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Title:

Serum metabolomic adaptations following a 12-week High-Intensity Interval Training combined to citrulline supplementation in obese older adults

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ABSTRACT

Physical activity and nutrition play important roles in preventing adverse health outcomes that accompany aging. It has been shown that high-intensity interval training (HIIT) combined with citrulline (CIT) supplementation can improve physical and functional capacities. The aim of this study was to evaluate serum metabolites following a 12-week HIIT combined or not with CIT in obese older adults, and to correlate the metabolic changes with clinico-biological parameters changes. Eighty-six obese older adults completed a 12-week HIIT program combined with a 10 g daily supplementation of either CIT or placebo (PLA) during a double-blinded randomized interventional trial. Only participants with blood samples at T0 (before the intervention) and/or T12 (after the intervention) were included in our sub-analysis (HIIT-PLA -T0 : n = 44 and HIIT-PLA -T12 : n = 28; HIIT-CIT-T0 : n = 39 and HIIT-CIT-T12 : n = 42). Serum samples were analyzed by different liquid or gas phase chromatography methods coupled to mass spectrometry. Among the identified metabolites, 44 changed significantly following the 12-week intervention (Time effect), and 10 of them were more affected when HIIT was combined with CIT (Time×Supp effect). Arginine increased significantly due to the 12-week intervention. Correlation analyses demonstrated that decreased triglyceride (TG) (16:1/18:1/16:0) and aspartic acid significantly correlated with a reduction of adiposity-related parameters (fat mass, leg lean mass, leptin, total triglycerides and low-density lipoprotein). Arginine, TG (16:1/18:1/16:0) and aspartic acid might constitute biomarkers of cardiometabolic health and adiposity. Further studies are needed to confirm these associations and understand the underlying mechanisms.

HIGHLIGHTS:

- A 12-week intervention involving high-intensity interval training (HIIT) with or without citrulline (CIT) supplementation induced adaptations in the serum metabolome of obese older adults through significant changes in 44 metabolites.
- Changes in 23 metabolites were observed when a CIT supplementation was administered along with a 12-week HIIT intervention.
- TG (16:1/18:1/16:0) correlated with several adiposity parameters including leptin, triglycerides, legs lean mass.
- Aspartic acid correlated with several adiposity parameters including leptin, LDL cholesterol as well as android, arms and trunk fat mass.

KEYWORDS: HIIT, Aging, Obesity, Exercise, Nutrition

INTRODUCTION

Aging results from time-dependent genetic, molecular and physiological changes (1). Over the past decades, aging has become an important public health issue as it is associated with physical alterations (e.g. sarcopenia) that put older people at a higher risk of developing life-threatening diseases.

To promote healthy aging, physical activity is nowadays widely recognized as an effective intervention. High-intensity interval training (HIIT) confers benefit to the elderly by triggering positive cardiovascular adaptations such as reduced blood pressure, increased cardiac function, improved metabolic capacity (2) and reduced glycemia (3, 4). In addition, HIIT improves functional capacities (5), subjective wellbeing and objective physical fitness (6).

Older adults are becoming increasingly aware of the importance of nutrient supplementation (7). Citrulline (CIT), a non-essential amino acid active as L-citrulline, is present in the body and is a precursor of L-arginine, a substrate for NO synthase (8). It was suggested that CIT has an impact on the cardiometabolic health by providing: vasoprotective effects, anti-hypertensive effects, and protection against diabetic vascular dysfunction (9). From a metabolic view, CIT increases the mitochondrial biogenesis in skeletal muscle and improves adipose tissue lipolysis (9). In rats, it was demonstrated that CIT is able to favor fat mass reduction (10). Moreover, CIT supplementation provided a protective effect on age-related LTP (Long Term Potentiation) impairment in mice due to its antioxidant properties (11). Recently, many research studies in the exercise science domain focused their interests on the CIT supplementation combined to exercise. This amino acid revealed an effect on fatigue and performance in mice by lengthening the time to exhaustion and by improving the performance (12). In addition, it has been shown that a CIT supplementation increases blood flow in older adults (13) and decreases blood pressure (14), and this by increasing the NO bioavailability (9) which leads to smooth muscle vasodilation of the vessels.

The effect of CIT combined with HIIT intervention was investigated in dynapenic-obese older adults and showed positive effects on functional capacity (walking speed) and muscle function (upper muscle strength) (15). In obese older men and women, adding CIT to HIIT resulted in a greater increase in muscle strength and a significant decrease in total fat mass (16). Altogether, these results emphasize the importance of combined non-pharmacological interventions (nutrition and exercise) for a healthy aging and obesity prevention. However, to the best of our knowledge, the clinical manifestations and general biological parameters of HIIT combined with CIT are essentially described, omitting the biomolecular actors. Additional experiments are needed to complete our understanding on the mechanisms through which non-pharmacological approaches contribute to healthy aging.

Metabolomics provides a snapshot of the current metabolic status of an organism and allows the monitoring of changes in metabolite profiles, from their identification to their quantification. In animal models such as rats, the circulating metabolome is strongly affected by exercise and aging (17). Additionally, a recent review reported that metabolite levels can be influenced by nutrition (18). Aging and exercise affect the rat urinary metabolome, and exercise attenuated the consequences of aging (19). Hence, these preclinical studies support the fact that aging as well as external interventions can significantly modify the metabolome.

