Original Communication

Glycine Propionyl-L-carnitine Modulates Lipid Peroxidation and Nitric Oxide in Human Subjects

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Abstract: *Objective:* To determine the efficacy of glycine propionyl-L-carnitine (GPLC) to decrease lipid peroxidation, elevate nitric oxide, and improve blood lipid profiles in human subjects. *Methods:* Thirty untrained, normolipidemic subjects performed eight weeks of supervised aerobic exercise while supplementing GPLC at one of two doses (1 or 3 grams daily of PLC + glycine) or placebo, following random assignment in a double-blind manner. Fasting blood samples were analyzed at rest for malondialdehyde, nitric oxide, and lipids before and after the intervention. *Results:* Malondialdehyde was decreased ($p<0.05$) from pre- to post-intervention with 1 g GPLC (1.08 ± 0.24 vs. 0.69 ± 0.25 $\mu mol \cdot L^{-1}$) and 3 g GPLC (0.94 ± 0.18 vs. 0.66 ± 0.17 $\mu mol \cdot L^{-1}$), but did not change statistically ($p>0.05$) with placebo (1.12 ± 0.21 vs. 1.03 ± 0.23 $\mu mol \cdot L^{-1}$). Nitric oxide was increased ($p<0.05$) from pre- to post-intervention with 3 g GPLC (21.34 ± 2.27 vs. 29.46 ± 3.61 $\mu mol \cdot L^{-1}$), but did not change statistically ($p>0.05$) with 1 g GPLC (23.22 ± 4.13 vs. 26.24 ± 4.32 $\mu mol \cdot L^{-1}$) or placebo (24.31 ± 3.90 vs. 26.14 ± 4.11 $\mu mol \cdot L^{-1}$). No main effects or interaction effects were noted for blood lipids ($p>0.05$). *Conclusion:* GPLC supplementation combined with eight weeks of aerobic exercise decreases lipid peroxidation and elevates nitric oxide, but does not further improve blood lipid profiles in normolipidemic subjects.

Key words: Carnitine, exercise, lipids, lipid peroxidation, malondialdehyde, antioxidant, nitric oxide

Introduction

In the United States it is estimated that more than 71 million people have one or more forms of cardiovascular disease (CVD) [1], resulting in a large economic burden and a significant number of deaths annually. A large portion of these conditions involve arterial disease, which is a progressive disorder characterized by the accumulation of lipids and dead cellular debris in the artery walls [2]. One of the likely precursors to atherosclerosis is dyslipidemia, a condition characterized by abnormal blood lipid levels typically involving elevated total and low-density lipoprotein (LDL) cholesterol and triglycerides, in addition to decreased high-density lipoprotein (HDL) cholesterol [3]. An equally important concern

is the oxidation of lipids, which appears to contribute to both the initiation and progression of atherosclerotic disease [4,5]. Nitric oxide, an important signaling molecule promoting vasodilation by acting on vascular smooth muscle, also plays a major role in vascular function [6].

Management of dyslipidemia, lipid peroxidation, and vasoreactivity is often done via use of pharmacological intervention. However, non-pharmacological and lifestyle approaches have been used with mixed success. Both dietary modification and aerobic exercise appear to be the first-line approach to management of arterial disease when medication is not chosen. In addition, a variety of nutritional supplements have been suggested to provide benefit in improving blood lipids, acting as antioxidants to decrease lipid peroxidation, and possibly acting as vasodilators to allow for enhanced circulation.

In this regard, the dietary nutrient carnitine has been used in both animals and humans with success. Carnitine is a natural component of mammalian tissue and is a necessary factor in the utilization of long-chain fatty acids within the mitochondria for energy production [7]. In theory, greater carnitine availability *in vivo* could lead to greater utilization of fatty acids as a fuel source, and over time improve lipid profiles as a result. Studies in which subjects with known disease (e.g., CVD and/or dyslipidemia) have used supplemental carnitine have noted favorable results in relation to blood lipids [8–16]. In particular, a significant lowering of triglycerides has been noted. No study to our knowledge has used otherwise healthy, normolipidemic subjects (i.e., total cholesterol <200 mg·dL^{-1}) in an attempt to further improve lipid profiles.

