear to burn calories even more efficiently. Exercise also helps you feel better about yourself, too, and is a great way to reduce stress in your life. We are asking that you commit 30 minutes/day, at least 3 times/week to physical activity outside of activities that you may already do as part of your daily routine. Here is an example of some activities that are inexpensive, easy, and hopefully fun for you to try!

Dancing - No need to even leave the house for this one. Involve your children or a friend in this one...simply put on the stereo and start moving! Vigorous dancing for only 20 minutes burns ~100 calories for someone who is 180 lbs.

Yoga and Pilates - There are many inexpensive video tapes on the market to guide you through this great stretching and strengthening activity; expensive classes at the gym are not necessary as long as you have the motivation to get it done. You will feel great afterwards; flexible and relaxed!

Swimming - Even a relaxed pace of swimming burns up to 3.5 calories/minute, but when you commit yourself to steady paced laps or water jogging, you can burn more than 7 calories/minute.

Exercise Equipment - Look in the classified section of the newspaper, this is a popular item that is sold at bargain prices! The advantage to some of these machines is that 1) certain activity goals you may want to reach can be programmed into the machine, and 2) some machines such as treadmills offer you the opportunity to catch up on reading while you are exercising.
Walking - There are many ways to increase your time spent walking. Try to increase the speed that you normally walk; brisk walking or climbing hills can burn more than 7 calories/minute. Here are a few ideas:
- If you make a telephone call, walk while you talk
- Park further away in store parking lots
- Avoid elevators, escalators, and buses when possible
- Choose the furthest entrance to your building at work; walk the long way
- Try to enlist a partner to walk with. This is an advantage for you in 2 ways: there is a commitment to walk with another person that you don’t want to let down, and walking with another while having a conversation makes the walk seem effortless and quick!

Setting Goals

Setting goals for your physical activity can be extremely effective for disciplining yourself, and can lead to permanent weight loss through lifestyle changes. Effective goals are:

1) Specific: “I will exercise more” is not specific enough to be entirely effective; a better goal is one that you can measure, such as “I will exercise today for 30 minutes”.

2) Realistic: Start with a small and attainable goal and work yourself up from there. Many exercise intentions have been sidelined because a goal set was not realistic enough for the individual to attain, and only frustration results.

3) Forgiving: If you unable to achieve all of your exercise goals one week, don’t let that lead to self-defeating thoughts and feelings. It is very important that you forgive yourself and continue with your physical activity routine as soon as possible.
Calcium

Recently published studies in leading medical journals suggest a link between dairy consumption and lower body weight. Data indicates that calcium may play a role in the body’s natural system for burning fat. Adequate calcium intake can also protect against osteoporosis, a crippling bone disease.

Make Calories Count

When you include dairy foods everyday as part of your reduced-calorie eating plan, you are not loading up on empty calories. Milk, cheese and yogurt are nutrient-rich foods that naturally provide calcium, protein and other essential vitamins and minerals for good health. Include at least three servings of milk, cheese or yogurt everyday. In an effort to reduce calories, be sure to enjoy low-fat or fat-free varieties when you can. You may have to experiment until you find the brands you prefer. While we recommend three low-fat servings from the dairy group everyday, you can further improve your calcium intake by also including other calcium-rich foods such as fortified orange juice, nuts, dark green vegetables, and tofu.

<table>
<thead>
<tr>
<th>Dairy Products &amp; Alternatives</th>
<th>Serving Size</th>
<th>Calcium (mg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Milk: whole, 2%, 1%, skim</td>
<td>1 cup</td>
<td>300</td>
</tr>
<tr>
<td>Soy milk, calcium fortified</td>
<td>1 cup</td>
<td>300</td>
</tr>
<tr>
<td>Yogurt: plain or with fruit</td>
<td>1 cup</td>
<td>450</td>
</tr>
<tr>
<td>Swiss cheese</td>
<td>1 oz</td>
<td>270</td>
</tr>
<tr>
<td>Cheddar cheese</td>
<td>1 oz</td>
<td>205</td>
</tr>
<tr>
<td>Frozen yogurt</td>
<td>½ cup</td>
<td>105</td>
</tr>
<tr>
<td>Calcium-fortified orange juice</td>
<td>1 cup</td>
<td>300</td>
</tr>
<tr>
<td>Tofu, firm or soft</td>
<td>3 oz</td>
<td>60 - 250</td>
</tr>
<tr>
<td>Broccoli</td>
<td>½ cup (cooked)</td>
<td>45</td>
</tr>
</tbody>
</table>

How Much Calcium Do You Need?

<table>
<thead>
<tr>
<th>Age Group</th>
<th>Dietary Reference Intakes (DRI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0-6 months</td>
<td>210 mg/day</td>
</tr>
<tr>
<td>6-12 months</td>
<td>270 mg/day</td>
</tr>
<tr>
<td>1-3 years</td>
<td>500 mg/day</td>
</tr>
<tr>
<td>4-8 years</td>
<td>800 mg/day</td>
</tr>
<tr>
<td>9-18 years</td>
<td>1300 mg/day</td>
</tr>
<tr>
<td>Adults 19-50 years</td>
<td>1000 mg/day</td>
</tr>
<tr>
<td>Adults 51+ years</td>
<td>1200 mg/day</td>
</tr>
</tbody>
</table>
33 Trim & Tasty Snack Ideas

Incorporating dairy snacks between meals is a healthy way to keep hunger in check and stay energized throughout the day. In addition, enjoying 3-A-Day of Dairy --- 3 servings of milk, cheese or yogurt each day --- as part of a reduced-calorie weight loss plan can help people lose more weight by burning more fat than just by cutting calories alone.

**Milk**

1. Rise and Shine:
   Get a mid-morning boost with a cold glass of fat-free milk and sliced peaches.
2. Strawberry-Sicle:
   Mix lowfat strawberry milk with fresh strawberry slices and freeze in a popsicle container.
3. Flavor on-the-Fly:
   Fat-free flavored milks are perfect anywhere you go — just take, shake and sip!
4. Choco-Raspberry Chug:
   Blend a cup of fat-free milk with frozen raspberries and sweetened cocoa.
5. Sweet Milk Steamer:
   Microwave a cup of lowfat milk and honey.
6. Banana Split Blenders:
   Blend lowfat strawberry milk with banana slices and ice.
7. Morning Mocha:
   Mix a cup of reduced-fat milk with a teaspoon of instant coffee and sweetened cocoa.
8. A Sip of Spice:
   Warm a cup of milk and mix with Chai tea to warm up the afternoon.
9. Graham Dunk:
   Dip a few graham crackers into a cold glass of lowfat milk.
10. Fruity Splash:
    Blend your strawberry milk with fresh bananas and ice.
11. Vanilla-Berry Bowl:
    Enjoy lowfat vanilla milk with a bowl of fresh berries.

**Cheese**

12. Salsa Roll-Up:
    Roll Monterey Jack cheese into a whole-wheat tortilla and dip in salsa.
13. Cheddar Crunch:
    Mix 1/4 cup of Cheddar cheese, shredded with popcorna and pretzels.
14. Veggie Wraps:
    Wrap Colby Jack cheese around spears of asparagus.
15. Cherry Tomato Cruncher:
    Top wheat crackers with reduced-fat Mozzarella cheese and cherry tomatoes.
16. Cheese & Crackers:
    Layer lowfat American cheese and smoked turkey onto crackers.
17. Seafood Spread:
    Mix canned salmon or crab with Ricotta or Mozzarella cheese and eat on pita wedges.
18. Cheesy Tostada:
    Top a tostado with fat-free refried beans and reduced-fat shredded Pepper Jack cheese.
19. Chicken Melt:
    Melt a slice of reduced fat Colby Jack cheese over canned chicken on an English muffin.
20. Cheese Kabobs:
    Alternate small slices of apples and reduced-fat Cheddar cheese on skewers.
21. Lettuce Wraps:
    Wrap a slice of Swiss cheese, turkey, and Dijon mustard in lettuce leaves.
22. String by String:
    Pack string cheese into your bag for a post-workout energizer.

**Yogurt**

23. Tropical Smoothie:
    Blend fresh orange slices with strawberry yogurt and ice.
24. Cucumber Salad:
    Mix diced cucumber and a cup of lowfat plain yogurt, mint and a pinch of salt and pepper and spread on whole-wheat pita wedges.
25. Mango Mixer:
    Enjoy a tropical treat by blending mango, plain lowfat yogurt, and a splash of pineapple juice.
26. Parfait Platter:
    Layer granola and fresh fruit with your favorite lowfat yogurt.
27. Simple Shortcake:
    Try pieces of angel food cake with a cup of strawberry yogurt for a heavenly treat.
28. Berry Blast:
    Mix blackberry yogurt with fresh blueberries or raspberries.
29. Go Nuts:
    Top lowfat vanilla yogurt with a few crushed pecans and cinnamon.
30. Honey Fruit Dip:
    Mix fat-free yogurt with a dollop of honey for a sweet fruit dip.
31. Yo-on-the-Go:
    Enjoy your favorite flavor of drinkable yogurt at the office or on the go.
32. Mocha Muffin:
    Stir chocolate syrup into a cup of coffee-flavored yogurt and freeze.
33. Yogurt Pops:
    Freeze squeezable yogurt for a quick and creamy popsicle.

