GLYCINE SUPPLEMENTATION TO IMPROVE INSULIN SENSITIVITY IN HUMANS

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NUTRITION SCIENCES

ABSTRACT

The main purpose of this pilot study was to investigate the insulin sensitizing effect of glycine as a dietary supplement in insulin resistant (IR) normoglycemic subjects (N= 10), and to determine significant changes in insulin sensitivity and lipid profile after four weeks of glycine supplementation. Descriptive statistics were used to describe the basic characteristics of the study population. A paired t-test was used to determine differences between insulin sensitivity and lipid profile pre- and post- intervention, considering the estimation of HOMA-IR and Matsuda- index scores.

Results showed that glycine supplementation might improve triglyceride (TG) levels in European Americans; and low-density lipoprotein cholesterol (LDL-c) concentrations in women after a four-week of intervention. Although, this work reports interesting results, these findings did not provide sufficient evidence to demonstrate that glycine supplementation may improve insulin sensitivity and lipid profile in insulin-resistant (IR) normoglycemic subjects. The implementation of a larger investigation with sufficient power is necessary to fully test our hypothesis and further explore these findings.

Keywords: glycine, insulin resistance, insulin sensitivity, lipid profile, HOMA-IR, Matsuda-index.

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"The steps of a righteous man are ordered by the Lord" Psalms 37:23

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INTRODUCTION

The prevalence of insulin resistance (IR) and the development of type 2 diabetes mellitus (T2DM) are increasing in the United States (U.S.) and worldwide at an alarming rate (1). Globally, it is estimated that 366 million people have diabetes, and 280 million have impaired glucose tolerance (IGT). Diabetes and its complications have become major causes of morbidity and mortality in the U.S. (1, 2, 16).

IR is usually defined as the inability of insulin to stimulate glucose uptake into skeletal muscle (2, 15). IR can be detected prior to the development of T2DM, and is associated with a cluster of risk factors that include relative hypertension, glucose intolerance, upper body fat distribution, dysfibrinolysis, and dyslipidemia [characterized by high triglycerides, low high-density lipoprotein cholesterol (HDL-c), and high low-density lipoprotein cholesterol (LDL-c)] (6,17). This trait cluster, known as the metabolic syndrome, is present in an estimated 35% of adults in the U.S. (19), and is a powerful risk factor not only for the future development of T2DM but also for cardiovascular diseases.

IR is believed to be a center pathophysiological process toward T2DM, the metabolic syndrome, and cardiovascular disease (19, 33). The likelihood of developing IR and the metabolic syndrome can be attributed to several factors, including genetic abnormalities, fetal abnormalities, visceral adiposity, and abnormal macronutrients metabolism (33, 37). Although, previous studies have shown that there is a relationship

between excess adiposity and the development of IR (7, 18, 44), general adiposity is not the only environmental change factor explaining the variability in IR in non-diabetic individuals (7, 16, 18).

Recent studies have shown associations between circulating levels of several amino acids (AAs) with excess adiposity (4,7,8,) and with insulin sensitivity (8,44), suggesting that AAs play a role play a role in the development of IR. For example, several studies have demonstrated that there is a relationship between the branched chain AAs (BCAA), particularly leucine/ isoleucine (Leu/Ile), and the development of IR (3,45), and a recent study reported that other AAs, including glycine, may also play a predominant role in the prevention of T2DM (23). Although, modifications in AA concentrations may result from intrinsic and extrinsic factors (4, 7, 8), the exact mechanisms by which IR and AAs are linked remain unclear.

Among the different AAs emerging as relevant contributors to insulin-related outcomes, glycine appears to be one of great interest. Previous studies involving metabolic syndrome patients have suggested that glycine may play a protective role against the development of IR, particularly in the presence of excess adiposity (23) and genetic predisposition IR (8, 44). A recent work involving individuals with varying degrees of insulin sensitivity examined the relationships between circulating AA levels and the gold standard measure of insulin sensitivity, glucose disposal rate (GDR) via hyperinsulinemic-euglycemic clamp technique (11). This study identified glycine as the one with the strongest relationship with GDR in non-diabetic and diabetic subjects.

In addition, insulin sensitive subjects had the greatest concentrations of circulating glycine (11). These data suggest that glycine, an AA that could easily and practically be provided as a nutritional supplement, may serve as a potential therapeutic agent to improve insulin-related outcomes among individuals (11).

Regarding the above observations, we proposed that glycine supplementation may improve insulin sensitivity, especially in insulin-resistant subjects. Glycine has several beneficial effects that would justify its clinical use. Most important, it can be administered in the diet without major adverse effects, as suggested by Rosse et al. (36) and Carvajal-Sandoval et al. (35). For instance, only minors digestive symptoms, including mild abdominal pain and soft stools, were reported in volunteers, who were administered 9 grams per day of glycine on an empty stomach during the daytime (Inagawa et al., 2006) (28).

Purpose of the study

The purpose of this pilot study was to demonstrate that glycine supplementation may improve insulin sensitivity in IR normoglycemic subjects. A positive outcome of this study could help to expand the usage scope of glycine as a dietary supplementation to safely reduce the prevalence rate of IR. Our work could also lead to a better therapy for and prevention of IR and T2DM.

Objective of the study

The overall goal was to establish the insulin-sensitizing effects of glycine as a dietary supplement. Our study involved testing IR normoglycemic subjects to assess the improvement in insulin sensitivity and blood lipid profiles after a four-week period of glycine supplementation.

Research hypothesis and specific aim of the study

Our study is based on the hypothesis that dietary glycine supplementation over a four-week period does significantly improve insulin sensitivity and lipid profile in normoglycemic subjects, thus favorably modulating glycemic homeostasis and fat metabolism. The specific aim was to conduct a pilot study that would evaluate whether glycine supplementation might improve insulin sensitivity and lipid profile. To test our hypothesis , over a four-week period, glycine was added to the usual diet of IR subjects, and its effects on insulin sensitivity and lipid profile were assessed. An oral glucose tolerance test (OGTT) was performed to assess the impact on glycemic homeostasis and insulin sensitivity. Blood lipids were checked to evaluate the effects on fat metabolism.

REVIEW OF LITERATURE

Human insulin resistance

Epidemiology

In the U.S. the prevalence of IR in the general population is estimated to be 35% (17, 19). IR is also common among non-diabetic individuals (15, 19), and may be associated with a higher risk of cardiovascular diseases in both non-diabetic and diabetic populations (15, 16). According to the American Heart Association (AHA), high rates of IR are reported in Africans Americans, in elderly persons, and in individuals with high blood pressure, obesity, physical inactivity, and vascular disease (2, 17, 33).

Pathophysiology

Glucose is the body's major source of energy. Facilitated by the hormone insulin, glucose is transported into cells (the muscles, fat, and liver cells) from the blood to be used as energy for a range of cellular functions. In addition, insulin converts excess glucose into glycogen for storage in the liver and muscle (5).

In people with IR, the body's cells fail to respond to the normal actions of the hormone insulin (2, 5). As a result, circulating levels of insulin and glucose are raised, and this hyperglycemia triggers the production of more insulin. IR and hyperglycemia can lead to T2DM and the metabolic syndrome/ IR syndrome (15, 16).

IR can lead to a cascade of events, including defects in signaling pathways. Glucose transporter type 4, also known as GLUT-4 (found in adipose tissues and striated muscles) is responsible for transporting glucose into the cells after binding insulin to its receptors (2,5). However, in the IR states, fewer numbers of GLUT-4 are translocated to the cell surface by insulin, leading to a decrease in glucose uptake rates. Therefore, the triglycerides (TG) stored in fat cells are broken down to provide free fatty acids (FFA) as the source of energy (2, 5). Previous studies have shown that defective intracellular signaling impaired glucose transport may be both inherited and acquired (2, 5). Also, these abnormalities may be the cause of most IR states and other biochemical events (5).

Measurement of insulin resistance

It is of interest to accurately quantify insulin sensitivity in individuals at risk for metabolic and cardiovascular conditions. The hyperinsulinemic euglycemic glucose clamp technique is the "gold standard" method for assessing insulin sensitivity, as it directly measures the effects of insulin in promoting glucose utilization under steady-state conditions (18, 19). It is an established method that provides precise quantitative measures of IR. However, the glucose clamp technique is rarely used in large investigations because it is expensive, laborious, and requires an experienced staff (42, 43).

Instead, surrogate indices have been developed for assessing IR/ insulin sensitivity. These surrogate measures use either fasting blood samples or samples obtained during oral glucose tolerance tests (OGTTs). Also, indices of insulin sensitivity are commonly used in epidemiological and clinical studies. They include the homeostasis

model assessment of insulin resistance (HOMA-IR), the Matsuda index, the fasting insulin level (FIL), the quantitative insulin sensitivity check index (QUICKI), the Avignon index, the Stumvoll index, and the new simple index assessing insulin sensitivity using oral glucose tolerance test (SIisOGTT) (39, 40, 41).

These alternatives for estimating insulin sensitivity are less labor intensive and are inexpensive in comparison with the hyperinsulinemic euglycemic glucose clamp technique. There is no universally accepted threshold that defines IR. Studies have demonstrated that covariates including age, gender, and ethnicity may affect the accuracy of the surrogate measures of IR to identify individuals with cardio metabolic risk (40, 41)

Strategies to improve insulin resistance

Improving insulin sensitivity requires lifestyle modifications, including dietary changes, weight reduction, increased physical activity, smoking cessation, and drug therapy (33). Previous studies have demonstrated that, except drug therapy, other approaches that require lifestyle change are difficult to implement (33). Potential non-pharmacological therapy pitfalls include lack of motivation, and weight loss can be challenging for patients with comorbid conditions who are physically inactive with to achieve and maintain (33).