Aside from the potential positive effects on blood lipids, carnitine has also been reported to have antioxidant properties in both animal [17–21] and man [22–24]. These effects appear mediated by a reduction in xanthine oxidase activity [19], a free-radical scavenging activity [21], and/or a regulation of metabolic reactions [20]. Moreover, the propionyl ester of L-carnitine, propionyl-L-carnitine (PLC), has been noted recently to increase blood nitric oxide production in a population of patients with peripheral arterial disease [25].

Propionyl-L-carnitine appears to have the highest affinity for carnitine acetyltransferase and possesses protective effects against reactive oxygen species (ROS)-induced oxidation [20]. This form of carnitine has recently been combined with the amino acid glycine in a unique molecular bonded form called glycine propionyl-L-carnitine (GPLC). Previous reports indicate that glycine independently promotes positive effects on lipid peroxidation [26,27], vasodilation [28], and blood lipids [29,30]. It is unknown whether the combination of glycine and PLC would foster synergistic effects on these parameters. Therefore, the purpose of this investigation was to determine the efficacy of GPLC to decrease lipid peroxidation, elevate nitric oxide, and improve blood lipid profiles in human subjects.

Materials and methods

Subjects

Thirty-two subjects between the ages of 18 and 44 were enrolled for participation in this study and completed all phases of the intervention. Subjects were classified as untrained, meaning that they had not participated in structured physical activity for a minimum of six months prior to enrollment in the study. Prospective subjects were excluded if they were current smokers, currently taking antioxidant supplements, or if they had any orthopedic, cardiovascular, or metabolic problems that would affect their ability to perform sub-maximal and maximal exercise. The subjects' eligibility was determined by verbal self report as well as the completion of detailed health history, drug and dietary supplement usage, and physical activity questionnaires. After being informed of the procedures, potential risks, and benefits associated with the study, subjects were asked for both written and verbal informed consent based on the procedures approved by the Institutional Review Board for the use of Human Subjects of the University of Memphis (protocol approval # H05-88-01).

Baseline testing and graded exercise test

During the first visit to the lab, subjects underwent various anthropometric assessments (e.g., height, weight, body fat percentage) and familiarization sessions for the equipment that was used in subsequent testing. Resting heart rate and blood pressure measures were taken after ten minutes of rest in an isolated room.

After the initial visit was completed, the subjects reported to the lab in the morning after an overnight fast of at least eight hours. No strenuous physical activity was to be performed by the subjects for at least 48 hours before the testing session. The subjects

underwent a maximal graded exercise test (GXT) while expired gases were collected and analyzed using a SensorMedics Vmax 229 metabolic system (Viasys Healthcare, Yorba Linda, CA) for determining peak oxygen consumption (VO_{2peak}). The treadmill test consisted of the standard Bruce protocol, in which all subjects exercised until exhaustion. The maximal heart rate achieved during this GXT was used to design the individualized exercise prescriptions for the subjects. The GXT, in addition to measurement of all anthropometric variables, was conducted again following the eight-week intervention period in order to determine potential changes in these variables.

Supplementation

Following the conclusion of the above initial tests, subjects were provided with study instructions and were randomized in double-blind manner to one of following three conditions plus aerobic exercise: Placebo (n=10); GPLC at 1 g·day^{-1} (n=10); GPLC at 3 g·day^{-1} (n=12). The GPLC is a USP grade nutritional supplement consisting of a molecular bonded form of PLC and the amino acid glycine (GlycoCarn™, Sigma-tau HealthScience S.p.A, Rome, Italy). The dosage of PLC was provided at either 1 or 3 g·day^{-1}. The glycine content was equal to 348 mg in the 1 g·day^{-1} treatment and 1044 mg in the 3 g·day^{-1} treatment. For ease of reporting throughout this manuscript, we refer to the two dosages as simply 1 and 3 g·day^{-1} to reference the actual PLC content. Capsules were provided to subjects in unlabeled bottles every two weeks. All capsules were identical in appearance. In all conditions subjects ingested 6 capsules daily at two separate times (morning and evening) to allow for the above dosages. Following randomization all subjects began the aerobic exercise program as described below.