Eating Low-Fat with Lean Meats

- **Fish and shellfish** — Fish and shellfish is lower in fat, saturated fat, and calories than most meats and poultry. Great for grilling!
- **Poultry** — This includes chicken, Cornish hen, turkey, and ground turkey. The whiter the meat, the less fat and calories it contains.
- **Lean beef** — Terms such as round, sirloin and loin usually equal a leaner cut of meat. These cuts may require longer, slower cooking time. Buy "choice" cuts or "select" grades of beef rather than "prime." Choose lean or extra lean ground beef (no more than 15 percent fat).
- **Lean ham and pork** — Tenderloin and loin chop are the best pork choices. Ham and Canadian bacon are higher in sodium than other meats, but are still considered a lean meat choice.
- **Wild game** — Rabbit, venison, pheasant and duck (without skin) are low in fat and are great choices.
- **Processed sandwich meats** — These include low-fat turkey, chicken, turkey ham, turkey pastrami or lean boiled ham. However, beware of the high sodium content of these meats.

Serving size

- 3 oz. cooked (4 oz. raw) lean meat, poultry or fish

A three-ounce portion of meat, poultry or fish equals:

- About the size of a deck of playing cards
- 2 thin slices of lean roast beef (each slice 3" x 3" x 1/4")
- 1/2 of a chicken breast or a chicken leg with thigh (without skin)
- 3/4 cup of flaked fish

**WHY EAT LEAN MEAT?**

- **High Protein Source**
- **Rich Iron Source**
- **Contains B-12 Vitamins**

Ideas for Ordering and Cooking Lean Meat

1. When ordering lean meat in restaurants make sure that it has been prepared with either dry or moist heat methods; poached, steamed, grilled, baked, broiled.
2. To reduce saturated fat and calorie intake, avoid meat that has been battered and fried, slathered in butter, or blanketed in creamy sauces.
3. Experiment with leaner cuts of meat in your favorite recipes to replace higher fat choices.
4. Use cookware such as crock pots (for slow cooking lean cuts of beef), rice steamers (works great with shellfish) and good Teflon® skillets (eliminates the need for oil) for good results with lean meat.

Iron

• A mineral abundant in most lean meats; an iron deficiency can cause anemia, fatigue, difficulty concentrating, and a resistance to healing.
• Recommendations: 18.0 mg (ages 18 – 51), 8.0 mg (age 51+) daily.

- Raisins, broccoli, spinach, and kidney beans are good sources of iron, however, iron that comes from animal (called Heme iron) is absorbed more efficiently.

Sources of Iron

- Raisins, 2/3 cup
- Broccoli, 1 cup
- Spinach, 1/2 cup
- Kidney Beans, 1 cup
- Egg, 1 each
- Halibut, 3 oz
- Turkey, white meat, 3 oz
- Chicken, 3.5 oz
- Pork, 3 oz
- Beef, 3 oz
- Beef Liver, 3.5 oz

mg of Iron

SOURCES: Rutger’s Department of Health Education. April 2005.
**Cheesy Pepper Quesadillas***

Prep/Total Time: 30 min.

1 T margarine, melted
2 whole wheat flour tortillas (8 inches)*
½ C shredded Monterey Jack cheese
½ C shredded reduced-fat cheddar cheese
¼ C julienne strips sweet yellow pepper
¼ C julienne strips sweet red pepper
¼ C julienne strips red onion
Garlic salt, optional
Salsa and sour cream, optional

Brush margarine on each side of tortillas. Place on an ungreased baking sheet. Sprinkle half of each tortilla with 1 T Monterey Jack and 1 T cheddar cheese. Top each with sweet peppers, onion, garlic salt if desired and remaining cheese. Fold tortillas over filling.

Bake at 450 F for 10-12 minutes or until golden brown and cheese is melted. Cut into wedges. Serve with salsa and sour cream if desired. Yield: 2 servings.

**Nutrition Facts**: 1 serving (prepared with reduced-fat margarine, reduced-fat cheeses and calculated without optional ingredients)

**Calories**: 254; **Fat**: 14g (7g saturated fat); **Cholesterol**: 30mg; **Sodium**: 348mg; **Carbohydrate**: 21g; **Fiber**: 2g; **Protein**: 15g.


**OPTIONAL**: Add 3 oz cooked shredded chicken breast to tortilla before baking
Red Pepper Pilaf

Roasting or broiling sweet red peppers brings out the flavor and adds a smoky note. Combine them with rice and you get a pilaf that’s both colorful and tasty.

3 C bullion
1 C brown rice
1 large sweet red pepper
1 onion, chopped
Freshly ground black pepper

Set aside ½ C of the bullion. Bring the remaining 2½ C to a boil and stir in the brown rice; cover, reduce heat and cook 40-45 minutes, or until tender but not mushy.

Meanwhile, cook the onion in the reserved ½ C bouillon until softened, about 10 minutes. Roast the pepper over a gas flame or under a broiler until the skin is charred. Place it in a paper bag to steam. Remove the seeds, stem and most of the skin and dice the pepper. When the rice is cooked, stir in the red pepper and onion. Season to taste with ground black pepper.

Yield: 4 servings

From Fat Free Flavor Full
**Whole Wheat Pesto Pasta***

2 C uncooked whole-wheat noodles*
2 T pesto sauce
1/3 C sun dried tomatoes
1 lb skinless chicken breast
1 T olive oil
2 C fresh broccoli florets

Soak sun dried tomatoes in 1 C very hot water. Meanwhile, sauté chicken in oil, cool and cut into bite-size pieces. Cook noodles according to package directions; drain and stir in pesto sauce.

Microarray broccoli on high until just crisp tender, about 2.5 minutes. Drain tomatoes, chop, and add to pasta along with broccoli and chicken. Serve immediately.

**Yield:** 4 servings

From the kitchen of Sami Heim
**Corn-fetti Salad**

1 C corn kernels (frozen or fresh)
1 can chick peas, drained
(or 2 C cooked chick peas)
1 C celery, diced
½ C onion, chopped
½ C green pepper, chopped
2 T pimento, chopped
¼ C non-fat Italian dressing

Thaw frozen corn or cook fresh briefly in boiling water. Combine the corn with the remaining ingredients. Chill and serve.

**Yield:** 4 servings

from *Fat Free Flavor Full*
**Amy’s Whole-Grain Cereal**

1 C wheat flakes  
1 C barley flakes  
1 C brown rice  
½ C millet  
½ C wheat bulgur  

Combine grains together. Add grains to 8 C boiling water. Cover and simmer for ~15 minutes.

Add **any or all** of the following: brown sugar, vanilla, raisins, slivered almonds, sliced apples, sliced bananas, and cinnamon. Simmer for 5 more minutes, or when water is absorbed. Grains should be slightly crunchy, but add more water and cover for a while is a softer texture is preferred, or if grains are sticking to bottom of pan (they usually do).

**SUGGESTION:** This amount makes a **lot**. You can put this whole combined amount of dry grains in a sealed container, and cook amount desired whenever needed. Just make sure ratio of water to grains is 2:1. Some of these grains can only be found at a health food store.

from the kitchen of Amy Ciccarella
**Chicken and Rice with Meat**

1 T margarine  
½ lb extra lean ground beef  
1 t allspice  
½ t ground cinnamon  
1/8 t ground black pepper  
2 ¾ C hot water  
1½ t salt  
½ lb skinless, boneless chicken breast halves  
1 C uncooked brown rice  
½ C pine nuts

Melt butter in large skillet over medium heat, and cook ground beef until evenly brown. Season with allspice, cinnamon, and pepper; continue cooking 1 minute. Pour in hot water, season with salt, and place chicken in skillet. Cover, cook 25 minutes, until chicken juices run clear.

Remove chicken from skillet, and shred. Return to skillet, and mix in the rice. Cover, and continue cooking 40-45 minutes, until rice is tender, and liquid has been absorbed.

In a separate skillet over medium heat, cook and stir the pine nuts 5 minutes, or until lightly browned. Sprinkle pine nuts over the beef, chicken, and rice mixture to serve.

**Turkey Fajitas**

1 lb turkey breast cutlets or slices, cut into $\frac{1}{2}$-inch strips  
$\frac{1}{2}$ C fresh cilantro, chopped  
1 clove garlic, minced  
$\frac{1}{2}$ t cumin  
$\frac{1}{4}$ t chili powder  
1/8 t reduced-sodium soy sauce  
1/8 t Worcestershire sauce  
2 t vegetable oil  
1 red bell pepper, cut into 1/8 x 2-inch pieces  
1 green bell pepper, cut into 1/8 x 2-inch pieces  
2 C onion, thinly sliced and separated into rings  
3 T limejuice  
8 whole wheat flour tortillas  
Optional:  
  reduced calorie sour cream  
  guacamole  
  salsa or Pico de Gallo sauce

Combine turkey, cilantro, garlic, cumin, chili powder, soy sauce and Worcestershire sauce. Cover and refrigerate 1 hour.  
Stir-fry turkey mixture in 1 t oil for 4 minutes, or until turkey is no longer pink. Remove from skillet and set aside.  
Add remaining t oil to skillet. Stir fry red and green peppers 2 minutes or until slightly softened. Add onion; cook stirring constantly, until vegetables are crisp-tender.  
Return turkey strips to skillet. Pour limejuice over mixture and stir to combine. Remove from heat and serve immediately in flour tortillas.  
GARNISH with sour cream, guacamole and Pico de Gallo sauce if desired.  
Yield: 4 servings  
From [http://www.eatturkey.com/recipe/recipe.cgi/2/10540](http://www.eatturkey.com/recipe/recipe.cgi/2/10540)
Cheesy Pepper Quesadillas*

Prep/Total Time: 30 min.
1 T margarine, melted
2 flour tortillas (8 inches)*
½ C shredded Monterey Jack cheese
¼ C shredded reduced-fat cheddar cheese
¼ C julienne strips sweet yellow pepper
¼ C julienne strips sweet red pepper
¼ C julienne strips red onion
Garlic salt, optional
Salsa and sour cream, optional

Brush margarine on each side of tortillas. Place on an ungreased baking sheet. Sprinkle half of each tortilla with 1 T Monterey Jack and 1 T cheddar cheese. Top each with sweet peppers, onion, garlic salt if desired and remaining cheese. Fold tortillas over filling.