Recently, effective insulin-sensitizing drugs were made available on the market to treat patients with T2DM or pre-diabetes. These medications include two major classes of pharmaceuticals, biguanides (metformin) and thiazolidinediones (TZD). Metformin is the only medication recommended by the American Diabetes Association (ADA) for T2DM prevention, especially in younger patients (18). Unlike metformin, TDZ improves

glucose disposal by skeletal muscle by 30% to 200% (18, 33). Other insulin-sensitizing drugs include antihypertensive drugs and pravastatin (33). IR can lead to a variety of serious health disorders, including pre-diabetes, diabetes, the metabolic syndrome, cardiovascular disease, and stroke (19, 37).

Prediabetes

Prediabetes is a condition in which glucose tolerance, fasting glucose, or both are impaired (1, 16). The risk of developing IR increases in people with glucose intolerance (16), independent of diabetes status. Two hundred and eighty (280) million individuals worldwide have impaired glucose tolerance (fasting glucose: 110-120 ml/dl), have a 10% yearly risk of progressing to T2DM (1, 16), and have a greater risk of developing cardiovascular disease (11% increases in 10 years), which can lead to heart attack or stroke (16, 19). It was estimated that, in 2009, 78 million Americans had prediabetes (12).

Diabetes

IR is present in the majority of patients with T2DM, and plays an important role in the pathophysiology of diabetes (2, 15). T2DM is now a pandemic and shows no signs of abatement (2).The prevalence of T2DM in the world was estimated at 285 million (6.4%) in 2010, among adults aged 20 -79 years old, and this value is predicted to rise to around 439 million (7.7%) by 2030 (15). About 90-95% of diabetic individuals are diagnosed with T2DM, evidently considered the most predominant form of the disease.

The insulin resistance syndrome / the metabolic syndrome

The IR syndrome is defined as a state of IR that is characterized by hypertension, raised levels of triglycerides, excess accumulation of abdominal fat, and decreased levels of high density lipoprotein (HDL), or good cholesterol (18, 19). Considerable focus has been put on the IR syndrome for several decades because of its association with an increased risk for T2DM, cardiovascular disease, and stroke (17, 18).

In clinical practice, the diagnosis of the metabolic syndrome is based on findings from the physical examination and clinical laboratory data. According to guidelines from the AHA, the metabolic syndrome is diagnosed when a patient has at least three of the following five conditions: fasting glucose ≥100 mg/dl, blood pressure ≥130/85 mm Hg, TG ≥150 mg/dl, HDL-C < 40 mg/dl in men or < 50 mg/dl in women, waist circumference ≥102 cm (40 inches) in men or ≥88 cm (35 inches) in women; if Asian American ≥90 cm (35 inches) in men or ≥80 cm (32 inches) in women (18, 33, 34).

Most recent studies have shown that vascular inflammation, fibrinolysis, and disorders of thrombosis may be included in the IR syndrome (31, 33). Ford et al.'s work demonstrated that the metabolic syndrome is highly prevalent (35%) in the U.S.; therefore, this fact has serious implications regarding public health (6).

Plasma lipids and lipoproteins

Plasma lipids

Abnormal levels of plasma lipids or dyslipidemia are common in clinical practice; therefore, they cannot be overlooked. Previous epidemiological studies have described the role of dyslipidemia as a major risk factor for cardiovascular disease (33), and coronary heart disease is the principal cause of death in all western societies (8). Lipids play an important role in all living cells. They are essential components in the structure of cell membranes. Lipids are organic compounds that are insoluble in water but soluble in organic solvents. They are involved in metabolic and hormonal pathways (31).

The four major forms of lipid in the plasma are FFA, free and esterified cholesterol, triglycerides, and phospholipids (8, 31). FFA may be free, non-esterified (NEFA) or can be esterified with glycerol to form triglycerides. Free cholesterol is responsible for hormones and the bile acid synthesis, and the formation of the membrane, whereas, esterified cholesterol is essential for transport and storage of the sterol (8). TG comprise FFA and glycerol. Fats and oils are major constituents of TG. They are transported to the liver and adipose tissue in the form of lipoproteins. Phospholipids are complex lipids that comprise a phosphate group (8)

Plasma lipoproteins

Lipoproteins are the form in which lipids circulate in the blood (31). Lipoproteins vary in size, composition, and function. The five major types of lipoproteins include chylomicrons, very-low density lipoprotein (VLDL), intermediate-density lipoprotein

(IDL), low-density lipoprotein (LDL), or "bad cholesterol", and high-low density lipoproteins (HDL), or "good cholesterol."

Chylomicrons are the largest lipoproteins that transport dietary cholesterol and TG from the intestine to peripheral tissues (liver, adipose, cardiac, and skeletal muscle tissue). IDL and VLDL carry endogenous triglycerides from the liver to the body's cells. In contrast, LDL is involved in the transfer of cholesterol to the peripheral tissues, and HDL removes the cholesterol from cells to sites of degradation and excretion principally in the liver (8, 31).

The measurement of lipids and lipoproteins requires that blood be obtained after a 12-hour fast for more interpretable results. The recommended values of these different test results are as follows: The total cholesterol (TC) $\leq$ 200 mg/dl, triglycerides (TG) $\leq$ 150mg/dl, HDL cholesterol $\geq$ 40mg/dl, LDL cholesterol $\leq$ 100mg/dl for patients who are at high risk of heart disease, and $\leq$ 130mg/dl for low-risk patients (4). Several studies have demonstrated that elevated levels of serum lipids are associated with an increased risk of cardiovascular disease, including coronary artery disease, heart attack, and death (8, 31). In IR states, the major lipoprotein abnormalities include decreased HDL, reduced LDL particle size, and high TG levels (5, 37).

Effect of glycine intake on human disease

Amino Acids

Amino Acids are the building blocks of proteins. AAs are defined as organic compounds composed of amine and carboxylic acid functional groups (4, 7). Scientists have identified 20 AAs, 10 of which are produced by humans and are classified as non-

essential AAs. These include asparagine, aspartic acid, alanine, cysteine, glycine, glutamic acid, glutamine, proline, serine, and tyrosine. The other 10 essential AAs cannot be produced by the human body; they are required in the diet. The essential AAs comprise arginine, histidine, isoleucine, leucine, lysine, methionine, phenylalanine, threonine, tryptophan, and valine (7, 22).

Recent research suggests that AAs may be important in the development of IR, as circulating levels of several AAs have been associated with obesity and insulin sensitivity (22, 44). Garvey et al.'s work has examined the relationship between circulating AAs and IR in humans using the hyperinsulinemic euglycemic glucose clamp technique for assessing insulin sensitivity. The study found that glycine had the strongest positive correlation with glucose disposal rate, while leucine/ isoleucine had the strongest negative correlation (11).

Glycine

Glycine is the most widely available of the 20 AAs in the body and has the simplest molecular structure. Glycine can be synthetized from common metabolic intermediates in all living cells (21). Glycine is not essential to the human diet, as illustrated by the fact that 45 g of glycine can be synthesized in the body, while only 3-5 g of glycine are derived from diet per day (21, 23). It is also found in many high-protein foods, such as fish, meats, and dairy products. Previous studies have used glycine supplementation as a nutrition intervention to treat various diseases in human and animal models, as described below.

Glycine supplementation to treat various diseases

A recent work found that glycine is associated with increased insulin sensitivity; giving rise to the hypothesis that glycine may have a protective role against the development of IR, particularly in the presence of increased adiposity, genetic predisposition to IR, and overall metabolic dysfunction (11). The study's aim was to examine the relationships between circulating amino acid levels and the gold standard measure of insulin sensitivity, glucose disposal rate (GDR) via hyperinsulinemic-euglycemic clamp technique among individuals with varying degrees of insulin sensitivity. The research findings suggest that glycine levels are strongly positively associated with insulin sensitivity, and individuals who were insulin sensitive had the highest level of circulating glycine. While not indicative of cause-and- effect, these observations support favorable glycemic responses with greater concentrations of circulating glycine.

El Hafidi et al. conducted an experiment on 28-day-old sucrose-fed rats in which 1% glycine was added to the rats' drinking water for four weeks. Their work suggested that glycine may protect against increased circulating non-esterified fatty acids (NEFA), fat cell size, intra-abdominal fat accumulation, and blood pressure in sucrose-fed rats (10). Glycine supplementation can also suppress the inflammatory response in animal models of ischemia reperfusion, injury, and transplantation (20).

Moreover, Kamara et al.'s work suggested that glycine plays a protective role against the development of IR, particularly when associated with obesity and metabolic perturbations in metabolic syndrome patients (9). Recently, glycine supplementation (100

mg/kg/day) was administered to uncontrolled T2DM patients with severe deficient synthesis of glutathione (GSH) (29). Following dietary supplementation of GSH precursors, AAs (glycine and cysteine), GSH synthesis and concentrations increased significantly, and plasma oxidative stress and lipid peroxides decreased significantly (29).

In 1933, Harris et al. reported that glycine was administrated at a dose of 7.5 - 25g/day to treat patients with myopathies (23). Later, in 1976, glycine was also tested in children with isovaleric academia at a dose of 250 mg/kg/24h (24). In 1998, glycine was used in the management of seizures in 3-phosphoglycerate dehydrogenase deficiency, and the addition of 200 mg/kg/d of glycine to up to 500 mg/kg/d of L-serine resulted in the complete disappearance of seizures (25). It was reported in 2001 that a 5 g dose of glycine ingested orally in the morning increased insulin secretory responses without changes in insulin action in healthy first-degree relatives of T2DMpatients (26). Marchini et al. also studied glycine supplementation (0.2 g/kg/d) on protein turnover in obese women (27). Recently, Bannai et al. investigated glycine supplementation (3 g at bedtime) on subjective daytime performance in partially sleep-restricted healthy volunteers (30).

METHODS

Overview

Data in this study were collected from the glycine's research protocol No. 9309179. The principal investigator was Dr. W. Timothy Garvey. The study received approval from the Institutional Review Board (IRB) of the University of Alabama at Birmingham. Informed written consent was obtained from all subjects.

Subjects

The pilot study was conducted at the University of Alabama at Birmingham (UAB) in the Department of Nutrition Sciences. The study took place from April through July 2013. Participants were living in metropolitan Birmingham. They were recruited from the outpatient clinics and from a registry of previous participants in Dr. Garvey's studies.