Aerobic exercise program

All subjects performed eight weeks of supervised aerobic exercise (stationary cycling and walking/jogging) in the Cardiorespiratory/Metabolic Laboratory at the University of Memphis. The training intensity and duration began at the lower limit of the American College of Sports Medicine (ACSM) recommendation (55% heart rate reserve for 30 minutes) and progressed to higher levels over the eight-week period (85% heart rate reserve for 45 minutes). The training intensity was given to subjects in the form of a "target" heart rate range. The specific numbers were based on the maximal heart rate achieved during their GXT, in addition to their resting heart rate. Heart rates (via Polar heart rate monitors) and ratings of perceived exertion (RPE) were recorded at three times during each exercise session by research assistants to assure that subjects were training at the appropriate intensity. Exercise logs were also maintained by research assistants who supervised each exercise session. Throughout the study period subjects were reminded of their "target" heart rate range and their target RPE, and the importance in achieving these numbers was stressed.

Blood collection and biochemistry

Fasting venous blood samples amounting to about 20 mL were taken from subjects via needle and vacutainer on the morning of the GXT, both before and after the intervention. These blood draws occurred after a 10-minute rest period. Following collection, blood samples were processed accordingly and the plasma/serum for analysis of malondialdehyde, nitric oxide, and lipids was immediately stored at -80°C until analyzed. Blood was also collected and processed for analysis of complete blood count (CBC) and metabolic panel data, and transported to Laboratory Corporation of America for analysis within 24 hours of collection using automated clinical analyzers (CBC—Coulter LH750; metabolic panel— Roche/Hitachi Modular).

Malondialdehyde was analyzed in plasma following the procedures of Jentzsch et al [31] using reagents purchased from Northwest Life Science Specialties (Vancouver, WA). Specifically, 250 μL of sample were added to microcentrifuge reaction tubes with the addition of 10 μL of butylated hydroxytoluene (BHT) in methanol to minimize *ex vivo* lipid peroxidation. 250 μL of 1 M phosphoric acid and 250 μL of 2-thiobarbituric acid reagent was added to each reaction tube and mixed thoroughly. Samples and reagents were incubated for 60 minutes at 60°C in a water bath. Following incubation, tubes were removed and immediately centrifuged at 10,000 x g at 4°C for 5 minutes. The reaction mixture was transferred to a cuvette and the absorbance read using a spectrophotometer at both 535 and 572 nm to correct for baseline absorption. Malondialdehyde equivalents were calculated using the difference in absorption at the two wavelengths. Quantification was per-

formed with a calibration curve using tetramethoxypropane in a stabilizing buffer.

Nitric oxide was analyzed in plasma using a commercially available assay kit (Caymen Chemical, Ann Arbor, MI). Following conversion of nitrate to nitrite using nitrate reductase, Greiss reagent was added which converts nitrite into a deep purple azo compound. The absorbance was then detected photometrically at 540 nm.

Assays for total cholesterol, HDL cholesterol, and triglycerides were performed using plasma following standard enzymatic procedures using a microplate assay as described by the reagent manufacturer (Thermo Electron Clinical Chemistry, Louisville, CO). Standard curves for all assays were developed for determination of unknown samples, and known normal and abnormal samples were used for quality control purposes. Prior to HDL cholesterol analysis, precipitation of apolipoprotein B containing lipoproteins [very low-density lipoprotein (VLDL), LDL, and Lp(a)] was performed using phosphotungstic acid, coupled with centrifugation, and HDL cholesterol was measured in the supernatant. LDL cholesterol was calculated using the Friedwald equation as follows: LDL cholesterol = TC – HDL cholesterol – (triglycerides/5). All assays were performed in triplicate on first thaw.

Dietary records

All subjects were instructed to maintain their normal diet during the study period and to complete food records to allow for nutrient intake assessment during the first and eighth week of intervention. Therefore, subjects completed seven-day records both at the start and conclusion of the intervention period. Subjects were given specific instructions regarding the recording of portion sizes and quantities, in addition to viewing food models in order to enhance precision. Upon return of the records, research assistants reviewed each entry with subjects to confirm accuracy. These procedures were followed according to the instructions of a Department Registered Dietician. Records were analyzed for total calories, protein, carbohydrate, fat, vitamin C, vitamin E, and vitamin A (Diet Analysis Plus, version 5.0, ESHA Research, Salem, OR).

Statistical analysis

All dependent variables were analyzed using a 3 (condition) x 2 (pre- and post-intervention) ANOVA. Significant interactions and main effects were further analyzed using Tukey's *post hoc* tests. All analyses were performed using JMP statistical software (SAS Institute, version 4.0.3, Cary, NC). Statistical significance was set at $p \leq 0.05$. The data are presented as mean ± SEM.