In human, unique metabolomic signatures have been related to biological aging through profiling of novel biomarker metabolites, such as amino acids, fatty acids, acetylcarnitine, sphingolipids and nucleotides (20). However, few studies have investigated the impact of exercise on the age-related metabolic dysregulation. In obese older adults, the serum metabolome adaptations were evaluated following 12 weeks of HIIT and moderate-intensity continuous training (21). The results revealed interesting associations between several metabolites and several clinical and biological parameters which could be potentially interesting for a healthy aging (21). Metabolomics can also be used as a non-invasive method to monitor training status, as revealed by studies comparing highly trained athletes to low-activity individuals following submaximal endurance exercise intervention (22). Moreover, step reduction intervention in overweight adults unveiled an increase in circulating metabolites (glutamine, carnitine and creatine), making them possible biomarkers for early detection of metabolic dysregulation in older individuals (23). Of note, untargeted metabolomics may also be used to detect the compliance with dietary interventions in clinical studies (24). Hence, exercise and nutrition interventions, considered as non-pharmacological interventions, were found to induce changes in the metabolome.

Altogether, these findings support that metabolomics can be used as a minimally invasive method to monitor the impact of exercise and diet. Driven by this consideration, we decided to investigate potentially health-related shifts in the circulating metabolome in the context of a clinical trial that combines HIIT and CIT in obese older adults.

MATERIAL AND METHODS

Study protocol overview

This study is a sub-analysis from a double-blinded randomized interventional trial ((16); RCT NCT02417428). Participants were recruited for the main study through social communication in Montréal (Canada). In order to participate in the study, participants had to meet the following criteria: 1- autonomous (being able to follow the exercise program), 2- obesity (BMI between 30 and 40 kg / m^2) or a waist circumference greater than 102 cm for men and 88 cm for women or fat mass (%; total or android or gynoid) equal or superior to 27% in men and 40% in women; 3- stable weight (± 5 kg) for 6 months; 4- non-smokers and moderate drinkers (max: 15 g/day of alcohol) ; 5- no history of stroke ; 6- inactive (less than 2 hours of structured physical activity per week); 7- no involvement in a vigorous exercise program for at least 12 months; 8- ability to understand French; 9- postmenopausal for women (over 60 consecutive months without menstruations). Exclusion criteria were the following: 1- presence of metal implant (pacemaker); 2- asthma requiring oral steroid treatment; 3-use of medication that could affect metabolism or cardiovascular function; 4- use of anticoagulants (only for participants undergoing biopsies); 5- use of hormonal-replacement therapy. Participants with diagnosed (untreated) neurological, cardiovascular, lung diseases or cognitive disorders were excluded.

During the 12-week intervention, all participants followed an exercise intervention (HIIT, 3 times/per week; 30 min/session) and took blindly and daily 10 g of supplementation (CIT or placebo (PLA); (more details were described previously (15)). A computer-generated randomization procedure stratified the randomization by blocks of four (since there are 2 groups: PLA and CIT). Each individual's container containing PLA or CIT was prepared and identified with a letter code by an independent technician. The nature of the supplement of each container was written in a sealed envelope which was securely stored in a locked container. The double-blinding was performed in a way evaluators and participants were kept blind about the nature of the supplementation until the end of the protocol.

Anthropometric parameters (weight, height, BMI, circumferences), body and muscle composition (DEXA; XCT-3000 QPCT), muscle function (upper and lower body muscle strength; leg muscle power), functional (Timed Up and Go, SPPB, unipodal balance) and aerobic (6 min walking test) capacities, serum glucose, insulin, High-density Lipoprotein (HDL) and Low-density Lipoprotein (LDL) cholesterol, Insulin-like Growth Factor Binding Protein-3 (IGFBP-3), leptin and adiponectin and Homeostatic Model Assessment of Insulin Resistance (HOMA-IR), were assessed pre (T0) and post (T12) intervention as described previously (15). Triglycerides (TG) were measured at the McGill

University Health Centre clinical laboratory. The study approval was obtained by the Ethics Committee of the *Université du Québec à Montreal (UQAM)* (#2014_e_1018_475). Prior to the interventions, all participants provided their informed consent in written after receiving the necessary information concerning the study.

Metabolomics profiling

Blood samples were collected by a physician in fasting state (at least 8 hours) before and after the 12-week intervention. Serum samples were used to analyze blindly serum metabolomics at Gustave Roussy Cancer Campus facility (Villejuif, France) using mass spectrometers coupled to multiple different liquid or gas phase chromatography methods. More specifically, a 7890B gas chromatography (Agilent Technologies, Waldbronn, Germany) coupled to a triple quadrupole 7000C (Agilent Technologies, Waldbronn, Germany) was used to detect intracellular metabolites (targeted analysis). Bile acids metabolomics were obtained using a UHPLC/MS - RRLC 1260 system (Agilent Technologies, Waldbronn, Germany) coupled to a Triple Quadrupole 6410 (Agilent Technologies). Short chain fatty acids, oxylipin and lipids metabolomics were assessed by a UHPLC/QUAD+ - RRLC 1260 system (Agilent Technologies, Waldbronn, Germany) coupled to a 6500+ QTRAP (Sciex, Darmstadt, Germany). Polyamines metabolomics were quantified using a UHPLC/QQQ - RRLC 1260 system (Agilent Technologies, Waldbronn, Germany) coupled to a Triple Quadrupole 6410 (Agilent Technologies). Finally, intracellular metabolites (untargeted analysis) were identified using UHPLC/Q-extractive MS - Dionex Ultimate 3000 UHPLC system (Thermo Scientific) coupled to a Q-Exactive (Thermo Scientific). Each method details have been described previously (25).