After obtaining informed consent, 10 volunteers were selected to participate in the study based on several criteria. Specifically, women and men between the ages of 20 and 65 were recruited. In addition, the study group included European- American (EA) and African- American (AA) non-diabetic individuals diagnosed with one or more ATPIII risk factors for metabolic syndrome (waist circumference > 40 inches in men and > 35 inches in women; fasting glucose $\geq$ 100 mg/dl; TG > 150 mg/dl; HDL-c < 50 mg/dl in

women and < 40 mg/dl in men; blood pressure ≥ 130/ ≥ 85 mmHg) were sequentially enrolled.

Except for extremes of body mass index (BMI) (< 21 and > 42kg/m2); volunteers were recruited over a broad range of body weight to ensure inclusion of both lean and obese subjects. All volunteers had normal count blood cells (CBC) /platelets / prothrombin time (PTT), liver function tests, creatinine / blood urea nitrogen (BUN), and thyroid status. Volunteers were not engaged in regular exercise as this is known to improve insulin sensitivity (16, 18).

Volunteers were asked to maintain their usual diet. No volunteers were on lipid-lowering drugs, anti-hypertensive drugs, or any medications known to alter glucose or fat metabolism. Pre-menopausal females were studied after pregnancy was ruled out by serum beta human chorionic gonadotropin (HCG).

Study protocol

The study consisted of three phases. Phase 1: the baseline visit consisted of (a) health history, physical examination (vital signs, height, weight, BMI, waist / hip circumference), electrocardiography (ECG), and clinical laboratory tests (CBC, electrolytes, BUN, creatinine, liver function tests); and (b) a pregnancy test for pre-menopausal females.

Phase 2: took place a week after the Phase 1 visit. Subjects were asked to return after they qualified based on the tests above. Eligible subjects were given glycine powder and instructed to dissolve 5 g (one teaspoon) in half a cup of water or fruit juice (if it was

100% juice without added sugar) and consume with each of three meals for a total of 15 g/day with their usual diet.

Phase 3: took place two weeks after Phase1. Subjects remained on glycine supplementation for an additional two-week period. During this period, the following tests were done: (a) a 24-hour urine collection for measurement of creatinine, protein, urea nitrogen, and c-peptide; (b) a three-hour 75 g OGTT with blood obtained for measurement of glucose, insulin, and FFA; (c) anthropometric measurements; (d) indirect calorimetry. After four weeks, the evaluation performed at baseline was repeated for every subject. All phases described in this work were completed within four to five weeks, and procedures were conducted in the morning after a 10-hour fast.

To promote improved quality control, only trained personnel, including two registered nurse study coordinators, were involved in the study, and meticulous care was taken with respect to sterility and technique to minimize the risks of vessel puncture. Subjects with a low red blood cell count or iron deficiency were excluded, and blood drawing did not exceed 400 ml from each patient over the period of study. Volunteers were regularly called and emailed to check on their compliance with glycine supplementation and also to record any complaints or adverse effects.

Data collection

Data were collected from April through July 2013. The following data were collected at the baseline (week 1), week 2, and week 4 after intervention:

Anthropometrics

BMI was calculated as body weight in kilograms divided by the square of height in meters (kg/m2). BMI provided a measure of generalized adiposity. Fat distribution was assessed by waist and hip circumferences (cm) using a tension-controlled tape measure by Novel Products (Rockton, IL). Weight was measured using a weight scale in pounds, and height was measured using a height measuring board in English units (inches and feet).

Blood assays and OGTT

Plasma glucose was measured by glucose oxidase method using a glucose analyzer (YSI 2300; Yellow Springs Instruments, Yellow Springs, OH). Serum insulin levels were measured using an electrochemiluminescence immunoassay (Roche Diagnostics, Mannheim, Germany).

A 75- g, three-hour OGTT was performed with measurements of blood glucose and insulin levels obtained at 0, 30, 60, 90, 120, and 180 minutes. These measurements established the category of glucose tolerance. A conventional lipid panel (HDL-c, LDL-c, total cholesterol, and TG) was measured in fasting blood using an enzymatic assay. LDL-c is not measured directly, it is estimated from the following Fried Ewald equation: LDL = Total cholesterol - HDL-c – Triglycerides /5 (9).

Indirect calorimetry

Energy expenditure and substrate oxidation rates are measured using an open-circuit indirect respiratory calorimeter (Deltratrac, DATEX, Helsinki), using a ventilated

hood technique and under resting conditions (the individual is required to be completely at rest during a 30-minute period).

Insulin sensitivity

Insulin sensitivity was assessed in all subjects using HOMA-IR (derived from fasting plasma insulin and glucose levels) and the Matsuda index (derived from glucose and insulin levels obtained during oral glucose tolerance tests). Insulin sensitivity indices were calculated by the following equations (39, 40, 41):

HOMA-IR: [fasting plasma glucose level (mg/dl) × fasting insulin level (μunit=mL)]/ 405. Lower HOMA-IR values indicate greater insulin sensitivity (i.e., less IR). Matsuda index = 10.000/square root of × [(fasting plasma glucose × fasting insulin level) × (mean glucose × mean insulin during OGTT)]. Higher Matsuda index values indicate greater insulin sensitivity (i.e., less IR).

Other variables

Information was also collected on socio-demographic background (e.g., age, education level, employment status, and marital status), reproductive history, family history of diabetes, and medical history (e.g., high blood pressure).

Data analysis

All quantitative analyses were performed using SAS® 9.2 (SAS Institute, Cary, NC,). Descriptive statistics were used to describe subjects' basic characteristics, including means, standard deviation (SD), and range. Analyses were stratified by gender and race at baseline, which were used as controlling variables. Our hypothesis was tested by using a paired t-test to determine significant differences between Si and serum lipids before and after glycine intervention in participants. Given that this was a pilot study, an effect was considered to have occurred if there was a trend of 0.15 ($p < 0.15$) or less probability that these effects occurred by chance.

RESULTS

Characteristics of participants

Ten volunteers had originally consented to participate in the study (participant rate was 100%). During the investigation, one participant was dropped from the study; he complained of dysentery-like symptoms (high temperature, cold, and diarrhea) after taking glycine powder. This participant was immediately replaced by another volunteer. Therefore, a total of 10 non-diabetic 'participants completed the study from pre-intervention to post-intervention (four weeks).

Four (40%) were EA, and six (60%) were AA. Four (40%) were male, and six (60%) were female. The mean age of the study subjects was 50.5 ± 1.9 years with a range of 42-58 years. On average, subjects were obese, with a mean BMI of 35 ± 1.8 kg/m2. The mean total cholesterol was normal, at 173.2 ± 17.9 mg/dl. OGTT, HOMA-IR, and Matsuda-index results were available in all 10 subjects.

Characteristics of participants at baseline by gender

Of female participants, 60% were younger than 50 years old (mean age ± SD was 46.8 ± 4.95 years), while 40% of male participants were younger than 60 years old (mean age ± SD was 56 ±1.82 years). Female were younger than male participants ($p = 0.0083 < 0.15$). In addition, the findings showed that females were more insulin sensitive than males at baseline (5.01 ± 1.94 µU/ml vs. 4.69 ± 0.87 µU/ml), whereas males were

more insulin resistant than females (2.78 ± 1.79 μU/ml vs. 2.11 ± 0.49 μU/ml). However, these differences were not statistically significant (respectively $p = 0.77$; $p = 0.39$). Other variables, including BMI, blood pressure, plasma lipids, FBG, and FIL, were not statistically significant (Table 1).

Table 1: Descriptive statistics at baseline by gender

Characteristics	Female (n= 6) Mean (SD)	Male (n = 4) Mean (SD)	P values
Age (years)	46.8 (4.95)	56 (1.82)	**0.0083**
BMI (kg/m2)	35.8 (6.23)	34.01 (5.22)	0.65
Cholesterol (mg/dl)	167.33 (69.71)	182 (36.77)	0.71
TG (mg/dl)	114.33 (57.24)	174.5 (159.45)	0.41
LDL (mg/dl)	114.67 (30.87)	110.75 (30.47)	0.85
HDL (mg/dl)	64.33 (33.68)	36.75 (14.15)	0.17
RQ	0.91 (0.064)	0.93 (0.19)	0.74
Waist circumference (cm)	38.7 (5.34)	44.5 (5.95)	0.16
Carbohydrates (g)	217.33 (72.96)	255.83 (141.34)	0.58
Fat (g)	44.59 (31.88)	45.6 (77.73)	0.97
FBG (mg/dl)	91.5 (9.41)	84.5 (20.74)	0.48
FIL (µU/ml)	9.28 (1.63)	13.5 (7.31)	0.19
Systolic (mmhg)	121.67 (13.84)	132.5 (18.81)	0.32
Diastolic (mmhg)	71 (26.71)	80.75 (4.86)	0.49
HOMA-IR	2.11 (0.49)	2.78 (1.79)	0.39
Matsuda-index	5.01 (1.94)	4.69 (0.87)	0.77

Characteristics of participants at baseline by race

TG levels were statistically higher in EAs compare to TG levels in AAs (209 ±143 vs 91.33 mg/dl ± 34.88 mg/dl) ($p = 0.08$). Furthermore, AAs were more insulin resistant than EAs (2.69 ± 1.42 µU/ml vs. 1.92 ± 0.36 µU/ml); whereas EAs and AAs were identically insulin sensitive (4.88 ± 0.60 vs. 4.88 ± 2.01). However, these means were not statistically different ($p = 0.33$ and $p = 0.99$, respectively). Other variables, including BMI, blood pressure, plasma lipids, FBG, and insulin levels, were not statistically significant (Table 2).