Results

Of the 32 subjects completing the intervention, two subjects were excluded from the data analysis due to baseline hyperlipidemia (total cholesterol >200 mg·dL^{-1}). This was done in an attempt to maintain a homogenous sample of normolipidemic subjects. The subjects remaining in the analyses were assigned to the placebo condition (n=9), 1 gram GPLC condition (n=10), and 3 gram GPLC condition (n=11). Compliance was measured for supplement intake, percentage of training completed, and percentage of target heart rate obtained. The percent of supplement compliance (assessed via capsule counts upon bottle return every two weeks) for the placebo group, the 1 gram GPLC group, and 3 gram GPLC group was 97.3 ± 2.6, 94.4 ± 2.4, and 95.5 ± 2.5 respectively. The percentage of training completed (session number) in the placebo group was 85.5 ± 3.3, while the 1 gram GPLC group and 3 gram GPLC group completed 90.7 ± 2.9 and 87.7 ± 3.1 percent of the training, respectively. The percentage of target heart rates within the prescribed intensity range was as follows: placebo group: 92.0 ± 5, the 1 gram GPLC group: 93.9 ± 4.5, and the 3 gram GPLC group: 88.8 ± 4.7. No statistical differences were noted between conditions for supplement compliance ($p = 0.7175$), percentage of training completed ($p = 0.4978$), or percentage of target heart rate obtained ($p = 0.7357$).

Descriptive and dietary variables

No statistical interactions or main effects were noted for any descriptive variable ($p > 0.05$). Data are shown in Table I. No statistical interactions or main effects were noted for any dietary variable ($p > 0.05$), with the following exceptions. The vitamin E intake of the placebo condition was lower than both GPLC conditions ($p = 0.027$), while the total kilocalorie intake of the 1 gram GPLC condition was si-

Table I: Descriptive characteristics of subjects before and following an eight-week intervention of aerobic exercise and placebo or GPLC supplementation

Variable	Placebo Pre	Placebo Post	1 g·day^{-1} GPLC Pre	1 g·day^{-1} GPLC Post	3 g·day^{-1} GPLC Pre	3 g·day^{-1} GPLC Post
Age (yrs)	28.25±1.98	28.25±1.98	26.10±2.21	26.2±2.19	26.56±1.38	26.67±1.33
Height (cm)	167.31±2.74	167.31±2.74	168.7±3.80	168.7±3.80	168.33±2.32	168.33±2.32
Weight (kg)	77.25±7.95	77.50±8.05	76.92±5.64	76.32±5.71	69.56±5.81	68.81±5.31
Body fat (%)	25.30±1.44	24.06±1.15	25.76±2.70	23.58±2.48	27.13±2.49	25.64±2.09
BMI (kg·m^{-2})	27.24±2.06	27.32±2.09	26.72±1.28	26.52±1.35	24.47±1.83	24.21±1.61
Waist to Hip	0.787±0.027	0.774±0.027	0.776±0.026	0.783±0.026	0.756±0.015	0.746±0.012
Rest Heart Rate (bpm)	65.5±2.64	64.00±4.28	69.10±1.98	67.40±3.61	70.44±2.80	68.00±3.44
Resting SBP (mm Hg)	113.50±3.02	111.50±4.84	114.40±3.51	111.80±3.09	113.78±1.81	109.78±3.29
Resting DBP (mm Hg)	69.50±3.60	68.00±4.66	73.90±2.88	69.20±2.64	71.56±3.40	72.44±2.74
VO_{2peak} (ml·kg^{-1}·min^{-1})	31.25±1.70	32.44±1.37	31.02±2.98	33.64±2.94	29.01±2.47	30.63±2.51

No statistically significant differences were noted for any variable from pre- to post-intervention or between conditions ($p > 0.05$). Values are mean±SEM.

Table II: Dietary data of subjects during weeks one and eight of an eight-week intervention of aerobic exercise and placebo or GPLC supplementation