Bake at 450 F for 10-12 minutes or until golden brown and cheese is melted. Cut into wedges. Serve with salsa and sour cream if desired. Yield: 2 servings.

Nutrition Facts: 1 serving (prepared with reduced-fat margarine, reduced-fat cheeses and calculated without optional ingredients)
Calories: 254; Fat: 14g (7g saturated fat); Cholesterol: 30mg; Sodium: 348mg; Carbohydrate: 21g; Fiber: 2g; Protein: 15g.

http://www.qctimes.com/internal.php?story_id=1047330&t=Food&c=13,1047330

OPTIONAL: Add 3 oz cooked shredded chicken breast to tortilla before baking
**Spinach Salad with Strawberries**

2 bunches fresh spinach leaves, washed, dried, and chilled
Honey Dressing (see recipe below)
1 C fresh strawberries, thickly sliced
1 T sesame seeds, toasted
1 small red onion, thinly sliced (optional)

Remove stems and veins from spinach and tear into bite-size pieces; place into large salad bowl. Pour Honey Dressing over spinach; toss gently. Add strawberries, sesame seeds, and onion. Toss again and serve.

**Honey Dressing**

2 T balsamic vinegar
2 T rice vinegar
1 T plus 1 t honey
2 t Dijon mustard
salt and pepper to taste

In a small jar with lid, combine all ingredients, cover and shake vigorously. Store, covered, in the refrigerator. Serve at room temperature.

From [http://whatscookingamerica.net/Salad/spinachstraw.htm](http://whatscookingamerica.net/Salad/spinachstraw.htm)
Pesto Pasta

2 C uncooked noodles
2 T pesto sauce
1/3 C sun dried tomatoes
1 lb skinless chicken breast
1 T olive oil
2 C fresh broccoli florets

Soak sun dried tomatoes in 1 C very hot water. Meanwhile, sauté chicken in oil, cool and cut into bite-size pieces. Cook noodles according to package directions; drain and stir in pesto sauce.

Microwave broccoli on high until just crisp tender, about 2.5 minutes. Drain tomatoes, chop, and add to pasta along with broccoli and chicken. Serve immediately.

Yield: 4 servings

From the kitchen of Sami Heim
Sweet Pepper Salsa Fish

1 lb fresh or frozen skinless fish fillets (about ⅜-inch thick)  
2 T cooking oil  
1 ½ C fresh mushrooms, quartered  
1 C coarsely chopped green and/or yellow sweet pepper  
1 small onion, halved and sliced  
1 C salsa  
fresh oregano

Thaw fish if frozen. Cut fish fillets into 4 serving-size portions, if necessary. Rinse fish; pat dry with paper towels; set aside.

In a large skillet heat 1 T oil. Cook mushrooms, peppers and onion for 5 minutes or just until tender. Remove vegetables with a slotted spoon; set aside.

Add the remaining oil to skillet. Add fish fillets. Cook over medium heat for 8-10 minutes or until fish flakes easily with fork, turning once.

Spoon cooked vegetables over fish fillets. Top with salsa.

Cover and cook over low heat about 2 minutes or until heated through. Garnish with fresh oregano, if desired.

Yield: 4 servings.

Nutrition Facts: Calories: 190; Total Fat: 10g; Saturated Fat: 1g; Cholesterol: 53mg; Sodium: 306mg; Carbohydrate: 8g; Protein: 21g.
Red Pepper Pilaf

Roasting or broiling sweet red peppers brings out the flavor and adds a smoky note. Combine them with rice and you get a pilaf that’s both colorful and tasty.

3 C bullion
1 C white rice
1 large sweet red pepper
1 onion, chopped
Freshly ground black pepper

Set aside ½ C of the bullion. Bring the remaining 2½ C to a boil and stir in the brown rice; cover, reduce heat and cook 30-40 minutes, or until tender but not mushy.

Meanwhile, cook the onion in the reserved ½ C bouillon until softened, about 10 minutes. Roast the pepper over a gas flame or under a broiler until the skin is charred. Place it in a paper bag to steam. Remove the seeds, stem and most of the skin and dice the pepper. When the rice is cooked, stir in the red pepper and onion. Season to taste with ground black pepper.

Yield: 4 servings

From Fat Free Flavor Full
**Chicken and Rice with Meat**

1 T margarine  
½ lb extra lean ground beef  
1 t allspice  
½ t ground cinnamon  
1/8 t ground black pepper  
2¾ C hot water  
1½ t salt  
½ lb skinless, boneless chicken breast halves  
1 C uncooked basmati rice  
½ C pine nuts

Melt butter in large skillet over medium heat, and cook ground beef until evenly brown. Season with allspice, cinnamon, and pepper; continue cooking 1 minute. Pour in hot water, season with salt, and place chicken in skillet. Cover, cook 25 minutes, until chicken juices run clear.

Remove chicken from skillet, and shred. Return to skillet, and mix in the rice. Cover, and continue cooking 20 minutes, until rice is tender, and liquid has been absorbed.

In a separate skillet over medium heat, cook and stir the pine nuts 5 minutes, or until lightly browned. Sprinkle pine nuts over the beef, chicken, and rice mixture to serve.

**Turkey Fajitas**

1 lb turkey breast cutlets or slices, cut into $\frac{1}{2}$-inch strips

$\frac{1}{2}$ C fresh cilantro, chopped

1 clove garlic, minced

$\frac{1}{2}$ t cumin

$\frac{1}{4}$ t chili powder

1/8 t reduced-sodium soy sauce

1/8 t Worcestershire sauce

2 t vegetable oil

1 red bell pepper, cut into 1/8 x 2-inch pieces

1 green bell pepper, cut into 1/8 x 2-inch pieces

2 C onion, thinly sliced and separated into rings

3 T limejuice

8 flour tortillas

Optional:

- reduced calorie sour cream
- guacamole
- salsa or Pico de Gallo sauce

Combine turkey, cilantro, garlic, cumin, chili powder, soy sauce and Worcestershire sauce. Cover and refrigerate 1 hour.

In a large non-stick skillet, over medium-high heat, stir-fry turkey mixture in 1 t oil for 4 minutes, or until turkey is no longer pink. Remove from skillet and set aside.

Add remaining t oil to skillet. Stir fry red and green peppers 2 minutes or until slightly softened. Add onion; cook stirring constantly, until vegetables are crisp-tender.

Return turkey strips to skillet. Pour limejuice over mixture and stir to combine. Remove from heat and serve immediately in flour tortillas.

Garnish with sour cream, guacamole and Pico de Gallo sauce if desired.

**Yield**: 4 servings

From [http://www.eatturkey.com/recipe/recipe.cgi/2/10540](http://www.eatturkey.com/recipe/recipe.cgi/2/10540)
APPENDIX D

WHOLE GRAIN STUDY
SAS STATISTICAL ANALYSIS PROGRAMS
SAS Code for Biochemical Data

%macro mix(y,code);
   data visit1(keep=subjectno &y._baseline);
      set grain;
      if visitno=1;
         &y._baseline=&y;
   run;

   data mrg(keep=subjectno visitno group &y &y._baseline y._change);
      merge grain visit1;
      by subjectno;
      if visitno=1 then delete;
         &y._change=&y - &y._baseline;
   run;

   proc sort data=mrg;
      by subjectno visitno;
   run;

   ods exclude all;
   run;

   *ods graphics on;
   run;

   proc mixed data=mrg;
      class subjectno group visitno;
      model &y._change = group visitno group*visitno &y._baseline / ddfm=kenwardroger residual;
   %end;
   %else %do;
      model &y._change = group visitno group*visitno &y._baseline / residual;
   %end;
      repeated visitno / subject=subjectno type=ar(1);
   lsmeans group*visitno / diffs cl alpha=%sysevalf(0.05/3);
   ods output lsmeans=lsmeans diffs=diffs;
   title1 "Whole Grain Study";
   title3 "Dependent Variable: &y._change";
   run;

