Section 3  Project Description:

Outline the overall goal(s) of the project in the space below; please be concise. Give enough information to assure that the purpose of the experiments and the techniques used are clear.

Purpose

It is well documented that obese individuals (BMI = 30) have a higher incidence of co-morbid conditions such as diabetes, dyslipidemia, and cardiovascular disease. One proposed physiological mechanism for this relationship is the inflammatory hypothesis. Higher levels of inflammatory factors have been reported in obese individuals. Inflammatory factors include pro-inflammatory cytokine IL-6 and C-reactive protein (Crp). IL-6 is believed to contribute to higher levels of blood lipids and stimulates the release of Crp from the liver. Serum Crp levels are measured to assess vascular inflammation and to predict atherosclerosis and diabetes (Lyons et al. 2003). In a study comparing obese and lean subjects, Kern and colleagues (2001) reported significantly higher levels of plasma IL-6 in obese subjects when compared to lean subjects and a strong relationship with obesity and insulin resistance. Esposito et al. (2003) examined the effect of lifestyle change by means of diet and physical activity counseling on circulating levels of IL-6 and Crp in obese women. The authors reported significantly lower serum concentrations of IL-6 and Crp in subjects receiving counseling when compared to control subjects. The authors concluded lowered bodyweight resulting from exercise and lifestyle counseling was connected to decreases in IL-6 and Crp.

The components of energy expenditure include basal energy expenditure/resting energy expenditure (REE) needs; activities of daily living (ADL) that include physical activity and exercise, and diet-induced thermogenesis (DIT). Differences in energy expenditure to meet basal needs and physical activity among different size individuals (i.e. lean, normal weight, overweight) have been documented. Whether or not differences exist in resting energy expenditure, weight loss rate, body composition, and biochemical changes among overweight females after following a prescribed diet (high protein, low fat or a high protein, high fat) is not clear. Some studies have investigated resting energy expenditure after dieting and weight loss. Barrows et al. (1987) investigated the long-term effects (4-6 months) of a very-low calorie liquid diet (VLCD) and subsequent realimentation (reintroduction of food) on resting energy expenditure in 15 obese females (mean body fat 46.8%) aged 30-54 years. REE significantly decreased following the VLCD and remained lower after the re-feeding period. In an eight-week study, Stallings et al. (1992) examined the effects of a low-calorie (800-850 kcal/day), high protein (2.0-2.5 g of protein/kg of ideal body weight/day) diet on weight loss and REE in seven healthy obese adolescents. The authors reported a significant decrease in percent ideal body weight and REE. However, no significant differences were found when REE was expressed as kcal/kg. Hendler and co-workers (1988) examined the effects of two weight loss diets on energy expenditure in 17 healthy obese subjects. Subjects were initially placed on a weight-maintenance diet and were then randomly assigned to either a very low calorie mixed diet (MD) providing 436 ± 2 kcal/d (41% protein, 55% carbohydrate, 4% fat) or a very low calorie, high protein (HP) diet providing 439 ± 5 kcal/d (95 ± 1% protein, 2 ± 1% carbohydrate, 3 ± 1% fat) for a 3-week period. Resting metabolic rate (RMR) significantly decreased in both the MD and HP group at the end of the 3-week period with no significant difference between the two diet groups.

Other studies have examined how diet composition affects energy expenditure and DIT. Schwartz et al. (1985) found that the thermic response to high fat feeding (85% kcal from fat) was lower than the thermic response to high carbohydrate feeding (85% kcal from carbohydrate) in both the obese and normal weight groups. In a study conducted by Westerterp-Plantenga and others (1997), DIT was measured in normal weight subjects after ingesting either a full fat (40% kcal from fat) meal or a low fat (26% kcal from fat) meal. The results found that the DIT was
significantly higher after ingestion of the reduced fat meal than the full fat meal. Similarly, Yerboeket-van de Venne and investigators (1996) found that energy expenditure was significantly lower after the consumption of a mixed diet (15% protein, 30% fat, 55% carbohydrate) and high fat diet (15% protein, 50% fat, 35% carbohydrate) versus a low fat diet (15% protein, 10% fat, 75% carbohydrate) in normal weight individuals. In contrast, Roust et al. (1994) found no significant differences in DIT between obese and non-obese individuals fed medium carbohydrate, low fat (30% fat, 50% carbohydrate) diet versus a high complex carbohydrate, low fat (27% fat, 53% carbohydrate) diet. Abbott et al. found no significant differences in energy expenditure when fed a high fat meal (42% fat, 43% carbohydrate) or a high carbohydrate meal (20% fat, 65% carbohydrate) between obese non-diabetic and obese diabetic Pima Indians.

The purpose of this study is to examine changes in REE, BW, % BF, lipid and glucose levels, and inflammatory markers in overweight college-aged females when assigned to different diet regimens for a six-week period without changes in daily activity.