Variable	Placebo Pre	Placebo Post	1 g·day^{-1} GPLC Pre	1 g·day^{-1} GPLC Post	3 g·day^{-1} GPLC Pre	3 g·day^{-1} GPLC Post
kcal	1819±135	1983±234	2212±177*	2077±167	*1808±175	1620±133
Protein (g)	75.88±8.65	80.63±10.65	88.30±10.81	90.10±8.81	75.22±10.53	65.67±4.77
% Protein	0.166±0.011	0.162±0.010	0.159±0.010	0.174±0.010	0.167±0.020	0.168±0.012
Carbohydrate (g)	228.38±21.85	237.88±46.09	258.60±24.34	228.30±21.59	241.89±19.62	201.11±21.70
% Carbohydrate	0.504±0.035	0.466±0.055	0.470±0.025	0.447±0.031	0.550±0.045	0.494±0.025
Fat (g)	65.63±7.96	70.75±9.59	80.80±5.54	91.00±10.69	77.56±10.66	61.67±7.13
% Fat	0.324±0.033	0.324±0.034	0.335±0.014	0.387±0.031	0.381±0.025	0.339±0.023
Vit.C (mg)	92.59±29.53	60.04±18.13	61.03±17.14	86.23±18.93	46.02±10.45	84.67±22.24
Vit. E (mg)	4.00±0.947*	4.10±0.888*	6.69±0.814	5.78±0.927	5.76±1.04	3.60±0.441
Vit. A (RE)	833.3±114.1	863.1±123.2	918.9±166.3	1081±220	866.35±98.58	597.1±90.2

*No statistical interactions or main effects were noted for any dietary variable ($p > 0.05$), with the following exceptions. The vitamin E intake of the placebo condition was lower than both GPLC conditions ($p = 0.027$) and the total kilocalorie intake of the 1 gram GPLC condition was significantly greater than that of the 3 gram GPLC condition ($p = 0.0452$). Values are mean±SEM.

gnificantly greater than that of the 3 gram GPLC condition ($p = 0.0452$). Dietary data are shown in Table II.

Blood-borne variables

Malondialdehyde was decreased from pre- to post-intervention with both 1 and 3 g GPLC ($p < 0.05$). While malondialdehyde was lower in the placebo condition following the intervention compared to pre-intervention, this decrease failed to reach statistical significance ($p > 0.05$). Nitric oxide was increased from pre- to post-intervention with 3 g GPLC

Table III: Blood malondialdehyde, nitric oxide, and lipid values of subjects before and following an eight-week intervention of aerobic exercise and placebo or GPLC supplementation

Variable	Placebo Pre	Placebo Post	1 g·day^{-1} GPLC Pre	1 g·day^{-1} GPLC Post	3 g·day^{-1} GPLC Pre	3 g·day^{-1} GPLC Post
Malondialdehyde (μmol·L^{-1})	1.12±0.21	1.03±0.23	1.08±0.24	*0.69±0.25	0.94±0.18	*0.66±0.17
Nitric Oxide (μmol·L^{-1})	24.31±3.90	26.14±4.11	23.22±4.13	26.24±4.32	21.34±2.27	*29.46±3.61
Total Cholesterol (mg·dL^{-1})	159.84±8.84	162.24±7.92	170.07±4.90	168.45±7.61	177.01±7.74	175.25±8.23
HDL Cholesterol (mg·dL^{-1})	48.80±6.54	52.99±6.81	52.5±3.98	55.7±4.38	55.96±4.99	62.40±7.32
Total Cholesterol: HDL	3.65±0.49	3.40±0.45	3.35±0.17	3.18±0.25	3.39±0.39	3.06±0.32
LDL Cholesterol (mg·dL^{-1})	95.64±8.79	97.22±8.26	102.13±3.33	98.99±7.78	108.40±7.94	103.03±8.01
Triglycerides (mg·dL^{-1})	77.03±10.73	70.18±9.85	77.16±9.13	68.78±6.88	63.25±7.78	49.15±7.61

* Denotes statistically significant difference from Pre value within same condition ($p < 0.05$). Values are mean±SEM.

Table IV: Complete blood count values of subjects before and following an eight-week intervention of aerobic exercise and placebo or GPLC supplementation