   *ods graphics off;
   run;

   ods exclude none;
   run;

   data lsmeans;
set lsmeans;
bon_p=min(1,probt*3);

cl='(','\mid trim(left(put(lower,6.1))))||','\mid trim(left(put(upper,6.1)))))||')';
format estimate 6.1 bon p pvalue6.4;
label probt="P-value (Unadjusted)"
bon_p="P-value (Bonferroni Adjusted)"
cl="95% Confidence Interval (Bonferroni Adjusted)"
run;

data diffs;
set diffs;
if visitno= visitno;
bon_p=min(1,probt*3);

cl='(','\mid trim(left(put(lower,6.1))))||','\mid trim(left(put(upper,6.1)))))||')';
format estimate 6.1 bon p pvalue6.4;
label probt="P-value (Unadjusted)"
bon_p="P-value (Bonferroni Adjusted)"
cl="95% Confidence Interval (Bonferroni Adjusted)"
run;

proc print data=lsmeans label;
var group visitno estimate ci bon p probt;
title1 "Whole Grain Study"
title3 "Dependent Variable: &y._change"
run;

proc print data=diffs label;
var group visitno _group _visitno estimate ci bon p probt;
title1 "Whole Grain Study"
title3 "Dependent Variable: &y._change"
run;
%mend mix;
ods pdf file="C:\data\Table2.pdf" run;
%mix(weight,1);
%mix(systolicbp,1);
%mix(diastolicbp,1);
%mix(waistcirc,1);
%mix(dxabodyfat,1);
%mix(cholesterol,1);
%mix(ldl,1);
%mix(ldl1,1);
%mix(ldl2,1);
%mix(ldl3,1);
%mix(ldl4,1);
%mix(major_peak_diam,1);
%mix(hdl,1);
%mix(triglycerides,1);
%mix(tc_hdl,1);
%mix(apo_ai,1);
%mix(apo_b,1);
%mix(gluc0,1);
%
mix(ins0,1);
%mix(gluc120,1);
%mix(ins120,1);
%mix(auc_glucose,2);
%mix(auc_insulin,2);
%mix(isi,1);
%mix(pai1,1);
%mix(crp,1);
%mix(ill,1);
%mix(ill6,1);
%mix(tnfa,1);
ods pdf close;
run;
SAS Code for Nutrient Data

```sas
data nut;
    set grain.recallnutrient;
    if subjectno=. then delete;

    /*calculate number of grams of fiber eaten for each 1000 calories*/
    fiber1000 = fiber / (kcal / 1000);
    solfiber1000 = solublefiber / (kcal / 1000);
    insolfiber1000 = insolublefiber / (kcal / 1000);

    /*log transform values for analysis*/
    logkcal = log(kcal);
    logcho = log(cholesterol);
    logfiber = log(fiber1000);
    solfiber1000 = log(solutefiber1000);
    loginsolfib = log(insolublefiber1000);
    logmg = log(magnesium);
    logns = log(sodium);
    logvitb6 = log(vitaminb6);
    logaddedsugar = log(addedsugar);
    logpufa = log(pu
```

```sas
proc sort data=nut;
    by subjectno visitno group;
run;

proc print data=nut;
run;

%macro mix(y);
    ods exclude all;
    run;

    *ods graphics on;
    run;

    proc mixed data=nut;
        class subjectno group visitno;
        model &y = group visitno group*visitno / ddfm=kenwardroger ual;
        repeated visitno / subject=subjectno type=ar(1);
        lsmeans group*visitno / cl;
        ate 'Control: Week 4-Week1' visitno -1 1 0 0
        group*visitno -1 1 0 0 0 0 0 0 / cl alpha=%sysevalf(0.05/9);
        estimate 'Control: Week 8-Week1' visitno -1 0 1 0
        group*visitno -1 0 1 0 0 0 0 0 / cl alpha=%sysevalf(0.05/9);
        estimate 'Control: Week 12-Week1' visitno -1 0 0 1
        group*visitno -1 0 0 1 0 0 0 0 / cl alpha=%sysevalf(0.05/9);
        estimate 'Grain: Week 4-Week1' visitno -1 1 0 0
        group*visitno 0 0 0 0 -1 1 0 0 / cl alpha=%sysevalf(0.05/9);
        estimate 'Grain: Week 8-Week1' visitno -1 0 1 0
        group*visitno 0 0 0 0 -1 0 1 0 / cl alpha=%sysevalf(0.05/9);
```
```
estimate 'Grain: Week 12-Week1' visitno -1 0 0 1
  group*visitno 0 0 0 0 -1 0 0 1 / cl alpha=%sysevalf(0.05/9);
estimate 'Grain-Control: Week 4-Week1' group*visitno 1 -1 0 0
  0 -1 1 0 0 / cl alpha=%sysevalf(0.05/9);
estimate 'Grain-Control: Week 8-Week1' group*visitno 1 0 -1
  0 -1 0 1 0 / cl alpha=%sysevalf(0.05/9);
estimate 'Grain-Control: Week 12-Week1' group*visitno 1 0 0
  -1 -1 0 0 1 / cl alpha=%sysevalf(0.05/9);
ods output lsmeans=lsmeans estimates=estimates;
title1 "Whole Grain Study";
title3 "Dependent Variable: &y";
run;

*ods graphics off;
run;

ods exclude none;
run;

data lsmeans;
  set lsmeans;
  ci='(|trim(left(put(lower,6.1))))||','||trim(left(put(upper,6.1)))))||')';
  format estimate 6.1;
  label estimate="LSMean"
    probt="P-value (Unadjusted)"
    ci="95% Confidence Interval";
run;

data estimates;
  set estimates;
  bon_p=min(1,probt*9);
  ci='(|trim(left(put(lower,6.1))))||','||trim(left(put(upper,6.1)))))||')';
  format estimate 6.1 bon_p pvalue6.4;
  label estimate="Difference in LSMeans"
    probt="P-value (Unadjusted)"
    bon_p="P-value (Bonferroni Adjusted)"
    ci="95% Confidence Interval (Bonferroni Adjusted)";
run;

proc print data=lsmeans label;
  var group visitno estimate ci;
  title1 "Whole Grain Study";
  title3 "Dependent Variable: &y";
run;

proc print data=estimates label;
  var label estimate ci bon_p probt;
  title1 "Whole Grain Study";
  title3 "Dependent Variable: &y";
run;
%mend mix;

ods pdf file="C:\data\Table3.pdf";
run;

%mix(kcal);
%mix(pctcarb);
%mix(pctpro);
%mlic(logpro);
%mix(pctfat);
%mix(pctsfa);
%mix(pctmufa);
%mix(pctpufa);
%mix(logpufa);
%mix(cholesterol);
%mix(logchol);
%mix(fiber1000);
%mix(logfiber);
%mix(solfiber1000);
%mix(logsolfib);
%mix(insolfiber1000);
%mix(loginsolfib);
%mix(
  %mix oqmg);
%mix(sodium);
  ogsodium); 
%mix(v 
  %mix ogvitb6);
%mix(addedsugar);
%mix(

ods pdf close;
run
SAS Code for Diet Satisfaction Data

data sat;
set grain.dsatfinal;
run;

proc sort data=sat;
by subjectno visitno group;
run;

proc print data=sat;
run;

%macro mix(y);
ods exclude all;
run;

*ods graphics on;
run;

proc mixed data=sat;
class subjectno group visitno;
model &y = group visitno group*visitno / ddfm=kenwardroger
residual;
repeated visitno / subject=subjectno type=cs;
lsmeans group*visitno / cl;
estimate 'Control: Week 4-Week1' visitno -1 1 0
  group*visitno -1 1 0 0 0 0 / cl alpha=%sysevalf(0.05/6);
estimate 'Control: Week 12-Week1' visitno -1 0 1
  group*visitno -1 0 1 0 0 0 / cl alpha=%sysevalf(0.05/6);
estimate 'Grain: Week 4-Week1' visitno -1 1 0
  group*visitno 0 0 0 -1 1 0 / cl alpha=%sysevalf(0.05/6);
estimate 'Grain: Week 12-Week1' visitno -1 0 1
  group*visitno 0 0 0 -1 0 1 / cl alpha=%sysevalf(0.05/6);
estimate 'Grain-Control: Week4-Week1' group*visitno 1 -1 0
  -1 1 0 / cl alpha=%sysevalf(0.05/6);
estimate 'Grain-Control: Week12-Week1' group*visitno 1 0 -1
  -1 0 1 / cl alpha=%sysevalf(0.05/6);
ods output lsmeans=lsmeans estimates=estimates;
title1 "Whole Grain Study";
title3 "Dependent Variable: &y";
run;

*ods graphics off;
run;

ods exclude none;
run;

data lsmeans;
set lsmeans;

ci='(||trim(left(put(lower,6.1)))||','||trim(left(put(upper,6.1))),)'|
format estimate 6.1;
label estimate="LSMean"
probt="P-value (Unadjusted)"
ci="95% Confidence Interval"

run;

data estimates;
set estimates;
bon_p=min(1,probt*6);

ci='('||trim(left(put(lower,6.1))))||','||trim(left(put(upper,6.1)))))||')';
format estimate 6.1  bon_p pvalue6.4;
label label="Comparison"
estimate="Difference in LSMeans"
probt="P-value (Unadjusted)"
    bon_p="P-value (Bonferroni Adjusted)"
    ci="95% Confidence Interval (Bonferroni Adjusted)"
run;

proc print data=lsmeans label;
var group visitno estimate ci;
title1 "Whole Grain Study";
title3 "Dependent Variable: &y";
run;

proc print data=estimates label;
var label estimate ci bon_p probt;
title1 "Whole Grain Study";
title3 "Dependent Variable: &y";
run;
%mend mix;

ods pdf file="C:\data\Table4.pdf";

%mix(health);
%mix(convn);
%mix(cost);
%mix(family);
%mix(preocc);
%mix(negasp);
%mix(prep);
%mix(dsatscor);

ods pdf close;
run;
APPENDIX E

MEAL STUDY
RECRUITMENT MATERIALS
Women with Irregular Menstrual Cycles or Elevated Hormone Levels wanted for a Nutrition Research Study

The purpose of this study is to determine how meals with different amounts of fat and fiber affect hormone levels.

Requirements for Participation

• Women with irregular menstrual cycles or elevated hormone levels
• Not currently pregnant
• Ages 19-40

Participation involves two 8-hour visits to the General Clinical Research Center at University Park. You will be given a meal to eat and small blood samples will be taken over the next 6 hours. Volunteers completing the study will receive compensation for their participation.

This study is under the direction of Dr. Richard S. Legro of the department of OB/GYN at the Penn State Hershey Medical Center

Interested Persons Please Contact:
Heather Katcher at 1-866-PSU-DIET or e-mail huk107@psu.edu

This research study has been approved by the Institutional Review Board (IRB) under federal regulations, at the Penn State Milton S. Hershey Medical Center.
Women with Irregular Menstrual Cycles or Elevated Hormone Levels wanted for a Nutrition Research Study!

The purpose of this study is to determine how meals with different amounts of fat and fiber affect hormone levels.

**Requirements for Participation**

- Women with irregular menstrual cycles or elevated hormone levels
- Not currently pregnant
- Ages 19 - 40

*Participation involves two 8-hour visits to the General Clinical Research Center at University Park. You will be given a meal to eat and small blood samples will be taken over the next 6 hours. Volunteers completing the study will receive compensation for their participation.*

This study is under the direction of Dr. Richard S. Legro of the department of OB/GYN at the Penn State Hershey Medical Center.

**Interested Persons Please Contact:**
1866-PSU-DIET (1-866-778-3438), leaving your name and phone number or e-mail huk107@psu.edu

This research study has been approved by the Institutional Review Board (IRB) under federal regulations at the Penn State Milton S. Hershey Medical Center.
Date completed__________

1. Full Name and Middle Initial_________________________________________________

2. Address: __________________________________________________________________
               __________________________________________________________________

3. Phone # Home_______________________ Work #_______________________________

4. Date of Birth______________ Age_______

5. State of Birth______________________ Marital Status______________________

6. Countries ancestors originated from (ethnic origin)____________________________

7. Allergies to medicine_______________________________________________________

8. Medications taken daily____________________________________________________

9. Height________________________ Weight__________________________

10. Dates of menstrual periods for last 3 months? LMP_____ PMP_____ PMP_____  
      G (# of times pregnant)___________ P (# of live births)__________________

11. How did you learn about this study? ______________________________

**Inclusion Criteria**

<table>
<thead>
<tr>
<th></th>
<th>YES</th>
<th>NO</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Women 19-40 years of age.</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2. Intermenstrual periods of $\geq 45$ days or $\leq 8$ menses per year.</td>
<td></td>
<td></td>
</tr>
<tr>
<td>3. In good general health</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

For the patient to be eligible for the study, criteria 1-3 must be marked “YES”.