Characteristics of participants before and after intervention

Though not statistically significant, TG levels were decreased after glycine intervention compared to TG levels at baseline (138.4 ±106.12 mg/dl vs 119.4 ± 83.42mg/dl; $p = 0.21$) (Table 3). Respiratory quotient (RQ) was not statistically significant after glycine intervention (0.915 ± 0.11 vs. 0.89 ± 0.08) ($p = 0.31$). On average, patients relied largely on carbohydrates (RQ = 0.9) as fuel (Table 3). FBG levels were significantly elevated in post-intervention compared with FBG at baseline based on the p value of <0.15 established a priori to define a trend for this pilot study (88.7 ±14.34 mg/dl vs. 99.32 ± 11.23 mg/dl; $p = 0.12$).

Fasting insulin levels (FIL) were not significantly higher in post-intervention compared with FIL at baseline (10.97 ±4.89 µU/ml vs. 15.52 ± 16.38 µU/ml; $p = 0.42$). However, mean insulin concentrations before and after intervention remained in the normal range (0-15 µU/ml). LDL-cholesterol levels were lower after glycine intervention

compared to the LDL-c levels at baseline, but it did not reach a statistical significance (109.3 ± 32.06mg/dl vs. 113.1 ± 29.03mg/dl; p = 0.28).

Mean HOMA-IR was slightly elevated after intervention compared to mean HOMA-IR at baseline (2.42 ± 0.82 vs.2.38 ± 1.15), but the difference was not statistically different (p = 0.92). Insulin sensitivity index (Matsuda index) was decreased after intervention (3.63 ± 1.75 vs. 4.88 ±1.54) compared to insulin sensitivity index at baseline, but it was not statistically significant (p = 0.16), which approached the value of p<0.15 established for a trend in this pilot study (Table 3).

Characteristics of participants before and after intervention after controlling by gender

After controlling by gender, LDL-c levels were significantly lower in female participants than in male participants (107.16 mg/dl ±33.39 vs. 114.66 mg/dl ± 30.87; p = 0.12). Also, Matsuda index was significantly reduced in females (2.95 ± 0.69 vs. 5.01mg/dl ± 1.94; p = 0.04) compared to males (Table 4).

Characteristics of participants before and after intervention after controlling by race

After controlling by race, TG levels were significantly decreased in EAs in post-intervention (209 mg/dl ± 143.79 vs. 161. 5 mg/dl ±123.40, p = 0.03). Matsuda index was significantly decreased in EAs (4.88 ± 0.6 vs. 3.35 ± 0.42; p = 0.03) (Table 5). Other variables, including BMI, blood pressure, plasma lipids, FBG, and FIL, were not statistically significant.

Table 2: Descriptive statistics at baseline by race

Characteristics	African Americans (n= 6) Mean (SD)	European Americans (n = 4) Mean (SD)	P values
Age (years)	51.5 (6.83)	49 (5.35)	0.56
BMI (kg/m2)	33.83 (4.81)	(36.96, 6.94)	0.41
Cholesterol (mg/dl)	177.67 (40.15)	166.5 (82.68)	0.77
TG (mg/dl)	91.33 (34.88)	209.0 (143.79)	**0.08**
LDL (mg/dl)	108.33 (34.06)	120.25 (21.92)	0.55
HDL (mg/dl)	44.67 (12.24)	66.25 (45.58)	0.29
RQ	0.94 (0.14)	0.87 (0.01)	0.34
Waist circumference (cm)	39.3 (4.67)	43.75 (7.39)	0.30
Carbohydrates (g)	257.27 (124.42)	195.93 (35.04)	0.37
Fat (g)	32.83 (63.45)	63.23 (16.97)	0.38
FBG (mg/dl)	93.83 (6.43)	81 (20.41)	0.18
FIL (µU/ml)	11.62 (6.12)	10 (2.75)	0.63
Systolic (mmHg)	131.5 (18.10)	117.75 (8.34)	0.19
Diastolic (mmHg)	69.33 (25.76)	83.25 (5.25)	0.32
HOMA-IR	2.69 (1.42)	1.92 (0.36)	0.33
Matsuda-index	4.88 (2.01)	4.88 (0.60)	0.99

Table 3: Before and after intervention

Characteristics	Baseline (mean, SD)	After intervention (mean, SD)	P values
Cholesterol (mg/dl)	173.2 (56.64)	181 (34.87)	0.46
TG (mg/dl)	138.4 (106.12)	119.4 (83.42)	0.21
LDL (mg/dl)	113.1 (29.03)	109.3 (32.06)	0.28
HDL (mg/dl)	53.3 (30.0)	44.2 (12.37)	0.37
RQ	0.915 (0.11)	0.89 (0.08)	0.31
Carbohydrates (g)	232.73 (100.07)	209.39 (97.69)	0.47
Fat ()	44.99 (50.78)	60.55 (44.99)	0.29
FBG (mg/dl)	88.7 (14.34)	99.32 (11.23)	**0.12***
FIL (µU/ ml)	10.97 (4.89)	15.52 (16.38)	0.42
HOMA-IR	2.38 (1.15)	2.42 (0.82)	0.92
Matsuda-index	4.88 (1.54)	3.63 (1.75)	0.16

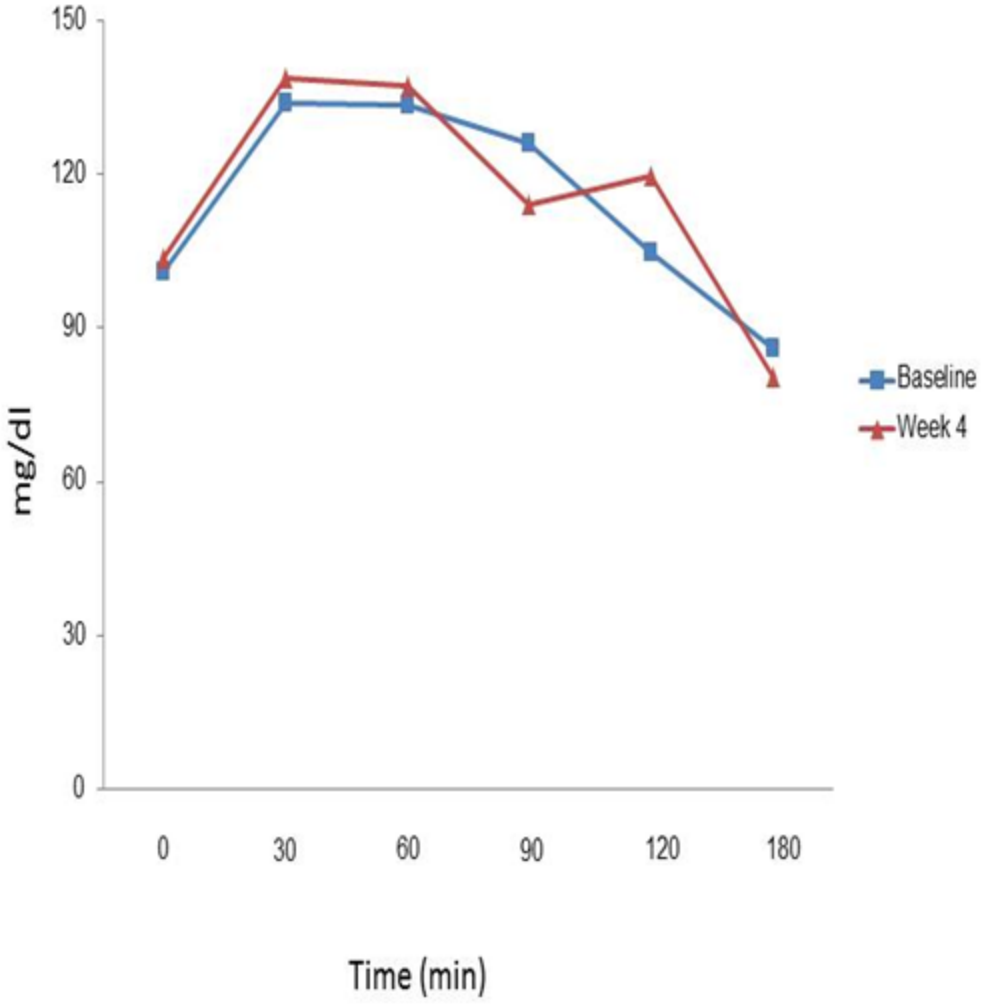
Table 4: before and after intervention after controlling by gender