Variable	Placebo Pre	Placebo Post	1 g·day^{-1} GPLC Pre	1 g·day^{-1} GPLC Post	3 g·day^{-1} GPLC Pre	3 g·day^{-1} GPLC Post
WBC (10^3 μL)	5.77±0.62	5.60±0.38	6.33±0.48	6.83±0.51	6.14±0.45	5.96±0.29
RBC (10^6 μL)	4.57±0.16	4.58±0.20	4.64±0.15	4.67±0.10	4.20±0.08	4.25±0.09
Hemoglobin (g·dL^{-1})	13.26±0.55	13.90±0.79	14.21±0.48	14.38±0.34	12.96±0.25	13.08±0.21
Hematocrit (%)	39.45±1.15	40.91±2.06	41.62±1.37	41.98±0.99	38.00±0.71	38.21±0.48
MVC (fL)	86.23±2.93	89.10±1.43	89.80±0.80	89.80±0.77	90.55±1.59	90.11±1.42
MCH (pg)	29.18±1.25	30.25±0.82	30.67±0.35	30.77±0.31	30.91±0.53	30.86±0.51
MCHC (g·dL^{-1})	33.58±0.40	33.95±0.38	34.15±0.17	34.26±0.12	34.12±0.19	34.22±0.19
RDW (%)	14.58±1.16	13.68±0.40	12.70±0.14	12.87±0.24	12.76±0.18	12.65±0.18
Platelets (10^3 μL)	271.87±32.56	313.20±41.04	271.10±22.78	249.40±17.53	265.11±10.43	264.11±12.55
Neutrophils (%)	51.75±4.25	49.16±3.72	53.90±2.97	53.80±2.50	55.33±3.77	52.33±4.08
Lymphocytes (%)	36.38±3.73	40.16±2.72	36.10±2.48	35.70±2.62	36.77±4.28	36.77±4.35
Monocytes (%)	7.50±0.78	7.16±0.70	7.60±0.70	7.20±0.44	5.66±0.62	7.88±1.05
Eosinophils (%)	3.75±0.64	3.00±0.73	2.30±0.44	2.90±1.39	1.66±0.37	2.22±0.72
Basophils (%)	0.63±0.18	0.50±0.34	0.20±0.13	0.40±0.16	0.55±0.17	0.78±0.14

No statistically significant differences were noted for any variable from Pre to Post intervention or between conditions ($P>0.05$). Values are mean±SEM.

($p < 0.05$), but did not increase statistically ($p > 0.05$) with 1 g GPLC or placebo. No main effects or interaction effects were noted for any measured blood lipid ($p > 0.05$). Data for malondialdehyde, nitric oxide, and blood lipids are presented in Table III. Data from the CBC (Table IV) and metabolic panel (Table V) revealed no change from pre- to post-intervention in any condition, nor were differences noted between conditions ($p > 0.05$).

Discussion

Data from the present investigation indicate that supplementation with GPLC in conjunction with eight weeks of supervised aerobic exercise 1) decreases lipid peroxidation, 2) increases nitric oxide, 3) does not further improve blood lipid profiles in normolipidemic subjects, and 4) appears safe and well tolerated at the dosages provided. Subject descriptive characteristics were similar between all three condi-

Table V: Comprehensive metabolic panel values of subjects before and following an eight-week intervention of aerobic exercise and placebo or GPLC supplementation

Variable	Placebo Pre	Placebo Post	1 g·day^{-1} GPLC Pre	1 g·day^{-1} GPLC Post	3 g·day^{-1} GPLC Pre	3 g·day^{-1} GPLC Post
Glucose (mg·dL^{-1})	88.28±2.95	91.28±2.93	90.60±2.05	90.70±2.42	84.44±1.46	86.44±2.06
BUN (mg·dL^{-1})	11.28±1.06	12.86±1.20	14.60±1.11	14.30±0.76	11.00±0.97	11.78±1.43
Creatinine (mg·dL^{-1})	0.84±0.06	0.91±0.07	0.81±0.07	0.85±0.06	0.67±0.03	0.73±0.03
BUN/Creatinine	14.14±1.95	14.85±1.98	19.40±2.43	17.60±1.49	16.66±1.59	16.00±1.61
Sodium (mmol·L^{-1})	138.86±0.36	138.86±0.67	138.30±0.61	138.10±0.59	138.22±0.36	137.88±0.71
Potassium (mmol·L^{-1})	3.91±0.07	4.10±0.09	4.21±0.12	4.05±0.08	4.07±0.12	3.96±0.08
Chloride (mmol·L^{-1})	104.10±0.48	104.14±0.79	102.20±0.59	101.90±1.00	102.55±0.41	102.33±0.62
CO_2 (mmol·L^{-1})	24.71±0.74	24.85±0.76	24.00±0.84	25.10±0.64	23.88±0.26	23.55±0.55
Calcium (mg·dL^{-1})	9.41±0.07	9.45±0.18	9.80±0.10	9.75±0.11	9.36±0.12	9.44±0.13
Protein (g·dL^{-1})	6.90±0.16	6.99±0.17	7.22±0.13	7.11±0.11	6.93±0.13	7.05±0.11
Albumin (g·dL^{-1})	4.10±0.03	4.12±0.17	4.31±0.06	4.24±0.09	4.21±0.12	4.30±0.07
Globulin (g·dL^{-1})	2.80±0.16	2.85±0.13	2.91±0.11	2.87±0.10	2.72±0.10	2.75±0.10
A/G Ratio	1.48±0.08	1.48±0.11	1.49±0.05	1.51±0.09	1.56±0.09	1.60±0.07
Bilirubin (mg·dL^{-1})	0.67±0.18	0.54±0.15	0.54±0.12	0.63±0.15	0.45±0.05	0.41±0.03
Alk Phos (IU·L^{-1})	64.61±0.47	71.14±4.36	68.10±9.17	65.80±8.87	58.00±6.84	58.00±6.60
AST (SGOT) (IU·L^{-1})	20.42±0.18	19.28±1.22	17.80±1.69	16.60±1.14	18.66±2.24	19.00±20.60
ALT (SGPT) (IU·L^{-1})	19.28±3.25	19.29±2.53	19.00±3.48	16.40±2.74	16.66±2.27	16.21±2.19