<table>
<thead>
<tr>
<th>Exclusion Criteria</th>
<th>YES</th>
<th>NO</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Current pregnancy or actively seeking pregnancy</td>
<td></td>
<td></td>
</tr>
<tr>
<td>3. Using oral contraceptives or other hormonal medication</td>
<td></td>
<td></td>
</tr>
<tr>
<td>4. Using lipid lowering medications or insulin sensitizing agents</td>
<td></td>
<td></td>
</tr>
<tr>
<td>5. Using medications that affect hormone levels (including GnRH agonists and antagonists, anti-obesity drugs and calcium channel blockers)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>6. Tobacco use</td>
<td></td>
<td></td>
</tr>
<tr>
<td>7. Anemia</td>
<td></td>
<td></td>
</tr>
<tr>
<td>8. Uncorrected thyroid disease.</td>
<td></td>
<td></td>
</tr>
<tr>
<td>9. Alcohol consumption of more than two drinks per day</td>
<td></td>
<td></td>
</tr>
<tr>
<td>10. Unusual meal patterns (including no breakfast, breakfast before 6 am or breakfast after 10am)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>11. Suspected or history of cervical carcinoma, endometrial carcinoma, or breast carcinoma</td>
<td></td>
<td></td>
</tr>
<tr>
<td>12. Hyperprolactinaemia (Prolactin &gt;25ng/ml)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>13. History of blood clotting disorder</td>
<td></td>
<td></td>
</tr>
<tr>
<td>14. Presence or history of diabetes mellitus</td>
<td></td>
<td></td>
</tr>
<tr>
<td>15. Existence of an intracranial lesion such as a pituitary tumor</td>
<td></td>
<td></td>
</tr>
<tr>
<td>16. Presence or history of thromboembolic disease</td>
<td></td>
<td></td>
</tr>
<tr>
<td>17. Presence or history of cerebral vascular disease</td>
<td></td>
<td></td>
</tr>
<tr>
<td>18. Past myocardial infarction</td>
<td></td>
<td></td>
</tr>
<tr>
<td>19. Presence or history of coronary artery disease</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

For the patient to be eligible for the study, criteria 1-19 must be marked “NO”.

Mailed appointment letter and consent form?  Y  N  Date mailed:___________
APPENDIX F

MEAL STUDY
INFORMED CONSENT
CONSENT FOR RESEARCH
Penn State College of Medicine
The Milton S. Hershey Medical Center
and
The Pennsylvania State University
University Park Campus

Title of Project: The effect of meals of varying fat and fiber content on postprandial plasma testosterone concentrations

Principal Investigator: Richard S. Legro, M.D.

Other Investigators: Heather Katcher, Romana Dmitrovic, M.D., William C. Dodson, M.D., Carol L. Gnatuk M.D.

Participant’s Printed Name: ____________________________

This is a research study. Research studies include only people who voluntarily choose to take part. This consent form gives you information about this research, which will be discussed with you. This consent form may contain words or procedures that you do not understand. You are urged to ask questions about anything that is unclear to you. Discuss it with your family and friends and take your time to make your decision. You will receive a copy of the signed and dated consent form to keep.

1. **Purpose of the Research:**
   You are being offered the opportunity to take part in this research because you have a condition known as Polycystic Ovary Syndrome (PCOS). This condition causes irregular periods, inability to get pregnant, excessive facial and body hair and hyperandrogenemia (increased levels of male hormones).

   This research is being done to find out if meals of different nutrient composition can have different effects on hormones, particularly testosterone. This study will examine whether meals high in fat or fiber affect blood levels of testosterone and other metabolic and reproductive hormones.

   Approximately 16 women are expected to take part in this research study at the Hershey Medical Center and University Park Campuses.
2. **Procedures to be Followed:**

**Screening Visit:** At the screening visit, the research coordinator will discuss with you all events that are involved in this study and any negative events that could potentially occur. You should feel free to ask questions at any time. If you are interested in participating in this research study, you will then be asked to sign this consent form.

**Screening Blood Draw**

A blood sample (2 tablespoons) will be taken to measure several reproductive hormones to determine whether you are eligible for this study. The study doctor will review the results of the screening visit. If you are eligible to continue in the study, we will ask that you provide a small blood sample (approximately two teaspoons) in the week before your first visit to determine what stage of the menstrual cycle you are in. We will check your progesterone levels to confirm that you are in the first (follicular) phase of your menstrual cycle. For the blood draw, you can either come to the GCRC or, if you live out of town or prefer to have your blood drawn in an alternate location, you will be sent blood collection and fed-ex materials. In this case, you would be given directions to have your blood drawn by a phlebotomist and to fed-ex your blood sample to Hershey Medical Center.

A progesterone level of less than or equal to 2 ng/dL will confirm that you are in the first phase of your menstrual cycle. If your progesterone level is greater than 2 ng/dL, you will be asked to reschedule your visit for the week following your next period.

A daily urinary ovulation test such as Clearblue Easy Ovulation Test will also be given or mailed to you to detect whether you ovulate during the study period. You will be asked to use the ovulation test each morning beginning the day after your blood draw for progesterone measurement, until completion of the study. If the kit does detect that you have ovulated, we will ask that you reschedule your next visit for the week after your next period.

**History and Physical**

You will be asked questions about your medical history and asked to fill out a food frequency questionnaire. You may decline answering specific questions. A physical exam will be performed and a pregnancy test will be administered to ensure that you are not pregnant. At this time we will also take measurements of height, weight, and waist circumference.

**Preparation for Study**

For the three days prior to the study day you will be asked to follow a standard diet consisting of 55% carbohydrate, 30% fat and 15% protein. You will be provided a sample meal plan and asked to follow the meal plan as closely as possible for the three days. You will be given a diet log and asked to record all foods and drinks that you have during these three days.
You will be asked to fast (no food or drink except for water) from 10 PM the night before and avoid strenuous exercise and alcoholic beverages for 24 hours before both study visits.

**Study Visit 1**

*Blood Draws*
You will have a small flexible tube inserted into your forearm vein by a nurse. This tube, called a catheter, will allow for blood samples to be taken without puncturing your skin with a needle each time. Once the catheter is in place, samples can be taken while you read, watch TV, etc. A blood sample (4 teaspoons) will be drawn to determine the concentrations of hormones in your blood before the meal.

*Test Meals*
You will then be given a meal and asked to consume it in 15 minutes. The meal will either consist of:
- (1) A sausage, egg and cheese sandwich on a croissant and whole milk
- (2) Yogurt, cereal, 1% milk and a fruit salad (banana and apple)
The meal that you receive on the first day will be determined randomly.

*Blood Draws*
For the next 6 hours, a small sample of blood will be taken from the catheter every ten minutes. You may request numbing cream on your skin to reduce the pain from the needle. We will draw approximately ½ teaspoon of blood every ten minutes and 4 teaspoons of blood at 30 minutes and every hour. A total of 20.5 tablespoons of blood will be drawn at each visit. This represents approximately 5.4% of the circulating blood volume of a 165 lb individual.

*Appetite Questionnaire*
Before and after the meal, you will be asked to rank your hunger and fullness on a scale ranging from not at all hungry or full to extremely hungry or full.

*Glucose Monitoring*
We will also monitor your glucose levels using the MiniMed continuous glucose monitoring system. A tiny plastic glucose sensor (less than 1mm wide and 1.25 cm long) will be inserted just under the skin of your lower back using an insertion device and a small needle, which will be removed immediately. The sensor sits just below the skin and you shouldn’t feel it at all. The sensor will be secured with a piece of tape to keep it in place. The glucose sensor works through an electrochemical reaction with glucose, which the sensor converts into electronic signals. The glucose sensor then sends these electronic signals through the attached cable to the monitor where these glucose values are stored. The glucose sensor will determine your glucose levels every 10 seconds, and will average and save these values every five minutes.
During the 6-hour period, you can read, work, listen to recorded music, and watch movies, however you must remain seated at all times except for bathroom visits. You will be given a meal following the last blood draw.

**Study Visit 2**

Upon completion of study visit 1, you will be asked to return 7 days later for the second study visit. The procedures during this visit will be identical to the first except you will not have to fill out the medical history or food frequency questionnaires. At your second visit, you will be given the meal that you did not receive on the first day. If you were given the sausage, egg, and cheese croissant meal at the first visit, then you will be given the yogurt, cereal and fruit meal at the second visit. If you were given the yogurt, cereal and fruit meal at the first visit, then you will be given the croissant and sausage meal at the second visit.

**DXA Scan**

If you are a participant at the Hershey Medical Center, a DXA scan will be performed at the end of your second visit. If you are a participant at the University Park campus, the DXA scan will be performed on the same day as your blood draw for progesterone measurement. The DXA scan, also known as dual x-ray absorptiometry, uses low dose x-ray of two different energies to distinguish between bone and soft tissue, giving a very accurate measurement of body composition. DXA is a painless, non-invasive test. DXA scan uses a pencil-beam to scan your body and compare it to standard objects. You will be asked to change into a gown or shorts and a tee shirt that will be provided. During this procedure, you will lie still and quiet on a padded table, but you will be able to breathe normally. The study lasts only a few minutes. The x-ray dose you will be exposed to is less than half of the exposure from a routine chest x-ray.