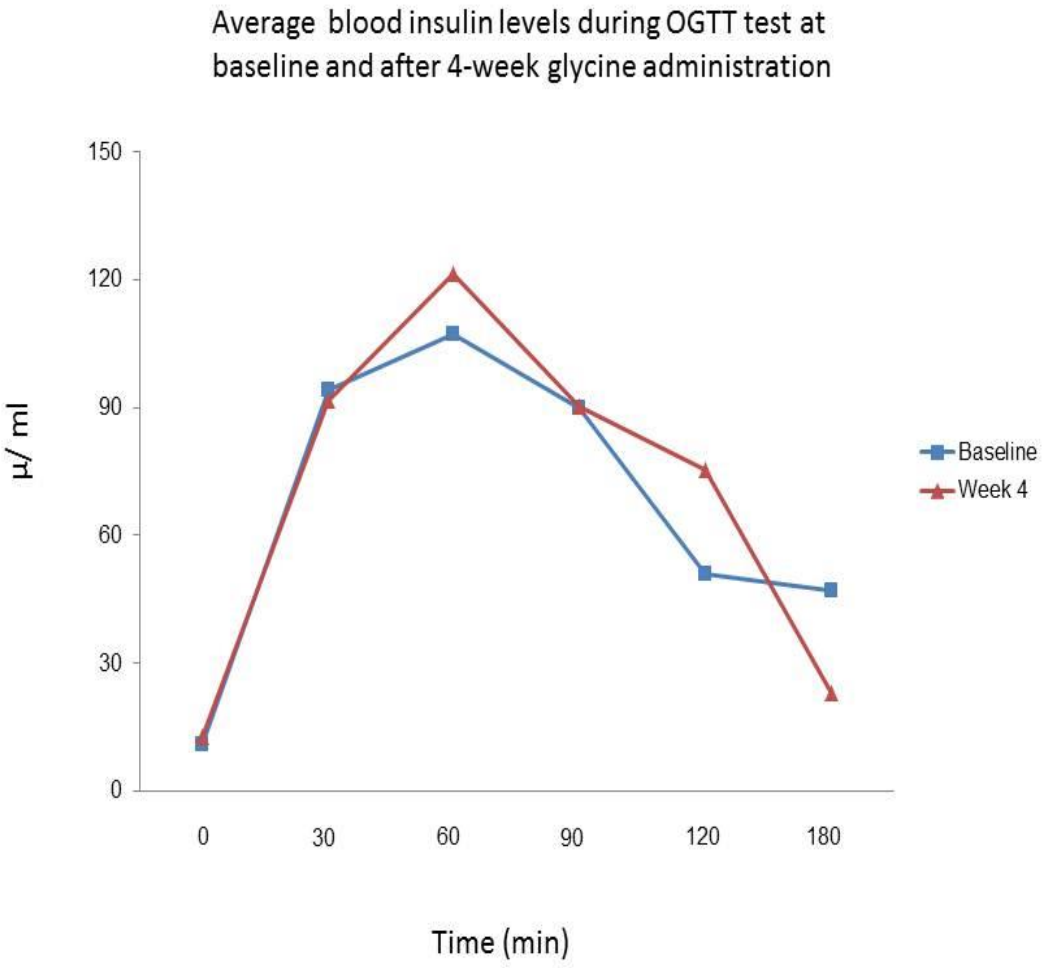
Variables	Gender	Baseline	After intervention	P values
LDL-c (mg/dl)	Female (n= 6)	114.66 (30.87)	107.16 (33.39)	**0.12**
	Male (n= 4)	110.75 (30.46)	112.5 (34.69)	0.7
Matsuda-index	Female	5.01(1.94)	2.95 (0.69)	**0.04**
	Male	4.69 (0.87)	4.65 (2.46)	0.98

Table 5: before and after intervention after controlling by race

Variables	Race	Baseline	After intervention	P values
TG (mg/dl)	AA	91.33 (34.88)	91.33 (38.01)	1
	EA	209 (143.79)	161.5 (123.40)	**0.03**
Matsuda-index	AA	4.88 (2.01)	3.81 (2.30)	0.47
	EA	4.88(0.60)	3.35(0.42)	**0.03**

Average blood glucose levels during OGTT test at baseline and after 4-week glycine administration




Average blood insulin levels during OGTT test at baseline and after 4-week glycine administration
150
120
90
60
30
0
µ/ ml
0
30
60
90
120
180
Time (min)
Baseline
Week 4

DISCUSSION

The primary purpose of the study was to determine whether glycine supplementation may improve insulin sensitivity and lipid profile in insulin resistant normoglycemic subjects. Glycine intake has been shown to decrease triglycerides levels and fat accumulation in rodents (10). Furthermore, a recent study demonstrated that glycine had the strongest relationship with glucose disposal rate (GDR), particularly in obese subjects (11). However, it remains unknown whether glycine supplementation of the diet could enhance insulin sensitivity in humans. Our study did not support significant improvements in lipid profile after four weeks of glycine intervention.

Our findings are not consistent with other published works on glycine administration in non-diabetic and diabetic patients. In these studies, glycine was found to play a protective role against the development of IR (20, 26, 27). However, ours is the first study that examined the effect of glycine in IR and non-diabetic patients and to enroll patients on the basis of one or more ATPIII risk factors for the metabolic syndrome.

When the mean values of the Matsuda- index at baseline (surrogate indice for assessing insulin sensitivity) were compared, we observed that women were more insulin sensitive than men (5.01 ± 1.94 µU/ml vs. 4.69 ± 0.87 µU/ml), whereas; males were more insulin resistant than females (2.78 ± 1.79 µU/ml vs. 2.11 ± 0.49 µU/ml), though it was not significant. These data are compatible with previous studies done on gender

differences and insulin sensitivity. Nuutila et al. found that women were more sensitive to insulin than equally fit men. According to them, muscle insulin sensitivity was almost 50% higher in women than in men. Several factors might explain these findings, including differences in fat distribution, uric acid concentrations serum triglycerides, and high-density lipoprotein cholesterol, between males and females (12).

Our study showed that TG levels were statistically higher in European Americans compared to TG levels in African Americans (209 ±143 vs 91.33 mg/dl ±34.88 mg/dl; p = 0.08) at baseline. This finding might be explained by the fact that different ethnic populations may have different body compositions (12, 13). This result is very interesting, as it shows that even with a small sample size such as ours (10 participants), we can detect differences between races.

Although, there was no statistical significance, AAs were found to be more insulin resistant than EAs, when the mean values of HOMA-IR were compared at baseline (2.69 ± 1.42 vs. 1.92 ± 0.36; p = 0.33). This result supports previous works that suggested that AAs are more insulin- resistant than EAs when FIL and HOMA-IR indices are used to assess insulin sensitivity (39). In addition, other studies have reported that AAs experience higher insulin concentrations compared with their EAs counterparts (39, 40).

TG levels were decreased after glycine intervention compared with TG levels at baseline (138.4 ±106.12 mg/dl vs 119.4 ± 83.42mg/dl), though the mean difference did not reach a statistical significance (p = 0.21). El Hafidi et al.'s work showed that glycine administration in rodents decreased TG levels and fat accumulation (10). Although

studies in animals can represent pre-clinical data relevant to humans, we should consider the differences in anatomy, function, and organ structure that exist between the two species. Therefore, applying information from animal studies to humans should be done with caution.

In this pilot, short-duration study, we observed no significant effect of glycine supplementation on insulin sensitivity or serum lipids after intervention, but we did observe a favorable effect on TG levels in EAs after intervention that approached statistical significance, when controlled for race (161. 5 mg/dl ± 123.40vs. 209 mg/dl ±143.79; p = 0.03).These results suggest that glycine supplementation may improve TG levels in EAs. This finding is interesting, as we observed that TG concentrations were significantly higher in EAs at baseline. Therefore, glycine might be beneficial in the treatment of various conditions associated with dyslipidemia. In addition, LDL-c levels were significantly decreased in female participants in post-intervention, when controlled for gender (107.16 mg/dl ±33.39 vs. 114.66 mg/dl ± 30.87; p = 0.12). However, our small sample size may undermine these findings.

FBG was significantly higher after intervention (p = 0.12).). We believe that a lack of dietary and behavioral intervention might explain this result, as patients were allowed to eat whatever they wanted. Though not statistically significant, mean HOMA-IR was slightly elevated in post-intervention, whereas, the mean insulin sensitivity index (Matsuda index) was decreased. Moreover, Matsuda index in women was decreased after intervention, when adjusted by gender, and decreased in EAs after intervention, when adjusted by race. A lower Matsuda index value indicates lower insulin sensitivity (i.e., higher IR). These discrepancies between surrogate indices values after intervention might

be related to insulin sensitivity affecting different organs. Thus, HOMA-IR indicates hepatic insulin sensitivity, while the Matsuda index reflects both hepatic and peripheral insulin sensitivity (40, 41).

Glycine supplementation was well tolerated without major adverse effects in our study. In an earlier study, minors digestive symptoms, including mild abdominal pain and soft stools, were commonly reported in volunteers, who were administered 9 g per day of glycine on an empty stomach (Inagawa et al, 2006). None of our volunteers experienced these complaints. Because glycine is effective and safe in humans, our results suggest that a larger trial with clinical end points is feasible.

Although our sample size was small, it was designed to detect a clinically significant effect of glycine on insulin sensitivity in order to proceed to a more elaborate randomized cross-over study. A larger sample size would have provided a more precise estimate of the treatment effect of glycine but not a more rigorous test of the research hypothesis.

Despite recent reports regarding the positive effect of glycine supplementation on insulin sensitivity in non-diabetic and diabetic patients (20, 26, 27), our pilot study did not corroborate these findings. This negative result may be explained by the use of the hyperinsulinemic-euglycemic clamp technique in previous studies, which directly measures the ability of insulin to promote glucose uptake in peripheral tissues (42, 43). Although our study used valuable indices (OGTT glucose and insulin values, HOMA-IR, and the Matsuda- index) to assess insulin sensitivity in non-diabetics subjects, results are more accurate with the hyperinsulinemic-euglycemic clamp technique.

Management of IR is based on improving insulin sensitivity with drugs or lifestyle interventions (18, 33). Our study participants were on a free-living diet. Other works have demonstrated that a high-carbohydrate diet may lead to both elevated plasma insulin and TG concentrations (7, 8). We believe that a modified diet combined with the glycine supplementation might improve the study outcomes.

Although, studies demonstrated that glycine may play a protective role against the development of IR, data from this study suggest that glycine might not be an effective intervention for IR in subjects with metabolic syndrome. Hence, a number of factors, including intrinsic (e.g., AA metabolism, protein metabolism, hormonal changes) and extrinsic factors (e.g., dietary intake, physical activity) (33, 37), may contribute to changes in AA concentrations in general and in glycine levels in particular. In addition, the underlying mechanisms linking IR and AAs have yet to be elucidated.

Limitations of the study

In contrast to prior studies on glycine supplementation in human and animal models, our findings suggest that glycine might not be an effective intervention for IR in non-diabetic subjects. However, these results should be taken with some caution considering the following limitations:

1) The study sample size was small; therefore, findings may not be reliable and cannot be generalized to the remaining patient population.
2) A four-week glycine intervention may not be sufficient to obtain favorable effect on the IR and lipids profile of non-diabetic patients.
3) A free-living diet prevented investigators from modifying participants' diets to improve the effect of glycine on insulin sensitivity in participants.
4) Our study population was, on average, metabolically healthy [means HOMA-IR (≤ 3) and Matsuda- index (≥ 3) were normal at baseline].