Statistical analyses

Quantitative results are expressed as mean ± SD. The delta changes (%) were calculated as ((T12-T0)/T0) × 100). Statistical significance testing of the measured metabolites was performed using multivariate and univariate analyses. As the multivariate analysis, principal component analysis (PCA) was conducted for a better visualization of the metabolomic data (R-packages *FactoMineR* and *factoextra*). The Levene's test was used to assess the homogeneity of variances. As the univariate analysis, a linear mixed-models

approach (R-package *nlme*) with a two-factor repeated measures ANOVA were then used to test the intervention effect (Time effect: T0 and T12), the supplementation effect (Supp effect: PLA and CIT) and their interaction (Time×Supp effect) on serum metabolomic adaptations. Post-hoc analyses were then done using simultaneous tests for general linear hypotheses (R-package *emmeans*) with a Bonferroni correction. Results were considered statistically significant when p-value < 0.05. Due to the high number of comparisons performed, the Benjamini Hochberg (BH) False Discovery Rate (FDR) was performed to correct for multiple comparisons (R-package *FSA*). FDR threshold was set at 0.05. Thereafter, the Grubbs' test was used to detect potential outliers on both the metabolites and the measured (clinical and serum) parameters. Based on these results, Pearson's correlation coefficient (linear correlation coefficients) was performed with and without outliers to assess the association between the metabolites delta changes and the clinico-biological parameters delta changes. Only correlations with Pearson's correlation coefficient above 0.35 and with p-value < 0.01 were considered. All statistical analyses were performed using the software R (3.6.2) (foundation for statistical computing, Vienna, Austria). Heatmaps of the metabolites were generated with the R-package *ggplot2* and the correlation graphs were drawn using the R-package *ggpubr*.

RESULTS

Participants

A total of 107 participants were eligible and recruited, but only 95 accepted to participate. These 95 individuals were randomized into 2 groups. Eighty-six participants completed the intervention However, only participants with extra blood samples at T0 (before the intervention) and/or T12 (after the intervention) were included in this metabolomic sub-analysis. In the HIIT-PLA group, 44 participants had blood samples before the 12-week intervention, and 28 participants had blood samples after the 12-week intervention. In the HIIT-CIT group, 39 participants had blood samples before the 12-week intervention, and 42 participants had blood samples after the 12-week intervention. The flow chart of the study is detailed in Figure S1 of the supplementary material.

To calculate delta changes (value at T12 minus value at T0, divided by value at T0, multiplied by 100) and to correlate changes in metabolites with alterations in clinico-biological parameters, only participants with serum samples both at T0 and T12 (Intervention: n=59) were included (HIIT-PLA: n=26 vs. HIIT-CIT: n=33).

Metabolomic profile adaptations following the 12-week intervention.

Serum metabolites were identified and quantified before and after the 12-week intervention. PCA analysis has been performed on all metabolites (Figure S2) and no significant changes in the metabolic profile were observed. In order to identify metabolites with more accuracy, a two-way repeated measures ANOVA was generated. Out of the 359 metabolites and ratios of metabolites analyzed, 44 displayed significant changes ($p < 0.05$) and are involved in different biochemical pathways (Table 1); 9 belong to the carbohydrate metabolism, 10 to the urea cycle, 10 to the tricarboxylic acid (TCA) cycle, 9 to the fat metabolism, and 6 to a variety of other metabolic circuitries. The ratios which were analyzed are ratios of metabolites that generate each other in a same metabolic cycle. Among these 44 metabolites and ratios of metabolites, 40 significantly varied due to the 12-week intervention (Time effect; Figure 1; data were normalized between 0 and 1) and 10 metabolites were affected differently and significantly (Time×Supp effect) according to the type of supplementation (PLA vs CIT; Figure S5).

Metabolomic changes following the 12-week intervention

For the carbohydrate metabolism, 5 metabolites and 4 ratios of metabolites were significantly changed. Acetic acid, glyceric acid, lactose, as well as ribose and ribulose significantly decreased. A significant increase was observed for xylitol, pyruvate/alanine, pyruvate/lactate, pyruvate/malate, and pyruvate/serine following the 12-week intervention.

For the urea metabolism, 5 metabolites and 4 ratios of metabolites were affected. Cadaverine, inosine and the ratio putrescine/ornithine significantly decreased. Arginine, citrulline, ornithine and the 3 ratios asparagine/aspartate, citrulline/arginine and citrulline/ornithine significantly increased following the 12-week intervention.

For the TCA cycle, 5 metabolites and 5 ratios of metabolites significantly varied. Fumaric acid, pyruvic and oxaloacetic acid, 2-oxoglutaric, fumarate/malate, oxaloacetic acid/malate and oxaloacetic acid/aspartate. Aspartic acid, 3-methylhistidine, citrate/oxaloacetic acid and aspartate/malate decreased following the 12-week intervention.

For the fat metabolism, a significant effect was observed for 6 metabolites. A significant decrease was observed for acetylcarnitine, margaric acid, TG (16:0/16:1/18:2) and TG (16:1/18:1/16:0), whereas a significant increase was observed for glycerophosphorylcholine and TG (18:2/18:2/22:0) following the 12-week intervention.

For other metabolic pathways (amino acid, nucleic acid, leucine and BCAA metabolisms), a significant effect was observed for 6 metabolites. 2-aminoadipic acid, guanosine, and ketoisovaleric acid significantly increased, while homoserine, 2-hydroxybutyric acid 2-oxovaleric acid significantly decreased following the 12-week intervention.