```
7 days
```

```
Study Day 1
8 Hours

Study Day 2
8 Hours
```

3. **Discomforts and Risks:**

   **High-Fiber Meal**

   A diet high in fiber may lead to gastrointestinal side effects such constipation, bloating and gas. In excessive amounts, high-fiber diets can also cause diarrhea. The amount of fiber that you will consume is well below the recommended daily amount and should not produce severe gastrointestinal distress. Over the course of the 8-hour study period study you will be asked to drink at least two cups of water to minimize constipation. Any other gastrointestinal
side effects due to increased fiber consumption should go away after a short period of time.

**High-Fat Meal**
A long-term diet high in fat is associated with increased LDL-cholesterol concentrations and increased risk of coronary artery disease, however, a high fat content from one meal imposes no serious risk.

**Blood Draws**
The discomfort associated with removing blood by venipuncture (by needle from a vein) is a slight pinch or pin prick when the sterile needle enters the skin. The risks include mild discomfort and/or a black and blue mark at the site of puncture. Less common risks include a small blood clot, infection or bleeding at the puncture site, and on rare occasions fainting during the procedure. The total amount of blood drawn at each visit will be approximately 303 mL or 20 tablespoons.

**DXA Scan**
The DXA bone density procedure exposes you to a small amount of x-ray radiation. The dose to your whole body will be approximately 1.5 mrem (mrem is a measure of your radiation dose). When averaged over your entire body, this amount of radiation poses no more risk than the natural background radiation (radiation that you are continuously exposed to from cosmic rays, radioactive materials present in the earth and building materials and radioactive materials normally present within your own body) that you receive each day from living in south-central Pennsylvania. For further comparison purposes, this is less radiation than you would receive from a routine chest x-ray or from cosmic rays during an airplane trip from Pennsylvania to California and back.

**Continuous Glucose Monitoring**
The risks associated with the MiniMed continuous glucose monitoring system are bleeding at the point of insertion, inflammation and infection. You will feel a slight pinch when the sensor is inserted, however, this should not cause you considerable pain. Since the sensor will only be inserted for a 7-hour period, infection is unlikely. The glucose sensor will be removed if any redness, pain, tenderness, or swelling develops at the insertion site.

**Topical Anesthetic Cream**
Numbing cream will not be used in those who have sensitivity to lidocaine. Eye contact should be avoided. When used, all sensations within the treated area are blocked. For this reason, unintentional trauma to the treated area, such as scratching, rubbing or exposure to hot or cold temperatures should be avoided until complete sensation has returned. During or immediately after application, mild swelling, skin redness or abnormal sensation may develop at the site of treatment.
4. **Possible Benefits:**
   a. **Possible benefits to the participant:**
      You will not benefit from taking part in this research study.
   
   b. **Possible benefits to society:**
      Information gained from this study may help discover more information regarding polycystic ovary syndrome, and how diet may be involved in its pathology and treatment.

5. **Other Options that Could be Used Instead of this Research:**
   You can elect not to participate in this study. If you decline to participate in this study, it will not affect any care or treatment you would normally receive from your regular doctor.

6. **Time Duration of the Procedures and Study:**
   If you agree to take part in this study, your involvement will last 9 days. You will be asked to come to the General Clinical Research Center at University Park or Hershey Medical Center 4 times. The screening visit will take approximately 15 minutes and the visit for progesterone measurement will take approximately 1 hour. Both study visits will take approximately 8 hours.

7. **Statement of Confidentiality:**
   a. **Privacy and Confidentiality Measures**
      Your research records and samples of your blood that are reviewed, stored, and analyzed at The Milton S. Hershey Medical Center (HMC) and Penn State College of Medicine (PSU) or at the University Park Campus will be labeled with a code number. The list that matches your name with the code number will be kept in a locked file in Dr. Legro’s office. The research records will be kept in a password-protected computer file and in file cabinets in locked rooms in Dr. Legro’s office. Your samples will be stored in a freezer and will be locked in Dr. Legro’s laboratory at Hershey Medical center.

      In the event of any publication or presentation resulting from the research, no personally identifiable information will be shared.

   b. **The Use of Private Health Information:**
      Health information about you will be collected if you choose to be part of this research study. Health information is protected by law as explained in the Privacy Notice. If you have not received this notice, please request a copy from the researcher. At The Milton S. Hershey Medical Center (HMC) and Penn State College of Medicine (PSU) and at the University Park Campus your information will only be used or shared as explained and authorized in this consent form or when required by law. It is possible that some of the other people/groups who receive your health information may not be required by Federal privacy laws to protect your information and may share it without your permission.
To participate in this research you must allow the research team to use your health information. If you do not want us to use your protected health information, you may not participate in this research.

Your permission for the use, retention, and sharing of your identifiable health information will continue indefinitely. If you consent to the collection of samples of your blood for future research, the period for the use of the sample is unknown. If you do not consent to the storage of samples of your blood for future research, they will be destroyed at the end of the research. If you choose to participate, you are free to withdraw your permission for the use and sharing of your health information and your samples at any time. You must do this in writing as indicated in the HMC Privacy Notice. Write to Dr. Legro and let him know that you are withdrawing from the research study. His mailing address is:

Milton S. Hershey Medical Center
Dr. Richard S. Legro, M.D.
P.O Box 850
500 University Drive H103
Hershey, PA 17033

If you withdraw your permission:
- We will no longer use or share medical information about you or your samples for the reasons covered by your written authorization, except when the law allows us to do so.
- We are unable to take back anything we have already done or any information we have already shared with your permission.
- We may continue using and sharing the information obtained prior to your withdrawal if it is necessary for the soundness of the overall research.
- We will need to keep our records of the care that we provided to you as long as the law requires.

The research team may use the following sources of health information:
- Personal health history
- Physical exam
- Blood sample results
- Food Frequency Questionnaire

Representatives of the following people/groups within HMC/PSU and University Park are allowed to use your health information and to share it with other specific groups in connection with this research study.
- The principal investigator, Dr. Richard S Legro, M.D.
- The HMC/PSU and University Park Institutional Review Board
- The HMC/PSU Human Subjects Protection Office and University Park Office for Research Protections
- The research team at the Hershey Medical Center and College of Medicine and at University Park
The people or groups listed in the above paragraph may share your health information with the following people/groups outside HMC/PSU and University Park for their use in connection with this research study. These groups, while monitoring the research study, may also review and/or copy your original research records.

- The Office of Human Research Protections in the U. S. Department of Health and Human Services
- National Institute of Child Health and Human Development
- Food and Drug Administration

8. **Costs for Participation:**
   The ultrasound, meals, and blood tests will be provided at no cost to you. Expenses for procedures done for study purposes during your participation in the study will be covered by the study and will not be billed to you or your insurance company.

   Every effort to prevent injury as a result of your participation will be taken. It is possible, however, that you could develop complications or injuries as a result of participating in this research study. In the event of injury resulting from this research, medical treatment is available but will be provided at the usual charge. It is the policy of this institution to provide neither financial compensation nor free medical treatment for research-related injury.

   Costs for the treatment of research-related injuries will be charged to your insurance carrier or to you. Some insurance companies may not cover costs associated with research studies. If for any reason these costs are not covered by your insurance, they will be your responsibility.

   You are not waiving any legal rights you may have by signing this form.

10. **Compensation for Participation:**
    You will be paid a total of $200 for your complete participation in this study. You will receive $75 for each visit and an additional $50 following completion of the study.

    If you are an employee of Penn State University, the compensation you receive for participation will be treated as taxable income and therefore taxes will be taken from the total amount. If you are not employed by Penn State University, total payments within one calendar year that exceed $600 will require the University to annually report these payments to the IRS. This may require you to claim the compensation that you receive for participation in this study as taxable income.

10. **Research Funding:**
    The institution and investigators are receiving funding from the National Institutes of Health to support the activities that are required to conduct this research.

11. **Voluntary Participation:**