CONCLUSION

The aim of the current pilot study was to identify whether glycine supplementation may improve insulin sensitivity and lipid profile in IR normoglycemic subjects over a four-week period. Although, our sample data did not provide sufficient evidence to demonstrate whether glycine supplementation in the diet could enhance insulin sensitivity in humans, we observed encouraging findings suggesting that glycine supplementation might improve TG levels in EAs, and LDL-cholesterol levels in women after a four-week intervention. Moreover, our study showed that TG levels were significantly higher in EAs compared to their AA counterparts at baseline, whereas AAs were found to be more insulin resistant than EAs at baseline, though it was not significant. These findings show that even with a small sample size (10 participants), there were aspects of the data that mimic patterns of the general population.

Several studies have used glycine supplementation as nutrition intervention to treat various diseases in human and animal models (10.11, 33, 34). However, these studies were longer in duration and larger in size, which might improve effectiveness. In the light of these facts and regarding the interesting findings that we observed, we believe that a larger and randomized cross-over design investigation with sufficient power is necessary to fully test the insulin-sensitizing effect of glycine in non-diabetic subjects with metabolic syndrome, and further explore these findings.

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APPENDIX

Investigator's Progress Report

Form version June 26, 2012

In MS Word, click in the white boxes and type your text; double-click checkboxes to check/uncheck.

x Continuing Review (Complete Items 1-11)
—OR—
☐ Final Report—all protocol-related activities are complete, including data analysis (Complete Items 1-10, and Item 12)

—FOR—

☐ Expedited Review
—OR—
☐ Convened (Full) Review

1. Dates

Today's Date	6/25/13
Starting Date of Project	11/20/12
Date of Last IRB Approval	9/19/2012

To help avoid delay, respond to all required items in the format provided, and include requested materials.
If previous approval expires before approval is officially re-issued by the Office of the IRB, all work on the protocol must cease.
The IRB recommends applying for continuing review 4-6 weeks before expiration of current approval. (See schedule.)

2. Principal Investigator (PI)

Name (with degree)	W. Timothy Garvey, M.D.	**Blazer ID**	garveyt
Department	Nutrition Sciences	**Division**	SHP
Office Address	Webb Bldg Room 232	**Office Phone**	934-6103
E-mail	garveyt@uab.edu	**Fax Number**	996-4082

PI Contact who should receive copies of IRB correspondence (Optional)

Name	Dana Y. Golson, RN, CDE	**E-mail**	ccnrn@uab.edu
Phone	996-4015	**Fax Number**	996-4082

Office Address (if different from PI)

3. UAB IRB Protocol Identification

Protocol Number F120831002

Protocol Title	Glycine Supplementation to Improve Insulin Sensitivity in Humans
Study Sponsor(s)	Dept of Nutrition Sciences
OSP Proposal Number (9 digits)	

Note. If the source or amount of funding for this project has

changed, include the new or revised funding application and provide the new OSP Proposal Number:	

4. Purpose

In two or three sentences, briefly summarize the purpose of this protocol, and related studies if applicable. Please use non-technical language, and write more for adults with general knowledge than for specialists.

▶ Recent research studies have demonstrated that Glycine may have a protective role against the development of insulin resistance. Our over goal is to establish the insulin sensitizing effects of Glycine. Other

5. Screened, entered, or otherwise accessed by the UAB Investigator(s). Include numbers for individuals, specimens, data records, charts, etc., as applicable to the protocol.

5.a. Number screened for study entry since the start of the project? (See 5.d.i.)	12
5.b. Number entered in study since the start of the project? (See 5.d.ii.)	12
5.c. Number entered in study since the last IRB review?	12

5.d. Complete the grids below to show how many have been screened and entered, along with their age or age range, gender, and race/ethnicity. Copy/paste the grids to repeat them for additional groups (e.g., controls, sub-studies) if needed.

Note. **If the research involves minors (<19 years of age), the PI must provide a separate, signed memorandum that either (a) confirms the previously assigned Children's Risk Level (CRL) number or (b) reassigns it and gives the reasons it has changed.**

5.d.i. Number Screened (Totals = 5.a.)

Race / Ethnicity	Male		Female	
	Age Range	Number Screened	Age Range	Number Screened
Caucasian	51-55	2	43-53	3
African American	55-57	3	42-59	4
Native American				
Asian				
Hispanic				
Other				

5.d.ii. Number Entered (Totals = 5.b.)

Race / Ethnicity	Male		Female	
	Age Range	Number Entered	Age Range	Number Entered
Caucasian	51-55	2	43-43	3
African American	55-57	3	42-59	4
Native American				
Asian				
Hispanic				
Other				

☐ Check the box at the left if the demographic information was not available (e.g., not collected for screening; collecting only specimens or data records and did not have access to the information)

6. Protocol Staff Listing

For each individual currently involved in the design, conduct, and reporting of the research, list the person's name, role in research, and CIRB status in the table below.

Copy/paste the table for each individual.

Financial Interests Related to the Research—Conflict of Interest (COI)

Human subjects research involving a disclosed financial interest on the part of any UAB employee or their immediate family is subject to IRB review following review by the UAB Conflict of Interest Review Board (CIRB). The following definitions apply: *Immediate family* means spouse or a dependent of the employee. *Dependent* is any person, regardless of his or her legal residence or domicile, who receives 50% or more of his or her support from the public official or public employee or his or her spouse or who resided with the public official or public employee for more than 180 days during the reporting period. *Financial Interest Related to the Research* means financial interest in the sponsor, product or service being tested, or competitor of the sponsor.

If one of the four items listed below is marked for an individual, a financial interest disclosure must be submitted to or currently on file with the CIRB. The IRB must receive a completed CIRB Evaluation before it will conduct its review.

COI 1 An ownership interest, stock options, or other equity interest related to the research of any value.

COI 2 Compensation related to the research unless it meets two tests:

- Less than $10,000 in the past year when aggregated for the immediate family.
- Amount will not be affected by the outcome of the research.

COI 3 Proprietary interest related to the research including, but not limited to, a patent, trademark, copyright, or licensing agreement.

COI 4 Board of executive relationship related to the research, regardless of compensation.

FULL NAME	CONFLICT OF INTEREST (COI)
W. Timothy Garvey, M.D., PI, Dana Y. Golson, RN, CDE; Armando Enriquez, MCCT, Miriam Rueger, RN, RD, CDE; Boni Epse Attobla M., Graduate student assistant	x None, or ☐ 1 ☐ 2 ☐ 3 ☐ 4 If any, is MOU in place? ☐ Yes ☐ No

7. Information Since the Date of Last IRB Review

- **Mark at least one checkbox to indicate the type(s) of information received since the Date of Last IRB Review.**
- **Please summarize each type of information, and provide details and copies as requested.**

7.a. You received multi-center trial reports that you have not previously forwarded to the IRB. Attach a copy and, in the space below, provide the date and source of report, and summarize the findings and any recommendations:	☐ Yes x No Multi-Center Trial Report
▶	
7.b. You received data and safety or other monitoring reports (e.g., DSMB, sponsor site visit). Even if you have already forwarded a copy to the IRB, attach a copy and, in the space below, provide the date and source of report, and summarize the findings and any recommendations:	☐ Yes x No Data Safety or Other Monitoring Report
▶	

7.c. You learned of literature published about this research. Attach the publication or provide its web address, and summarize the published findings here:	☐ Yes x No Published Literature
7.d. You learned of other relevant information regarding this research, especially about risks associated with the research. Attach a copy of the source and/or summarize below, and check "Other Information" at right. Check "Affects Willingness" also if this information might affect a participant's willingness to continue in the research, and describe the effects on participants here:	☐ Yes x No Other Information ☐ Yes x No Affects Willingness
7.e. You have received another type of information. Summarize the information here, including details relevant to participants:	☐ Yes x No Other Type of Information

8. Events Since the Date of Last IRB Review

Mark at least one checkbox to show event(s) that have occurred since the Date of Last IRB Review. Please summarize all events, and provide specific details and/or copies as requested.

8.a. One or more "reportable events" have occurred, which may constitute unanticipated problems involving risks to participants or others. Attach UAB Problem Report even if already reported to the IRB; attach UAB Problem Summary Sheet; provide brief narrative summary (2-3 sentences) of any trends or increases in frequency or severity noted, or enter "None noted" here:	☐ Yes x No Reportable Events (Table A)
8.b. Participants have experienced harms (expected or unexpected, serious or not serious) that do not meet the UAB IRB criteria for "reportable events." Attach UAB Problem Summary Sheet; provide brief narrative summary (2-3 sentences) of any trends or increases in frequency or severity noted, or enter "None noted" here:	☐ Yes x No Other Events (Table B)
8.c. You have had one or more problems obtaining informed consent. Briefly describe the problems here:	☐ Yes x No Consent Problems
8.d. You have received complaints about the research. Briefly describe the number and nature of the complaints:	☐ Yes x No Complaints

8.e. One or more participants withdrew, or were withdrawn from, the research.
Indicate here the number of withdrawals and the reason for each:

x Yes ☐ No
Withdrawals

▶ 2 total withdrawn: #1-the PI withdrew due to could not tolerate the Glycine supplement needed in the protocol, #1-the participant withdrew due to personal reasons at home.

8.f. Participants have experienced research-related benefits.
For example, "60% of participants in the treatment group appear to have reduced symptoms or reduced severity of symptoms, compared with 10% in the placebo group."
Briefly describe the benefits here:

x Yes ☐ No
Benefits

▶ The participants will not benefit directly from this study except possibly from the physical exam and clinical laboratory studies.

8.g. The risks, potential benefits, or both of this research have changed.
Briefly describe the changes here:

☐ Yes x No
Change in Risk or Benefit

▶

8.h. Events have occurred that relate to participant safety but do not fit into the categories listed above.
Briefly describe the events here:

☐ Yes x No
Other Events

▶

9. Protocol and/or Informed Consent Modifications

Check the applicable boxes to indicate modifications made since Date of Last IRB Review (Yes to 9.a.) or requested with this renewal (Yes to 9.b.). Please provide the details and materials requested.