To strengthen our feature selection criteria, we performed a false discovery rate (FDR) analysis (Tables S1-a and S1-b in the Supplementary Material). Following this analysis, 15 metabolites and ratios of metabolites revealed significant changes (Time effect; FDR < 0.05). These significant metabolites include: glyceric acid, xylitol and pyruvate/lactate (carbohydrate metabolism; Table S1-a); inosine and ornithine (urea metabolism; Table S1-a); fumaric acid, aspartic acid, pyruvic acid and oxaloacetic acid, 2-oxoglutaric acid, fumarate/malate, oxaloacetic acid/aspartate, and aspartate/malate belong to the (TCA cycle; Table S1-b); 2-aminoadipic acid, 2-oxovaleric acid and guanosine (other metabolic circuitries; Table S1-b).

Metabolomic changes for each group following the 12-week intervention

For the carbohydrate metabolism, acetic acid and pyruvate/malate significantly increased while lactose as well as ribose and ribulose significantly decreased in the HIIT-PLA group. Xylitol increased in the HIIT-CIT group. In both HIIT-PLA and HIIT-CIT groups, glyceric acid decreased and pyruvate/lactate significantly increased.

For the urea metabolism, urea, ornithine, arginine, citrulline and citrulline/arginine significantly increased while cadaverine, citrulline/ornithine and putrescine/ornithine significantly decreased in the HIIT-CIT group. In both HIIT-PLA and HIIT-CIT groups, inosine significantly decreased.

For the TCA cycle, pyruvic and oxaloacetic acid, oxaloacetic acid/malate significantly increased while aspartic acid, 3-methylhistidine and citrate/oxaloacetic acid significantly decreased in the HIIT-PLA group. Aspartic acid and fumarate/malate increased in the HIIT-CIT group. In both HIIT-PLA and HIIT-CIT groups, fumaric acid, 2-oxoglutaric acid and oxaloacetic acid/aspartate significantly increased and aspartate/malate significantly decreased.

For the fat metabolism, acetylcarnitine, margaric acid, TG (16:0/16:1/18:2) and TG (16:1/18:1/16:0) and undecanoic acid significantly decreased in the HIIT-PLA group. Glycerophosphorylcholine and TG (18:2/18:2/22:0) significantly increased in the HIIT-CIT group.

For other metabolic pathways (amino acid, nucleic acid, leucine and BCAA metabolisms), 2-hydroxybutyric acid decreased in the HIIT-PLA group. Homoserine significantly decreased while 2-aminoadipic acid and guanosine significantly increased in the HIIT-CIT group. In both HIIT-PLA and HIIT-CIT groups, 2-oxovaleric acid significantly decreased.

Correlations between serum metabolomic changes and shifts in clinico-biological parameters following the intervention

The clinico-biological parameters of the included participants are present in Tables S2-a and S2-b. Metabolomic delta changes (%) were correlated with clinical and biological parameters delta changes (%). We also performed the correlation analyses with all participants (Tables S3-a, S3-b, S3-c). In the interest of completeness, correlation analyses without outliers were performed (Tables S4-a, S4-b and S4-c). Significant correlations (p-value ≤ 0.01) were observed between changes in the serum abundance of several metabolites and specific clinico-biological adaptations resulting from HIIT alone (HIIT-PLA) or combined with CIT (HIIT-CIT).

TG (16:1/18:1/16:0) and aspartic acid correlated with several adiposity parameters

Changes in TG (16:1/18:1/16:0) and aspartic acid were significantly correlated with several adiposity-relevant parameters changes (Figures 2 and 3). With FDR analysis, the change in TG (16:1/18:1/16:0) did not appear significant anymore. However, since several correlations were observed between TG (16:1/18:1/16:0) and several adiposity parameters, this metabolite was considered. The change in aspartic acid appeared significant following the FDR analysis (FDR < 0.007).

Within the entire cohort of interpretable participants, TG (16:1/18:1/16:0) changes significantly correlated with leptin changes (r = 0.37, p < 0.01; Figure 2A) and triglycerides levels changes (r = 0.46, p < 0.001; Figure 2B). In the HIIT-PLA group, changes in TG (16:1/18:1/16:0) significantly correlated with changes in leg lean mass (r = -0.61, p < 0.001; Figure 2C). In the HIIT-CIT group, it positively correlated with changes in leptin changes (r = 0.67, p < 0.001; Figure 2D), changes in triglycerides levels (r = 0.49, p < 0.01; Figure 2E), changes in arm fat mass (r = 0.49, p < 0.01; Figure 2F), changes in android fat mass (r = 0.52, p < 0.01; Figure 2G) and changes in trunk fat mass (r = 0.46, p < 0.01; Figure 2H).

Within the entire cohort of interpretable patients, aspartic acid changes significantly correlated with changes in leptin (r = 0.39, p < 0.01). In the HIIT-CIT group, aspartic acid positively correlated with changes leptin (r = 0.63, p < 0.001), changes in LDL cholesterol (r = 0.51, p < 0.01), changes in android fat mass (r = 0.45, p < 0.01), changes in arm fat mass (r = 0.47 p < 0.01), and changes in trunk fat mass (r = 0.45, p < 0.01).