No statistically significant differences were noted for any variable from pre- to post-intervention or between conditions ($p > 0.05$). Values are mean±SEM.

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tions, with expected changes due to aerobic exercise training from pre- to post-intervention. Subject compliance with both the aerobic training prescription and supplementation was similar between conditions. All dietary variables were similar between conditions and across time, with the exception of a slightly lower vitamin E intake with the placebo condition compared to both GPLC conditions, and greater total kilocalorie intake with the one gram GPLC condition compared to that of the three gram GPLC condition (Table II). We do not believe that these very minor, albeit statistically significant differences influenced our results. For example, Hooper and colleagues [32] reported that small differences such as these are not associated with differences in blood lipid values. In relation to the antioxidant properties of vitamin E in preventing lipid peroxidation, studies have routinely found effects with dosages ranging from 400–1200I U·day^{-1}, which is far greater than the 2–3 mg differences noted between the placebo and GPLC conditions.

Lipid peroxidation, as measured by plasma malondialdehyde, was decreased to a greater extent in both GPLC conditions compared to placebo. Lipid peroxidation is an autocatalytic process linked to increased mitochondrial respiration and electron transport disturbances associated with oxygen uptake and energy production [33]. It involves degradation of polyunsaturated fatty acids and phospholipids through a chain reaction. With extensive damage, normal physiological function may be impaired (i.e., loss of membrane fluidity, increased membrane permeability with loss of cytosolic proteins, and alteration in enzyme function), and in extreme cases lead to cell death. Indeed, lipid peroxidation is thought to play an important role in the etiology of many pathological conditions such as cancer, atherosclerosis, and possibly other degenerative diseases. With regards to the specific lipid peroxidation end products, the measurement of malondialdehyde has been extensively used, and our lab has several recent published reports using this biomarker [34–37].

Recent studies have noted decreased lipid peroxidation as an adaptation to exercise [38,39] which agrees with our findings in the placebo condition (although not statistically significant). The addition

of GPLC to the aerobic training program further decreased lipid peroxidation, which is understandable based on previous evidence indicating antioxidant properties of both glycine [26,27] and carnitine [18–24]. It was recently demonstrated *in vitro* that L-carnitine has effective 1, l-diphenyl-2-picrylhydrazyl free radical (DPPH·) scavenging, hydrogen peroxide and superoxide anion scavenging, total reducing power, and metal-chelating activities [40]. Moreover, L-carnitine inhibited lipid peroxidation of linoleic acid emulsion to a greater extent than α-tocopherol and Trolox (a water-soluble analogue of tocopherol) [40]. These effects of carnitine are likely most responsible for our findings of decreased malondialdehyde with GPLC supplementation. To our knowledge this is the first study to demonstrate an antioxidant benefit of combination PLC and glycine supplementation in healthy human subjects. These findings may have implications for overall health, as lipid peroxidation is associated with impaired cellular function as described above. Furthermore, malondialdehyde has been reported to be positively correlated ($r=0.59$, $p < 0.001$) to homocysteine [41], a known risk factor for CVD. Hence, decreasing malondialdehyde levels appears important. It should be noted that while we report a benefit of GPLC supplementation in relation to lipid peroxidation, we do not know whether PLC or glycine would independently promote the same degree of change in our population of young, otherwise healthy subjects. Future investigations using these two nutrients in isolation, in comparison to combination therapy, are needed to address this issue. In addition, because we failed to include measurements of enzymatic (e.g., superoxide dismutase, catalase, glutathione peroxidase) and non-enzymatic antioxidants (e.g., thiols), we are uncertain of the specific mechanism allowing for the decrease in malondialdehyde with GPLC supplementation. Lastly, inclusion of additional biomarkers of oxidative stress such as protein and DNA oxidation should be considered in future work in this area. In this way, a better representation as to the overall oxidative status of the system can be ascertained.