Taking part in this research study is voluntary. If you choose to take part in this research, your major responsibilities will include compliance with visits and protocol instructions. You do not have to participate in this research. If you choose to take part, you have the right to stop at any time. If you decide not to participate or if you decide to stop taking part in the research at a later date, there will be no penalty or loss of benefits to which you are entitled. In other words, your decision to decline to participate in this research or to stop taking part in the research will not affect your medical care. Your research doctor may take you out of the research study without your permission. Possible reasons for this are: you become pregnant, you did not follow the instructions of the study doctor, you experience serious side effects, etc.

If you will be participating in another clinical trial at Hershey Medical Center or elsewhere while in this research, you should discuss the procedures and/or treatments with your physician or the investigators. This precaution is intended to protect you from possible side effects from interactions of research drugs, treatments or testing.

During the course of the research you will be informed of any new findings that may affect your willingness to continue participating in this research.

In the event that abnormal test results are obtained, you will be made aware of the results within 30 days and recommended to contact your private medical provider for follow-up.

12. Contact Information for Questions or Concerns:
You have the right to ask any questions you may have about this research. If you have questions or concerns or believe you may have developed an injury that is related to this research, contact Dr. Legro at 717-531-8478 or the OB/GYN resident on 24-hour call (717-531-8521).

If you have questions or concerns regarding your rights as a research participant, you may contact the research protection advocate in the HMC Human Subjects Protection Office at 717-531-5687 or the University Park Office for Research Protections at (814) 865-1775.

For more information about participation in a research study and about the Institutional Review Board (IRB), a group of people who review the research to protect your rights, please visit the HMC IRB’s Web site at [http://www.hmc.psu.edu/irb](http://www.hmc.psu.edu/irb). Included on this web site, under the heading “Links”, you can access the federal regulations for the protection of human research participants. If you do not have access to the internet, copies of these regulations are available by calling the HSPO at (717) 531-5687.

**Signature and Consent/Permission to be in the Research**
Before making the decision regarding enrollment in this research you should have:

- Discussed this study with an investigator,
- Reviewed the information in this form, and
- Had the opportunity to ask any questions you may have.
Your signature below means that you have received this information, have asked the questions you currently have about the research and those questions have been answered. You will receive a copy of the signed and dated form to keep for future reference.

**Participant:** By signing this consent form, you indicate that you are voluntarily choosing to take part in this research.

<table>
<thead>
<tr>
<th>Signature of Participant</th>
<th>Date</th>
<th>Time</th>
<th>Printed Name</th>
</tr>
</thead>
</table>

**Person Explaining the Research:** Your signature below means that you have explained the research to the participant/participant representative and have answered any questions he/she has about the research.

<table>
<thead>
<tr>
<th>Signature of person who explained this research</th>
<th>Date</th>
<th>Time</th>
<th>Printed Name</th>
</tr>
</thead>
</table>

(Only approved investigators for this research may explain the research and obtain informed consent.)

In addition the main part of the research study, there is an optional part of the research. You can participate in the main part of the research without agreeing to take part in this optional part.

**Storage of Leftover Blood Samples for Future Research Studies**
As part of this study, we are obtaining blood from you. If you agree, the research team would like to store leftover samples of your blood that is collected so that your blood can be studied in the future after this study is over. These future studies may provide additional information that will be helpful in understanding polycystic ovary syndrome, but it is unlikely that these studies will have a direct benefit to you. Neither your doctor nor you will receive results of these future research tests, nor will the results be put in your health record. Sometimes samples are used for genetic research about diseases that are passed on in families. Even if your samples are used for this kind of research, the results will not be put in your health records. It is possible that your blood might be used to develop products or tests that could be patented and licensed. There are no plans to provide financial compensation to you should this occur. If you have any questions, you should contact Dr. Legro at (717)531-8478.

Your leftover samples will be labeled with a code number and stored in Dr. Legro’s locked laboratory. If you consent to the collection of samples of your blood for future research, the period for the use of the samples is unknown. If you agree to allow your blood to be kept for future research, you will be free to change your mind at any time. You should contact Dr. Legro at (717)531-8478 and let him know you wish to withdraw your permission for your
blood to be used for future research. Any unused blood will be destroyed upon completion of the study and not used for future research studies.

You should initial below to indicate your preferences regarding the optional storage of your leftover blood for future research studies.

a. Your samples may be stored and used for future research studies to learn about, prevent, treat or cure polycystic ovary syndrome.
   ____ Yes  ____ No

b. Your samples may be stored and used for research about other health problems.
   ____ Yes  ____ No

c. Your samples may be shared with other investigator/groups without any identifying information.
   ____ Yes  ____ No

For Participants at Hershey Medical Center:

Optional Transvaginal Ultrasound

A transvaginal ultrasound is an option for participants at Hershey Medical Center. If you are participating at Hershey and choose to have an ultrasound, the ultrasound will be performed trans-vaginally on the morning of your first visit by inserting a plastic lubricated probe into your vagina for approximately 5 minutes. During this time, the probe will give off sound waves so that pictures of your ovaries and uterus will appear on a screen, and measurements of your ovaries and uterus will be taken. This may cause some temporary discomfort.

You should initial below to indicate whether you would like to have a transvaginal ultrasound performed.

_____ Yes  _____ No

Participant: By signing below, you indicate that you have read the information written above and have indicated your choices for the optional part of the research study.

________________________ __________ ______       _________________
Signature of Participant    Date  Time  Printed Name

Person Explaining the Research: Your signature below means that you have explained the optional part of the research to the participant/participant representative and have answered any questions he/she has about the research.

________________________ __________ ______       _________________
Signature of person who explained this research    Date  Time  Printed Name
APPENDIX G

MEAL STUDY
STUDY MATERIALS
Meal Plan

Breakfast

1 cup whole grain cereal
1 cup skim or 1% milk
1 banana or other fruit
or
½ cup oatmeal, cooked with 1 tsp brown sugar
1 cup calcium fortified orange juice

Snack
1 apple or other fruit

Lunch

1 Sandwich on two slices of whole grain bread:
3 ounces (3–4 slices) turkey breast
1 slice cheese
1 teaspoon mayonnaise
1 cup salad
1 tablespoon ranch style dressing

Snack
2 thin and crunchy granola bars

Dinner
3 ounce skinless chicken breast (size of a deck of cards)
1 cup of oriental style vegetables with 1 tsp olive oil
1 cup rice

Snack
1 cup skim or 1% milk
3 Chip’s Ahoy cookies
Energy and nutrient composition of the two test meals

<table>
<thead>
<tr>
<th></th>
<th>Amount</th>
<th>Energy (kcal)</th>
<th>Carbohydrate (g)</th>
<th>Protein (g)</th>
<th>Total Fat (g)</th>
<th>Saturated Fat (g)</th>
<th>Total Fiber (g)</th>
<th>Cholesterol (mg)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>High-fat, Low-Fiber Meal</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Jimmy Dean Sausage Egg and Cheese Croissant</td>
<td>1 (128g)</td>
<td>450</td>
<td>24</td>
<td>12</td>
<td>33</td>
<td>9</td>
<td>1</td>
<td>125</td>
</tr>
<tr>
<td>Whole Milk</td>
<td>8 oz.</td>
<td>150</td>
<td>11.4</td>
<td>8</td>
<td>8.1</td>
<td>5.4</td>
<td>0</td>
<td>33</td>
</tr>
<tr>
<td>Total</td>
<td></td>
<td><strong>600</strong></td>
<td><strong>35.4</strong></td>
<td><strong>20</strong></td>
<td><strong>41.1</strong></td>
<td><strong>14.4</strong></td>
<td><strong>1</strong></td>
<td><strong>158</strong></td>
</tr>
<tr>
<td>Percent Energy</td>
<td></td>
<td>24%</td>
<td>13%</td>
<td>62%</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Low-fat, High-Fiber Meal</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Honey Cluster Fiber 1 Cereal</td>
<td>55g</td>
<td>170</td>
<td>47</td>
<td>3</td>
<td>1</td>
<td>0</td>
<td>14</td>
<td>0</td>
</tr>
<tr>
<td>1% Milk</td>
<td>8 oz.</td>
<td>100</td>
<td>12</td>
<td>8</td>
<td>2.5</td>
<td>1.5</td>
<td>0</td>
<td>12.2</td>
</tr>
<tr>
<td>Apple</td>
<td>1 med (138g)</td>
<td>81</td>
<td>20</td>
<td>0.3</td>
<td>0.5</td>
<td>0.1</td>
<td>3.7</td>
<td>0</td>
</tr>
<tr>
<td>Banana</td>
<td>1 med (131g)</td>
<td>109</td>
<td>28</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>3.1</td>
<td>0</td>
</tr>
<tr>
<td>Dannon Yogurt + Fiber</td>
<td>8 oz.</td>
<td>140</td>
<td>26</td>
<td>8</td>
<td>0</td>
<td>0</td>
<td>6</td>
<td>0</td>
</tr>
<tr>
<td>Total</td>
<td></td>
<td><strong>600</strong></td>
<td><strong>133</strong></td>
<td><strong>20</strong></td>
<td><strong>4</strong></td>
<td><strong>1.6</strong></td>
<td><strong>26.8</strong></td>
<td><strong>12.2</strong></td>
</tr>
<tr>
<td>Percent energy</td>
<td></td>
<td>81%</td>
<td>13%</td>
<td>6%</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
APPENDIX H

MEAL STUDY
SAS STATISTICAL ANALYSIS PROGRAMS
**SAS Code for Analysis of Biochemical Data**