Describe your subjects.
Approximately thirty (10 HPLF group, 10 HPHF group, and 10 control group) overweight women ages 18-28 years will be recruited for the study through announcements in health and kinesiology classes and posted flyers around the Georgia Southern University campus. Subjects will be classified as overweight (25-29.9kg/m^2) based on their Body Mass Index (BMI), which is defined by the Centers for Disease Control (CDC) criteria. Subjects must be healthy and free from any known chronic disease.

Methodology (Procedures)
Blood Specimen Collection Procedures
Participants will report to the hematology room (2310) located at the Hanner Building in the Human Performance Laboratory. The participant will sit with their arm placed on a table devoted to blood collection. The researcher collecting blood is a registered nurse and faculty member in the school of Nursing. They will wear goggles and latex gloves during the collection procedure. A tourniquet will be applied to the upper arm of the participant to engorge the antecubital vein. A vein will be located and the area will be cleansed with a alcohol pad. A vacutainer system will be used with a 20-gauge 1” needle to access the vein. The needle will be inserted into the vein approximately 1/4” and stabilizing the vacutainer sheath. One at a time, three sealed vacutainer blood tubes will be inserted into the sheath to withdraw approximately 5cc of blood. Each sealed blood tube will be placed upright in a test tube rack for 30 minutes. A cotton ball is placed over the insertion site and the vacutainer needle is removed. The subject is instructed to hold pressure over the venipuncture site. Immediately, a non-removable needle cover (attached to the base of the needle) will be snapped into place. The entire vacutainer sheath and attached encased needle are placed in a puncture proof biohazard sharps container. A band aide is applied to the subject’s venipuncture site after clotting is ensured. If there are any blood droplets on the table, a 2X2 gauze is used to soak up the blood and disposed of in a biohazard container. Then, the area is cleansed with a disinfectant and allowed to remain wet for 30 seconds. Gloves are disposed of in a biohazard container. Blood samples will be centrifuged in plastic capped centrifuge tubes whenever possible, at 3000 x g for 15 min to separate plasma. Wearing protective clothing, gloves, and safety glasses, blood samples will be removed 5 minutes after they have been centrifuged. If broken, cracked, or damaged tubes are discovered, they will be directly placed in a biohazard container and the centrifuge will be cleaned with approved disinfectant solution. Plasma will be aliquotted into cryovials and stored at -80°C until analysis for circulating cytokine. Samples will be placed in a sealed cooler labeled with a Biohazard sticker and driven to the Chemistry building for analysis of cytokine concentration.

Plasma Cytokine Assessment
Samples will be placed in a sealed cooler labeled with a Biohazard sticker and driven to the Chemistry building for analysis of cytokine concentration. Plasma IL-6 cytokine concentrations will be determined
using commercially available enzyme-linked immunosorbent assay (ELISA) kits (R&D, Minneapolis, MN). This assay method employs the quantitative “sandwich” enzyme immunoassay technique. Wearing protective clothing, gloves, and safety glasses, plasma samples will be added to microtiter plates coated with monoclonal antibody specific for the cytokine according to manufacturer’s instructions. Cytokine present in the sample will bind to the immobilized antibody and after washing, an enzyme-linked polyclonal antibody specific for the specific cytokine is added to the wells. Following a wash to remove any unbound antibody-enzyme reagent, a substrate solution is added to the wells. After an incubation period, an amplifier solution will be added to the wells. This amplifier solution causes color development, which will be proportional to the amount of cytokine bound in the initial step. The color development will be stopped and the intensity of the color will be measured. Cytokine concentration will be determined by comparing the optical density (490 nm) of each sample to a standard curve determined using standards of appropriate cytokine. Cytokine concentration will be expressed as pg·mL⁻¹.

All micropipet tips used in this assay will be collected as biohazard and autoclaved prior to disposal. During periods of incubation or stirring, the microtiter plate will be covered with a plastic cover to avoid splashing. At the conclusion of the assay, all liquid waste (<34ml per plate) will be transferred to an absorbent material under the fume hood and, transferred to a biohazard waste container along with the plate and autoclaved prior to disposal.

**Plasma levels of glucose, Crp, and lipid profiles:** Blood samples will be shipped to Doctors Laboratory, Inc (Valdosta, GA) to determine blood glucose, Crp, and lipid profile levels.

**Resting Energy Expenditure:** REE will be measured using a metabolic cart (Viasys; Yorba Linda, CA). Viasys provides valid and reliable measurements of VO₂ and determination of resting energy expenditure.

**Body Composition:** Weight and height will be measured using a standard scale and stadiometer. BMI will be calculated using the following equation: weight ÷ (height in meters squared). Percent body fat will be determined using the BodPod system.