When considering values of clinico-biological parameters (Table S2-a and S2-b), time effect was observed for arm fat mass, leg lean mass and triglyceride levels. A Time×Supp effect was observed for

leptin and trunk fat mass. More specifically, intergroup differences were assessed as well. Arm fat mass significantly varied in all three groups. Android fat mass as well as trunk fat mass significantly improved in the HIIT-CIT group. Leg lean mass significantly differed in both the Intervention group and the HIIT-CIT group. Significant differences were obtained in the HIIT-CIT group for leptin. Triglycerides levels significantly differed in both the whole intervention group and the HIIT-PLA group.

DISCUSSION

General aims of the study

Metabolomic profiling was performed on serum samples from obese older adults who completed a 12-week HIIT combined or not with CIT supplementation. Our study aimed at unveiling metabolomic signatures accompanying these interventions, as well as their link to clinical and biological adaptations in obese older adults. We found that several metabolites increased or decreased due to the intervention (Time effect) or due to the interaction between HIIT and supplementation (Time×Supp effect). Interesting results were also observed when correlating these metabolites changes with several clinico-biological parameters. To the best of our knowledge, this is the first metabolomic study performed in an intervention combining exercise (HIIT) and CIT on a population of obese older adults.

Metabolome adaptations

Following the intervention, we observed quantitative shifts in specific metabolites. Interestingly, some of these changes were elicited by exercise alone or by the combination of exercise with CIT. In previous studies, exercise interventions were found to induce changes in the metabolome. In a study where an acute aerobic and anaerobic exercise in young adults was performed, the serum metabolome was altered following both types of exercise (26). Specifically, given that various metabolites of the fat metabolism were increased after aerobic exercise, this general finding is in line with our results since many metabolites of this same metabolism were also increased either following HIIT alone or following HIIT combined to CIT. However, the exact altered metabolites are not similar, which could be due to the different exercise modalities, age of participants as well as the CIT supplementation in our intervention. Another study involving a step reduction protocol (23) detected significant increases in several blood metabolites including glutamine, carnitine, creatine and methionine that were not affected in our study. This discrepancy might be explained by differences in exercise types, exercise intensity, and the

additional nutritional intervention (CIT supplementation in our protocol). Moreover, in a study comparing the serum metabolome of men before and after a 3h marathon, the metabolites that were significantly modified (including metabolites of the amino acid and fat metabolisms) were different than the metabolites that were significantly modified following our intervention (27). Moreover, in contrast to our results, serum glyceric acid increased in men after a marathon, and this discrepancy could be due to the different age of the participants and the different modalities of the exercise interventions including the frequency (the marathon being a one-time exercise whereas our HIIT intervention being a chronic exercise), and the different intensities (27). Therefore, the changes in the metabolome can be different regarding the modality of exercise intervention. It was previously established that following a 2-week sprint interval training in obese men, some metabolic parameters changed significantly immediately after exercise compared to baseline, but these changes were not significant anymore 72h following the intervention (28). When comparing the durations, their intervention is considered short-term whereas our intervention is considered long-term (12 weeks). Concerning the citrulline, it has been demonstrated that this amino acid's half-life increased linearly with increased doses (29). Since our blood sampling took place 1 week after the intervention and that citrulline effect was still apparent, this suggests that some adaptations occurred. Given that HIIT combined with CIT have previously been shown to induce changes in clinical and biological parameters in older adults (15, 16, 30), changes in metabolites could then be considered as molecular signatures of these clinico-biological changes.

Citrulline/arginine ratio as a possible biomarker of the intervention

Significant Time and Time×Supp effects were observed for arginine, which significantly increased following the intervention in the HIIT-CIT group. L-arginine is a substrate for nitric oxide (NO) synthase (8), and enhances cardiometabolic health by vasoprotective and antihypertensive effects (9). Citrulline also significantly increased following the intervention. Citrulline and arginine are interconnected as substrate and product in the same cycle, and hence balancing each other. Therefore, the increase of both arginine and citrulline might be due to the CIT administered to the participants. However, since the supplementation was stopped one week before the blood sample collection, we are then tempted to speculate that the increase of citrulline and arginine might be the result of an adaptation. We also calculated the citrulline/arginine ratio. Even though the change in this ratio did not appear significant anymore following the FDR analysis, this ratio might be interesting to evaluate. A significant increase was observed for this ratio in the HIIT-CIT group, meaning that citrulline is higher than arginine in this group. Since the body uses arginine to generate NO, it would be interesting to analyze the

relationship of the citrulline/arginine ratio and NO production (9). In the NO synthesis pathway, arginine is converted to citrulline by NO synthase (NOS) (31), which regulates responses to bacterial infection and endotoxic shock (32) as well as macrophage activation in inflammatory processes (33). A study aimed to evaluate the metabolic conversion between citrulline and arginine (34). The results obtained showed that oral L-citrulline supplementation increased the concentration of plasma L-arginine as well as the NO-dependent signaling in a dose-dependent manner (34). On the other side, when considering the effect of HIIT on NO metabolites (triggers of NOS) on rats, it has been demonstrated that HIIT increased the concentration of these metabolites (35). Hence, it is tempting to speculate that the increased citrulline/arginine ratio in our intervention which combined both HIIT and CIT may reflect an increased activity of NOS linked to health-improving NO generation.