In addition to the decrease in lipid peroxidation, we noted a statistically significant increase in nitric oxide in the 3 g GPLC group. Aerobic exercise alone has been reported to increase nitric oxide to a similar magnitude (~10%) as observed in our placebo and 1 g GPLC conditions [42,43]. Moreover, a recent report indicated that PLC given via intravenous (IV) infusion at a dosage of 6 grams per day to patients with peripheral arterial disease resulted in an increase from 14.5 ± 1.4 to 17.1 ± 1.2 $\mu mol \cdot L^{-1}$ [25]. The combination of aerobic exercise and GPLC at a dosage of 3 grams per day appears to mediate a further increase in nitric oxide, as seen in the present study. This may be partly explained by the actions of glycine to promote vasodilatation effects [28].

Lower nitric oxide is of clinical relevance because this molecule is responsible for mediating vasorelaxation in smooth muscle cells [44]. Our findings for nitric oxide may be partly associated with those for malondialdehyde. That is, while ROS production promotes vascular dysfunction [45], it is believed that such changes are partly mediated by impaired nitric oxide biosynthesis and action, which is directly affected by elevated levels of ROS [44]. Hence, decreasing oxidative stress (as indicated by lower levels of malondialdehyde) may be associated with normalization/enhancement of circulating nitric oxide. However, while malondialdehyde was decreased to a similar extent in both GPLC groups, nitric oxide was increased to a greater extent with 3 grams per day of GPLC. Therefore, our data do not indicate a direct relationship between lipid peroxidation values and nitric oxide, at least as measured in human blood samples. It is possible that even a low dosage of GPLC (1 gram per day) can promote favorable antioxidant benefits [40] resulting in a lowering of malondialdehyde, while higher dosages (3 grams per day) may be needed to significantly increase nitric oxide. Future study is needed in order to determine the impact of PLC and glycine alone, and in combination, with regard to lipid peroxidation, nitric oxide, and vascular function.

While we noted modest positive changes in blood lipids (e.g., decreased triglycerides), no variable underwent a statistically significant change for any condition. Prior investigations have noted positive adaptations in blood lipids following aerobic exercise (primarily a decrease in triglycerides and an increase in HDL cholesterol), and both carnitine and glycine supplementation. However, these previous studies have used humans or animals with hyperlipidemia, often with comorbidities such as CVD, diabetes, and hepatotoxicity. Such individuals/animals may experience a greater absolute change in blood lipids, making the detection of changes clearer. Moreover, the route of administration in many previous studies reporting positive outcomes has been intravenous rather than oral, often at dosages much greater than used in the present study [29,30]. These issues may help to explain our lack of statistically significant changes in the blood lipid parameters.

In addition to measuring the above variables, complete blood count and metabolic panels were included for each subject pre- and post-intervention to determine safety of supplemental GPLC. Data revealed no statistically significant differences for any variable from pre- to post-intervention or between conditions. These findings are in agreement with those of Rubin and coworkers [46] who reported no adverse outcomes in blood measures following three weeks of L-carnitine supplementation at a dosage of 3 g·day^{-1}. In addition to safety verified by blood measures, subjects reported no symptoms of gastrointestinal upset, which is one side effect previously noted with high-dose (e.g., >4 g·day^{-1}) carnitine supplementation [47].

Conclusion

When combined with an eight-week program of supervised aerobic exercise, we report a decrease in lipid peroxidation with both one and three grams per day of GPLC, and an increase in nitric oxide with three grams per day of GPLC, with no improvement in blood lipids in a population of normolipidemic subjects. This is the first study to note antioxidant benefits and elevations in nitric oxide with combined PLC and glycine supplementation in healthy human subjects.

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