9.a. Previous Modifications

Since the last IRB review, have you made modifications to the protocol, consent process, or consent document?
If Yes, have the modifications been approved by the IRB?

xYes ☐No

xYes—Provide a copy of each amendment form stamped "Approved" by the IRB during this approval period.
☐No—In the space below, justify making the modification without prior IRB approval:

▶

9.b. Modifications Requested With This Renewal

Are you requesting IRB review of changes to the protocol (e.g., procedures, personnel, recruitment)? If so, check "Yes" and describe them in the space below. If adding personnel, indicate role in research, provide full name and UAB department/division, and address conflict of interest.

x Yes ☐ No
Protocol Changes

▶ Please remove Sandra Krause, RN and Kerry Lok, Research Assistant, as personnel on this protocol.

Are you requesting IRB review of changes to the consent process and/or form(s)? If so, check the applicable "Yes"

☐ Yes x No
Consent Process Changes

box and, in the space below, describe the changes.	☐ Yes x No Consent Document Changes

If the changes affect the consent form(s), indicate the number of consent-assent forms used for this protocol, and describe the changes to each form:
(a) describe all changes to IRB-approved forms and the reasons for them;
(b) describe the reasons for the addition of any materials (e.g., addendum consent); and
(c) indicate either how and when you will reconsent enrolled participants or why reconsenting is not necessary.

Also, indicate the number of forms changed or added. For new forms, provide 1 copy. For revised documents, provide 3 copies:
- **a copy of the currently approved document (showing the IRB approval stamp, if applicable)**
- **a revised copy highlighting all proposed changes with "tracked" change**
- **a revised copy for the IRB approval stamp.**

▶

10. Gene Therapy, Gene Transfer, Recombinant DNA

If this study involves	☐ Gene therapy ☐ Gene transfer ☐ Recombinant DNA	☐ None of these
	Complete this item, and include memorandum with original signatures of Gene Therapy Review Panel addressing the risk-benefit ratio, any recommendations, and the CRL if applicable.	**Go to Item 11.**

10.a. Has the Panel's assessment of the risk-benefit ratio of this project changed? If yes, please explain below.	☐ Yes ☐ No Risk-Benefit Change

▶

10.b. Does the Panel have any recommendations regarding the protocol or the consent form? If yes, please explain below.	☐ Yes ☐ No Panel Recommendations

▶

Note. **If the research involves minors (<19 years old), the panel's memo must either confirm the previously assigned CRL number or reassign it and give the reasons it has changed.**

11. Continuing Review—Complete only if you want to renew IRB approval so that protocol-related activities can continue.

11.a. Accrual Status—Indicate whether the study is "NOT YET OPEN," "OPEN," or "CLOSED" (described below) and provide the details requested for that accrual status.

NOT YET OPEN: No individuals have been screened or entered.	☐ Not Yet Open
OPEN: The study could still enroll more individuals, add more specimens, review more records, etc. • **Attach a copy of the most recently approved consent form(s) OR note in the space below that the IRB has waived informed consent and/or use of a consent form.**	x Open

- **Describe plans for future accrual and/or enrollment here:**

▶ Continue to enroll and analyze data as it becomes available & progress towards the Place/Crossover Phase.

CLOSED: No more individuals will be enrolled, no more specimens or records will be added.	☐ Closed
If the study is closed, is a consent form being submitted for review? If "Yes," explain why in the space below.	☐ Yes ☐ No Closed & Consent Form
• **Indicate the date closed to accrual:**	Date Closed
• **Choose one status to describe accrued participants, specimens, records:**	Check **ONE** Status Below:
One or more is still receiving procedures as defined in the protocol (therapy, intervention, follow-up visits, etc.)	☐ On protocol procedure
All are off protocol-driven procedures, in long-term follow-up only	☐ In long-term follow-up
All are off protocol-driven procedures, in data analysis only	☐ In data analysis

▶

11.b. Describe any interim findings from this research. Please note that the IRB expects to receive findings on any protocol approved for 5 years.

▶ Pending analysis of laboratory findings.

12. Final Report—Complete only if you want to end IRB approval after all protocol-related data analyses are complete and no further work on the protocol will be done.

12.a. On what date were the final data analyses completed?	Final Date

12.b. Summarize the final findings from this protocol:

▶

12.c. Who will be responsible for managing and storing the data records, including any and all research-related electronic files and paper documents?

Name	
UAB Dept/Div, or Employer	
Work Address	
Daytime Telephone	

12.d. Describe the storage plan. How will data records be stored—on paper, computers, or both? How will they be protected from damage, unauthorized release, loss, and theft? How long will the data be stored?

▶

12.e. At the end of the storage period, will the data records be destroyed, archived, or transferred? Describe the plan in detail.	☐ Destroy ☐ Archive ☐ Transfer

▶

Note. **Specimens may be stored only if/as described in the IRB-approved protocol. Data records must be stored as described in the sponsor's protocol or contract if applicable, and/or in the UAB Health System Record Retention Policy. Anyone wishing to use these data or specimens for secondary research purposes or for purposes preparatory to secondary research must obtain prior IRB review and approval.**

Signature of Principal Investigator: _____

Date: _____

FOR IRB USE ONLY – Expedited Review

Change to Expedited Category Y / N

No change to IRB's previous determination of approval criteria at 45 CFR 46.111 or 21 CFR 56.111 ☐

_____

_____

_____

Signature (Chair, Vice-Chair, Designee)

Date

INITIAL TELEPHONE RECRUITMENT INFORMATION

Date:_____ **Study:**
_____

Source: Radio, Flyer, Newspaper, Reporter, Other _____ **Veteran**
_____

Initials _____ **Gender:** M F **Age:** _____ **Ht** _____ (ft/in)
Wt_____ (lbs)

Pregnant ?_____ Lactating? _____ Date participated in last study
_____

Medical History: Diabetes?

Heart Disease, Kidney Disease, Liver Disease, Stroke, CA, HTN, Seizures, Pancreatitis

Other?_____
_____

Medications: (Oral, Injectables,Inhalers, OTC, Vits, etc.)
_____

_____
_____

_____
_____

_____
_____

Allergies: NKDA
_____

If diabetic: Diagnosis Date _____ Actual years _____ Age of onset
_____

HBGM? Yes No FBS:_____ Glucose Meter Type:_____

Diabetes Medication Insulin?

_____
_____

_____
_____

_____
_____

Are you afraid of needles? _____
Is there any difficulty in drawing blood from your arm? _____

Do you exercise regularly?
_____

PHONE (H) _____ (0) _____ (Cell)
_____

COMMENTS:

_____

_____

_____

_____

_____

TITLE OF RESEARCH: Glycine supplementation to improve insulin sensitivity in humans

IRB PROTOCOL NUMBER: F120831002

INVESTIGATOR: W. Timothy Garvey, M.D.

SPONSOR: UAB Department of Nutrition Sciences

INTRODUCTION:

You are invited to be a participant in this study because you have one or more characteristics consistent with insulin resistance--obesity, impaired glucose tolerance (abnormal handling of a sugar load), high cholesterol or triglycerides (fats in the blood), and high blood pressure. In order to decide whether you wish to be a part of this research study you should know enough about its risks and benefits to make an informed decision. This consent form gives you detailed information about the research study that a member of the research team will discuss with you. This discussion should go over all aspects of this research including its purpose, the procedures that will be performed, any risks of the procedures, possible benefits, and possible alternatives. You will be asked if you wish to participate: if so, you will be asked to sign this form.

PURPOSE:

Insulin resistance can be detected prior to the development of Type 2 Diabetes and is a key risk factor for future diabetes. Approximately 30% of individuals in the US are estimated to have the insulin resistance syndrome. Insulin moves glucose (sugar) from the bloodstream into the muscle, where it is metabolized (used by the body). In many individuals, this process is impaired which increases the risk of developing diabetes, hypertension (high blood pressure), heart disease, and stroke (associated with hardening of the arteries). The investigator hopes to better understand the actual biochemical cause of this insulin resistance since this knowledge could be used to develop improved methods of treatment or prevention.

DESCRIPTION:

We expect about 20 participants to be eligible to participate in the study that will take place at the University of Alabama at Birmingham (UAB

EXPLANATION OF PROCEDURES:

This study consists of the following four phases: Pre-Phase, Phase 1, Phase 2, and Phase 3.

1. The following will take place during the Pre-Phase visit. This visit will take 45 minutes to 1 hour and15 minutes to complete.

    a. You will be asked questions regarding your overall health history (personal and family). A physician will perform a physical examination (vital signs, height, weight, BMI, waist/hip circumference) and EKG (a test that checks for problems with the electrical activity of your heart).

    b. You will have a venipuncture (needle stick) to obtain fasting blood samples for routine testing (electrolytes, complete blood count, thyroid level, liver/kidney function). We will use these test results to evaluate your general health status. A pregnancy test will also be performed if you are a female capable of becoming pregnant. The total amount of blood drawn for these tests will not exceed 30cc (2 tablespoons).

2. Once it is determined that you qualify for this study based on the tests above, you will come back in and the following will be take place during the Phase 1 visit. This visit will take approximately ½ day.

    a. You will be given Glycine (a nutritional supplement powder) and instructed to add 1 teaspoons (5 grams) dissolved in water and consumed with each of 3 meals for a total of 15 g/day to your usual diet. You will remain on Glycine for a 2 week period during Phase 1.