TG (16:1/18:1/16:0) and aspartic acid as possible markers for adiposity parameters follow-up

The metabolites TG (16:1/18:1/16:0) and aspartic acid, which are metabolites of fat metabolism and TCA cycle respectively, were significantly correlated with several adiposity-related parameters. In fact, TG (16:1/18:1/16:0) significantly decreased as a result of the intervention (Time effect) and the interaction (Time×Supp effect) especially in the HIIT-PLA group. The adiposity-related parameters changes with which TG (16:1/18:1/16:0) changes significantly correlated in at least one of the groups (Intervention, HIIT-PLA and/or HIIT-CIT) were changes in arm fat mass, android fat mass, trunk fat mass, legs lean mass, leptin and triglycerides. All of these parameters changes positively correlated with TG (16:1/18:1/16:0) changes except for leg lean mass which negatively correlated with this metabolite in the HIIT-PLA group. A significant positive correlation was obtained between TG (16:1/18:1/16:0) and total triglycerides measured by routine methods ($r = 0.54$, $p < 0.001$) in all participants (Table S5). Hence, changes in TG (16:1/18:1/16:0) might have some considerations as a biomarker to monitor changes in adiposity.

Aspartic acid significantly decreased as a result of the intervention in both the HIIT-PLA and the HIIT-CIT groups. Changes in adiposity-related parameters (arm fat mass, android fat mass, trunk fat mass, leptin and LDL cholesterol) were significantly correlated with aspartic acid changes in at least one of the groups. Thus, similar to TG (16:1/18:1/16:0), aspartic acid may be useful as a biomarker of changes in adiposity. A prior study reported a positive association between aspartic acid and total cholesterol (36), supporting our findings.

Limitations and future perspectives

Our study presents some limitations. The number of participants included in this trial was limited and somehow disequilibrated between the two groups (28 for PLA and 43 for CIT) due to absence of extra blood samples required for this ancillary study. Intermediate metabolomic analyses, for instance at mid-term of the 12-week-long intervention are missing. The serum metabolome cannot be representative of whole-body metabolisms, and additional analyses, for instance mass spectrometric metabolomics of muscle biopsies or non-invasive NMR-based metabolomic approaches are needed. Most importantly, the results obtained in this work require replication in an independent validation cohort undergoing similar interventions. To avoid missing any metabolite or metabolite ratio that could be of potential interest, results both with and without FDR analysis were presented. However, future studies are needed to confirm our results.

CONCLUSION

To our knowledge, this is the first study integrating serum metabolomics with an exercise intervention (HIIT) combined with a nutritional supplementation (CIT) in obese older men and women. The study led to the identification of putative new biomarkers reflecting a desirable clinical outcome. Forty-four metabolites from different biochemical pathways were affected by the intervention, and 2 of them (TG (16:1/18:1/16:0) and aspartic acid) correlated well with clinico-biological adaptations to the 12-week-long intervention. Additionally, the increase in arginine bioavailability and that of the citrulline/arginine ratio could indicate beneficial effects on cardiometabolic health and possibly on the immune-inflammatory system. Altogether, the serum abundance of 3 metabolites, arginine, aspartic acid and TG (16:1/18:1/16:0), might reflect positive health effects of HIIT combined with oral administration of CIT.

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Declaration of interest statement:

GK has been holding research contracts with Daiichi Sankyo, Eleor, Kaleido, Lytix Pharma, PharmaMar, Samsara, Sanofi, Sotio, Vascage and Vasculox/Tioma. GK is on the Board of Directors of the Bristol Myers Squibb Foundation France. GK is a scientific co-founder of everImmune, Samsara Therapeutics and Therafast Bio. GK is the inventor of patents covering therapeutic targeting of aging, cancer, cystic fibrosis and metabolic disorders.

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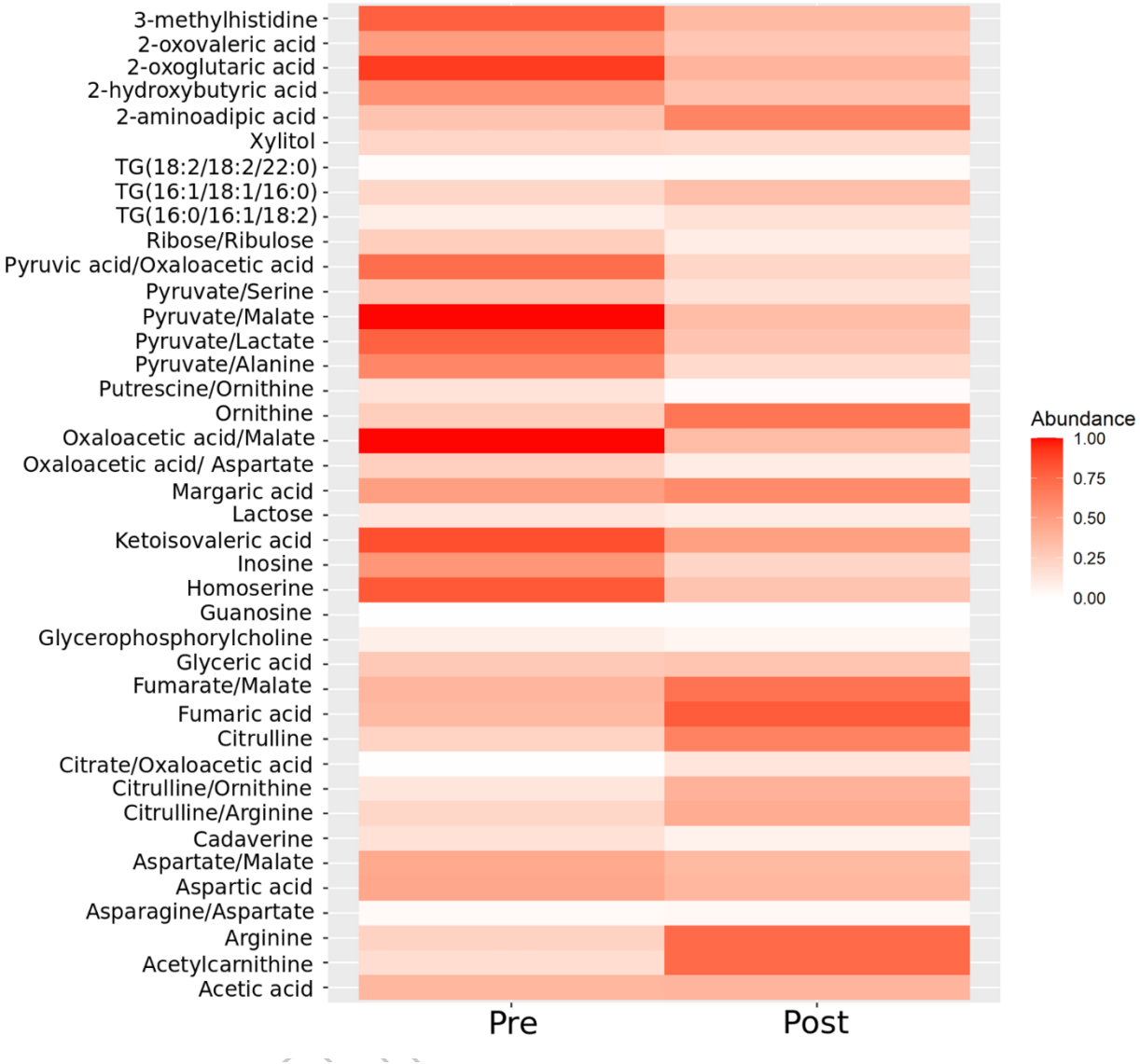