```sas
proc format;
    value grp_f 1='High Fiber --> High Fat'
                    2='High Fat --> High Fiber';
    value meal_f 1='Fiber'
                    2='Fat';
run;

data meal(drop=meal rename=(group=Sequence meal_new=Meal));
set in.meal_stud;
    FAI_percent=FAI*100;
    time_cont=time;
    if meal='Fiber' then meal_new=1;
    else if meal='Fat' then meal_new=2;
    if group=1 and meal='Fiber' then Period=1;
    else if group=1 and meal='Fat' then Period=2;
    else if group=2 and meal='Fat' then Period=1;
    else if group=2 and meal='Fiber' then Period=2;
format group grp_f. meal_new meal_f.;
run;

proc sort data=meal;
    by sequence subjectno period;
run;

data meal2;
set meal;
    *** Exclude 2 subjects that were ovulatory during the study ***;
    if subjectno in (59, 82) then delete;
run;

%macro glx(dsn=,y=,covar=,ttl3=,ttl6=);
proc means data=&dsn n mean stddev stderr min max maxdec=2;
    class sequence period meal time;
    var &y;
    title1 "2x2 Cross-Over Trial";
    title3 "&ttl3 Subjects 59 and 82 That Were Ovulatory During the Study";
    title5 "Dependent Variable: &y";
    title6;
run;

proc means data=&dsn n mean stddev stderr min max maxdec=2;
    class meal time;
    var &y;
    title1 "2x2 Cross-Over Trial";
    title3 "&ttl3 Subjects 59 and 82 That Were Ovulatory During the Study";
    title5 "Dependent Variable: &y";
    title6;
run;
```

---

**Note:** The code assumes that the dataset and variables are correctly defined elsewhere in the SAS environment. The `proc format` block defines format values for `grp_f` and `meal_f`. The `data` block reads the data and performs calculations and conditional statements to define new variables `meal_new` and `Period`. The `proc sort` block sorts the data by sequence, subject no, and period. The `data` block for `meal2` excludes subjects 59 and 82. The `%macro` block `glx` is used to run `proc means` with appropriate titles for analysis.
ods graphics on;
run;

proc glimmix data=&dsn;
  class subjectno meal period time;
  model &y=meal period time*time*meal &covar/ ddfm=kenwardroger;
  random period / subject=subjectno type=ar(1);
  random time / subject=period*subjectno type=sp(pow)
    (time_cont) residual;
  lsmeans time*meal / slicediff=(meal time) plot=meanplot
    (sliceby=meal join);
  ods exclude dimensions optinfo iterhistory
    convergencestatus fitstatistics;
  title1 "2x2 Cross-Over Trial";
  title3 "&ttl3 Subjects 59 and 82 That Were Ovulatory During
    the Study";
  title5 "Dependent Variable: &y";
  title6 "&ttl6";
run;

ods graphics off;
run;
%mend glx;

ods rtf
  file="C:/protocol_19542_diet_study_katcher/analysis/crossover3.doc ";
run;

%glx(dsn=meal,y=Testosterone,covar=,ttl3=Includes,ttl6=No Adjustment for Baseline Estradiol);
%glx(dsn=meal,y=FAI_percent,covar=,ttl3=Includes,ttl6=No Adjustment for Baseline Estradiol);
%glx(dsn=meal,y=SHBG,covar=,ttl3=Includes,ttl6=No Adjustment for Baseline Estradiol);
%glx(dsn=meal,y=Glucose,covar=,ttl3=Includes,ttl6=No Adjustment for Baseline Estradiol);
%glx(dsn=meal,y=Insulin,covar=,ttl3=Includes,ttl6=No Adjustment for Baseline Estradiol);

ods rtf close; run;

ods rtf
  file="C:/protocol_19542_diet_study_katcher/analysis/crossover3.doc";
run;

%glx(dsn=meal2,y=Testosterone,covar=,ttl3=Excludes,ttl6=No Adjustment for Baseline Estradiol);
%glx(dsn=meal2,y=FAI_percent,covar=,ttl3=Excludes,ttl6=No Adjustment for Baseline Estradiol);
%glx(dsn=meal2,y=SHBG,covar=,ttl3=Excludes,ttl6=No Adjustment for Baseline Estradiol);
%glx(dsn=meal2,y=Glucose,covar=,ttl3=Excludes,ttl6=No Adjustment for Baseline Estradiol);
%glx(dsn=meal2,y=Insulin,covar=,ttl3=Excludes,ttl6=No Adjustment for Baseline Estradiol);

ods rtf close; run;
SAS Code for Area Under the Curve Analysis

```sas
proc sort data=meal;
by subjectno meal time;

data meal4auc;
set meal;
by subjectno meal;
output;
/*~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~*/
/* proc expand will include a contribution for the last interval. */
/* for an accurate approximation of the interval, we need to make */
/* sure this last contribution is negligible. So I'll add an */
/* additional day value which is EXTREMELY close to the last value. */
/*~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~*/
if last.meal then do;
time=time+(1e-10);
output;
end;
run;

proc expand data=meal4auc out=auc method=join;
convert testosterone=auc_testosterone
fai_percent=auc_fai_percent
shbg=auc_shbg
glucose=auc_glucose
insulin=auc_insulin/
observed=(beginning,total) transformout=(sum);
  id time;
by subjectno meal;
run;

data auc(keep=subjectno meal sequence period auc:);
set auc;
by subjectno meal;
if last.meal;
run;

data auc;
merge auc meal;
by sequence subjectno period meal;
run;

proc sort;
  by sequence subjectno period;
run;

data auc2;
set auc;
*** Exclude 2 subjects that were ovulatory during the study ***;
if subjectno in (59,82) then delete;
run;
```
%macro mixed(dsn=,y=,covar=,ttl3=,ttl6=);

proc means data=&dsn n mean stddev stderr min max maxdec=2;
   class meal;
   var &y;
   title1 "2x2 Cross-Over Trial";
   title3 "&ttl3 Subjects 59 and 82 That Were Ovulatory During the Study";
   title5 "Dependent Variable: &y";
   title6;
run;

*ods graphics on;
run;

proc glimmix data=&dsn;
   class subjectno meal period;
   model &y=meal period &covar / ddfm=kenwardroger;
   random period / subject=subjectno type=cs residual;
   lsmeans meal / diff cl;
   ods select tests3 lsmeans diffs;
   title1 "2x2 Cross-Over Trial";
   title3 "&ttl3 Subjects 59 and 82 That Were Ovulatory During the Study";
   title5 "Dependent Variable: &y";
   title6 "&ttl6";
run;

*ods graphics off;
run;

%mend mixed;

ods rtf file="//FS/gcrc/hershey/legro/protocol_19542_diet_study_katcher/analyses/auc1.doc";
run;

%mixed(dsn=auc,y=auc_Testosterone,covar=,ttl3=Includes,ttl6=No Adjustment for Baseline Estradiol);
%mixed(dsn=auc,y=auc_FAI_percent,covar=,ttl3=Includes,ttl6=No Adjustment for Baseline Estradiol);
%mixed(dsn=auc,y=auc_SHBG,covar=,ttl3=Includes,ttl6=No Adjustment for Baseline Estradiol);
%mixed(dsn=auc,y=auc_Glucose,covar=,ttl3=Includes,ttl6=No Adjustment for Baseline Estradiol);
%mixed(dsn=auc,y=auc_Insulin,covar=,ttl3=Includes,ttl6=No Adjustment for Baseline Estradiol);
ods rtf close;
run;
ods rtf
file="//FS/gcrc/hershey/legro/protocol_19542_diet_study_katcher/analyses/auc3.doc";
run;

%mixed(dsn=auc2,y=auc_Testosterone,covar=,ttl3=Excludes,ttl6=No Adjustment for Baseline Estradiol);
%mixed(dsn=auc2,y=auc_FAI_percent,covar=,ttl3=Excludes,ttl6=No Adjustment for Baseline Estradiol);
%mixed(dsn=auc2,y=auc_SHBG,covar=,ttl3=Excludes,ttl6=No Adjustment for Baseline Estradiol);
%mixed(dsn=auc2,y=auc_Glucose,covar=,ttl3=Excludes,ttl6=No Adjustment for Baseline Estradiol);
%mixed(dsn=auc2,y=auc_Insulin,covar=,ttl3=Excludes,ttl6=No Adjustment for Baseline Estradiol);

ods rtf close;
run;
VITA

Heather I. Katcher

Education
2002 – 2007 Ph.D. Integrative Biosciences Graduate Program in Nutrition, The Pennsylvania State University, University Park, PA.
1998 – 2002 B.S. Psychobiology, Binghamton University, Binghamton NY.

Awards and Honors
- The Endocrine Society Meeting Travel Award – March 2007
- American Heart Association Predoctoral Fellowship – May 2005
- Vitamin D in the 21st Century Conference Travel Award, NIH - October 2003
- Life Sciences Consortium Graduate Fellowship - August 2002
- SUNY Scholarship for Academic Achievement, March 2001

Publications
- Katcher HI, Kunselman AR, Dmitrovic R, Demers LM, Gnatuk CL, Kris-Etherton PM, Legro RS. Prolonged reduction in testosterone concentration after a high fat, Western meal compared with a low-fat, high-fiber meal in women with polycystic ovary syndrome. In Preparation.

Abstracts and Presentations
- Katcher HI, Dmitrovic R, Kunselman A, Demers LM, Gnatuk CL, and Legro RS. Prolonged reduction in testosterone levels after a high fat meal compared with a high fiber meal in women with polycystic ovary syndrome. Oral presentation at The Endocrine Society Annual Meeting, Toronto, Canada, 6/2-6/5/07.