Glycine has a mild sweet taste. Glycine is the simplest and most widely available amino acid in the body. Amino acids are the building blocks of the proteins that are found in our bodies. It is also found in many high-protein foods, such as fish, meats, and dairy products.

b. A urine specimen will be collected for routine analysis. Then for a 24 hour period you will collect your urine, keeping all of it in a refrigerated jug. Urine protein, creatinine, albumin, urea nitrogen, c-peptide, and total volume will be measured to check your kidney function.

c. You will have a fasting oral glucose tolerance test (OGTT) performed. By way of a venipuncture (needle stick), a catheter (plastic tube) will be inserted in your arm vein and taped into place so that blood samples can be drawn through this tube

and used for testing on blood sugar and insulin levels. You will drink a beverage containing 75 grams of glucose (sugar). From the catheter that was placed in your arm vein, blood samples will be drawn over a period of 3 hours. The total amount of blood drawn for this test will not exceed 105cc (7 tablespoons).

d. You will have Anthropometric measurements performed. These measurements include thigh, waist, stomach, arm, chest, and leg circumference.

e. You will have a calorimetry test to determine how much energy your body uses and how your body metabolizes sugar and fat. You will have a 30 minutes rest period in bed. Then you will be asked to place your head in a special clear plastic ventilated hood. You will lay flat on your back and breathe normally. You will stay as still as possible under the hood for 30 minutes. The amount of oxygen you consume and the amount of carbon dioxide you produce will be measured from samples of air you exhale. If you become claustrophobic, you can easily remove the hood.

3. After 1 ½ to 2 weeks, you will come back in and the following will be performed during the Phase 2 visit. This visit should take about 30-35 minutes.

   a. You will have a venipuncture (needle stick) to obtain fasting glucose and insulin samples after completing 2 weeks of consuming Glycine (nutritional supplement powder). The total amount of blood drawn for this test will not exceed 30cc (2 tablespoon).

   b. A physician will perform a physical examination (vital signs, height, weight, BMI, waist/hip circumference).

   c. You will remain on the Glycine and continue to take (as previously instructed) for another 2 week period during Phase 2.

4. After 1 ½ to 2 weeks, you will come back in and the following will take place during the Phase 3 visit. This visit will take approximately ½ day.

   a. A urine specimen will be collected for routine analysis. Then for a 24 hour period you will collect your urine, keeping all of it in a refrigerated jug.

Urine protein, creatinine, albumin, urea nitrogen, c-peptide, and total volume will be measured to check your kidney function.

b. You will have a fasting oral glucose tolerance test (OGTT) performed. By way of venipuncture (needle stick), a catheter (plastic tube) will be inserted in your arm vein and taped into place so that blood samples can be drawn through this tube and used for testing on blood sugar and insulin levels. You will drink a beverage containing 75 grams of glucose (sugar). From the catheter that was placed in your arm vein, blood samples will be drawn over a period of 3 hours. The total amount of blood drawn for this test will not exceed 105cc (7 tablespoons).

c. You will have Anthropometric measurements performed. These measurements include thigh, waist, stomach, arm, chest, and leg circumference.

d. You will have a calorimetry test to determine how much energy your body uses and how your body metabolizes sugar and fat. You will have a 30 minutes rest period in bed. Then you will be asked to place your head in a special clear plastic ventilated hood. You will lay flat on your back and breathe normally. You will stay as still as possible under the hood for 30 minutes. The amount of oxygen you consume and the amount of carbon dioxide you produce will be measured from samples of air you exhale. If you become claustrophobic, you can easily remove the hood.

The total time expected to complete all of the phases described in this study will be about 4-5 weeks.

RISKS AND DISCOMFORTS:

Participation in this study may involve some risks or discomforts.

Glycine supplementation may cause digestive symptoms such as soft stool, stomach upset and mild abdominal pain.

The risks and discomforts associated with venipuncture include pain, bruising, and possible infection. The total amount of blood drawn during this entire study should not exceed 240cc (16 tablespoons).

Insertion of a plastic catheter in your vein will cause some pain when your arm is stuck with the needle. You could feel dizzy or faint. You could also experience minor discomfort having the catheter taped to your arm. A bruise may be left temporarily at the spot where your arm is stuck. There is a slight chance of infection, inflammation of your vein, and/or a blood clot formation; however, these risks are extremely rare.

During the oral glucose tolerance test (OGTT) you may experience headache, nausea, weakness, feeling light-headed, sweating, anxiety, hunger, or other symptoms. **If this happens, be sure to tell the person performing the test**. You will be monitored throughout the test to make sure that your blood glucose level does not drop too low**.**

Prior to each study visit, you will be required to fast overnight. This means that you will not eat or drink anything except water for 10 hours before the test begins in the morning and will remain fasted until the testing is completed. Possible side effects of fasting may include feelings of hunger, light headedness, and a drop in your blood sugar.

There are no known risks of the study procedures and Glycine consumption to a developing fetus. However, if you are a female capable of becoming pregnant, you must agree to use birth control (oral birth control pills or barrier method) for the duration of the study. You will be excluded if your pregnancy result is positive.

BENEFITS:

The investigator will inform you of all the results from the blood and urine tests and examinations. Otherwise, there will be no direct benefit to you for participating in this study. The investigator will learn more about the mechanisms that cause insulin resistance in human diseases such as high blood pressure, obesity, and diabetes. This knowledge may be useful in designing new effective therapies for treatment and prevention; therefore, society in general may benefit.

ALTERNATIVES:

The alternative is that you may choose not to participate in this research study.

CONFIDENTIALITY:

The information gathered during this study will be kept confidential to the extent allowed by law. However, the investigator, the research staff, the Office for

Human Research Protection (OHRP), the US Food and Drug Administration (FDA), and UAB's Institutional Review Board (IRB) will be able to inspect your records and have access to confidential information that identifies you by name. The results of the study may be published for scientific purposes; however, your identity will not be given out.

Information relating to this study, including your name, medical record number, date of birth, and social security number may be shared with the billing offices of UAB and UAB Health System—affiliated entities so that claims may be appropriately submitted to the study sponsor or to your insurance company for clinical services and procedures provided to you during the course of this study.

If any part of this study takes place at University of Alabama Hospital, this consent document will be placed in your file at that facility. The document will become part of your medical record chart.

You will be given information regarding your test results. With your permission, we will also share this information with your primary care physician.

VOLUNTARY PARTICIPATION AND WITHDRAWAL:

Whether or not you take part in this study is your choice. There will be no penalty if you decide not to be in the study. If you decide not to be in the study, you will not lose any benefits you are otherwise owed. You are free to withdraw from this research study at any time. Your choice to leave the study will not affect your relationship with this institution.

Dr. Garvey or a member of his research team may also choose to stop your participation in this study at any time, if it is decided to be in your best interest or if you do not follow instructions.

If you are a UAB student or employee, taking part in this research is not part of your UAB class work or duties. You can refuse to enroll, or withdraw after enrolling at any time before the study is over, with no effect on your class standing, grades, or job at UAB. You will not be offered or receive any special consideration if you take part in this research.

SIGNIFICANT NEW FINDINGS:

Dr. Garvey or his staff will notify you if any significant new findings develop during the course of this study that may affect your willingness to continue your participation in this research study.

COST OF PARTICIPATION:

There will be no cost to you from participation in this research study. The Glycine supplement and all study related tests and procedures will be provided at no cost. The costs of standard medical care will be billed to you/and or your insurance.

PAYMENT FOR PARTICIPATION IN RESEARCH:

You will receive $50 for the Phase 1 visit, $25 for the Phase 2 visit, and $75 for the Phase 3 visit. If you complete the entire study, you will receive a total of $150.00. If you drop out of the study before it is finished, you will be compensated for all completed visits.

PAYMENT FOR RESEARCH RELATED INJURIES:

UAB has not provided for any payment if you are harmed as a result of taking part in this study. If such harm occurs, treatment will be provided. However, this treatment will not be provided free of charge.

QUESTIONS:

If you have any questions about the research or a research related injury, W. Timothy Garvey, M.D. will be glad to answer them. Dr. Garvey's telephone number is (205) 934-6103. He may also be reached after hours by paging him at(205) 934-3411 (beeper #5358).

If you have questions about your rights as a research participant, or concerns or complaints about the research, you may contact the Office of the Institutional Review Board for Human Use (OIRB) at (205) 934-3789 or 1-800-822-8816. If you are calling the toll-free number, press the option for "all other calls" or for an operator/attendant and ask for the extension 4-3789. Regular hours for the Office of the IRB are 8:00 a.m. to 5:00 p.m. CT, Monday through Friday. You may also call this number in the event the research staff cannot be reached or you wish to talk to someone else.

STORAGE OF SPECIMENS FOR FUTURE RESEARCH:

Any blood (plasma and serum) sample obtained for research purposes through the procedures previously explained will be stored frozen in the Department of Nutrition Sciences at UAB and used for future research studies on insulin resistance, obesity, diabetes, and risk of cardiovascular disease**.** These tests are being done for research purposes only. You and your doctor will not be informed of the results. Your samples will be banked indefinitely or until no sample remains. These samples will be identified using a code number that can be linked to you. Measures are taken to protect the confidentiality of your samples. At any time, your samples will be destroyed upon your request. If you decide you want

your samples removed, you may contact Dr. Garvey at (205) 934-6103. The specimens obtained from you in this research may help in the development of a future commercial product. There are no plans to provide financial compensation to you should this occur.

Your biological samples and/or data collected from any of the procedures discussed above may be sent to researchers at other institutions who collaborate on similar studies. Your name or any other personal identifier will not be used. The sample and any data sent will only be labeled with coded numbers for purposes of identification.

The other investigators would not know your name and would not be able to link any information to you.

You do not have to agree to allow your blood specimens to be stored in order to be part of this study.

Please initial your choices(s) below:

_____I agree to allow my samples to be stored and used at UAB for future research on
insulin resistance, obesity, diabetes, and risk of cardiovascular disease.

_____ I agree to allow my stored samples to be shared with collaborating researchers at other institutions.

_____ I DO NOT agree to allow my samples to be stored and used for future research.

LEGAL RIGHTS:

You are not waiving any of your legal rights by signing this consent form.

SIGNATURES:

You will be given a chance to ask questions about this research study. All of your questions should be answered to your satisfaction. You should also feel free, at a later date, to contact Dr. Garvey or a member of his research team if you have any more questions.

Your signature below indicates that you agree to participate in this study. You will receive a signed copy of this informed consent.

_____

Signature of Participant
Date

_____

Signature of Investigator or Person Obtaining Consent
Date

_____

Signature of Witness
Date