Figure 1 Heatmap showing the normalized abundance of the metabolites and metabolites ratio with significant intervention effect (Time effect). Pre = Before the 12-week intervention. Post = After the 12-week intervention.

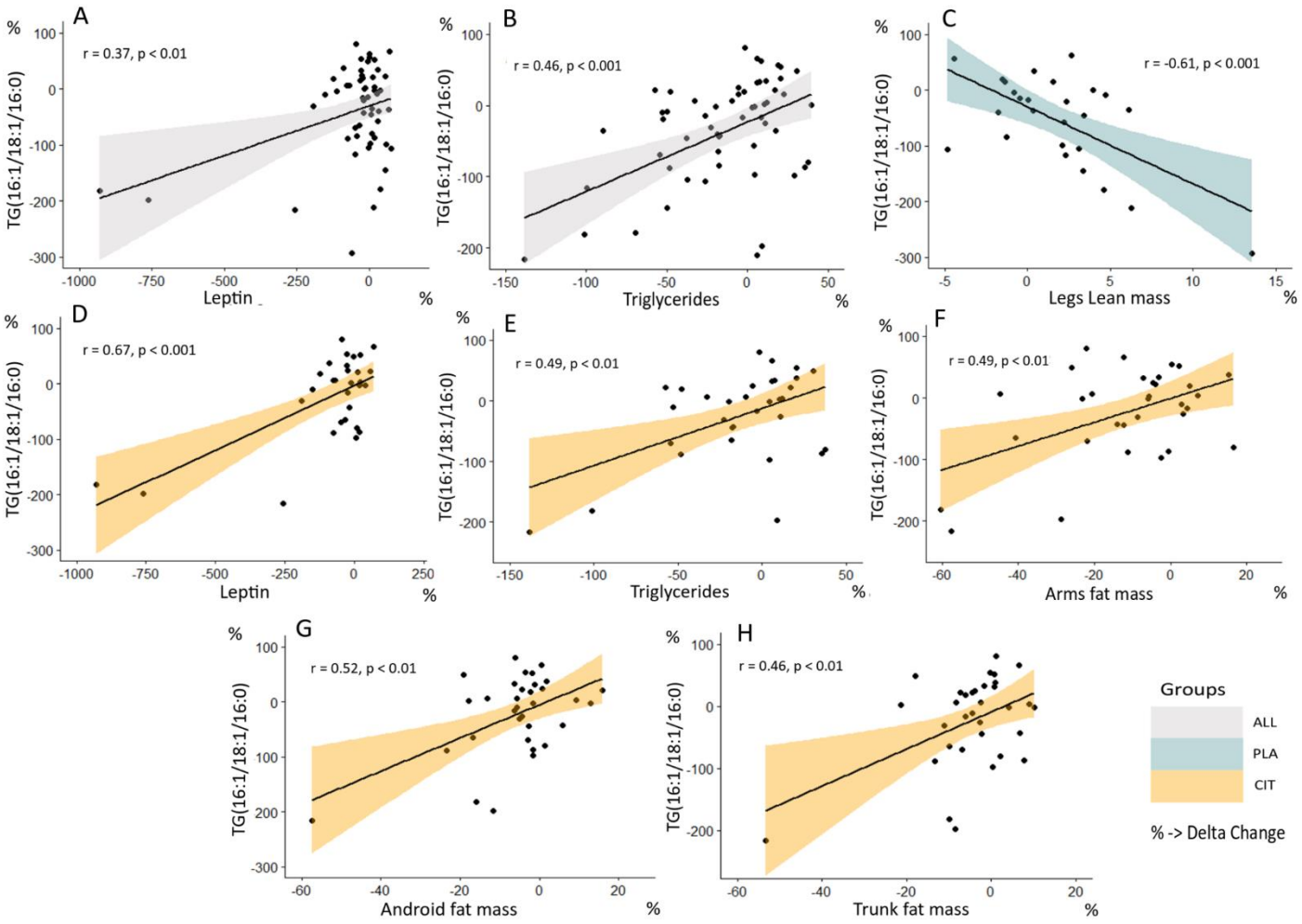

Figure 2: Significant correlations in the different groups between TG(16:1/18:1/16:0) delta change (%) and several adiposity parameters delta change (%).
Leptin (A and D). Triglycerides level (B and E). Legs lean mass (C). Arms fat mass (F). Android fat mass (G). Trunk fat mass (H). All = No supplement distinction. PLA = Placebo supplementation group. CIT = Citrulline supplementation group. Correlation analyses were performed using Pearson's correlation coefficient analysis; r = correlation coefficient.

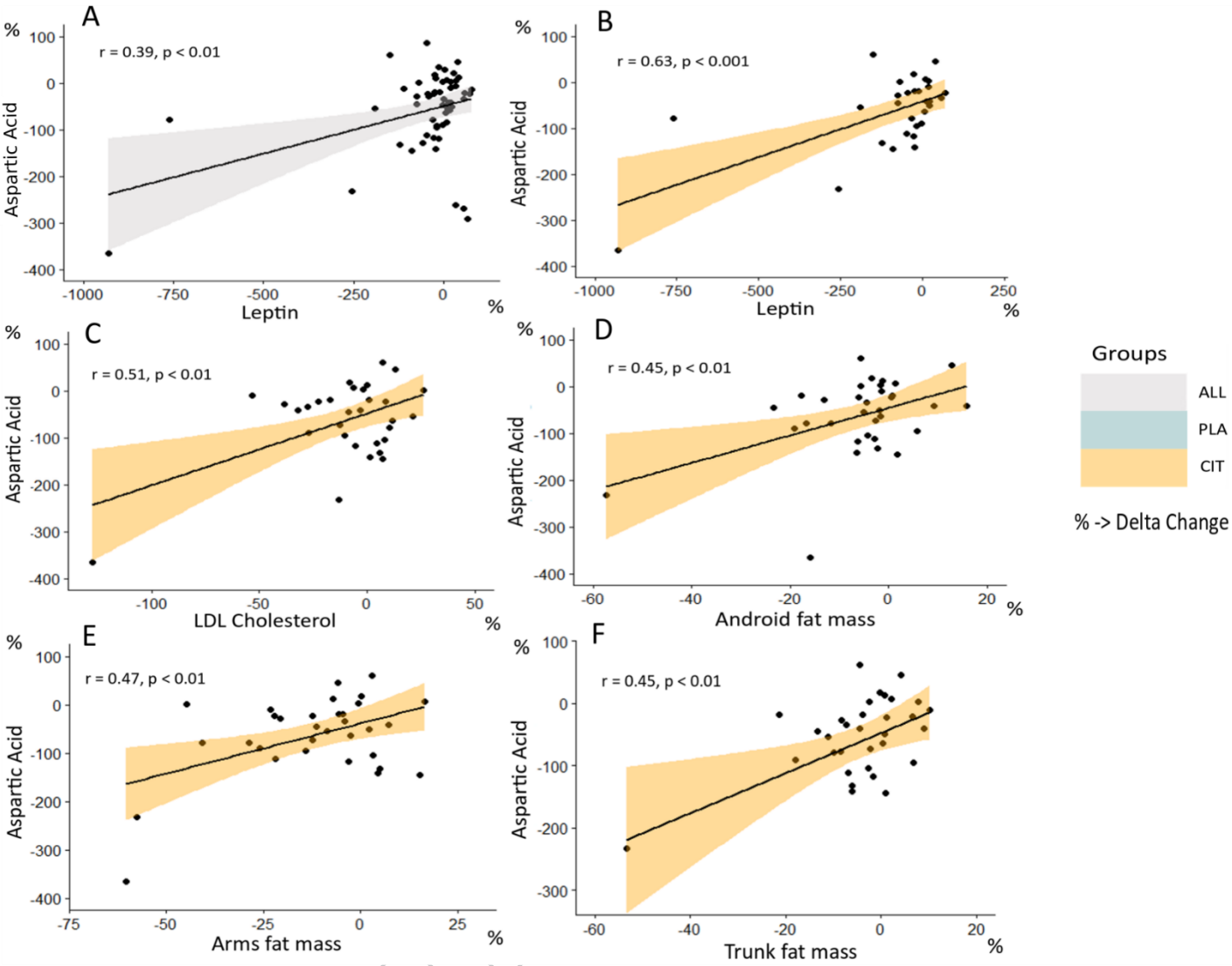

Figure 3: Significant correlations in the different groups between Aspartic acid delta change (%) and several adiposity parameters delta change (%).
Leptin (A and B). LDL cholesterol (C). Android fat mass (D). Arms fat mass (E). Trunk fat mass (F). All = No supplement distinction. PLA = Placebo supplementation group. CIT = Citrulline supplementation group. Correlation analyses were performed using Pearson's correlation coefficient analysis; r = correlation coefficient.

Table 1: Effect of a 12-week High-Intensity Interval Training (HIIT) combined to placebo (PLA) or citrulline (CIT) supplementation on metabolites of different metabolisms in obese older adults.

Metabolites (a.u) and ratios of metabolites	HIIT - PLA		HIIT - CIT		*p*-value		
	Pre (n = 44)	**Post** (n = 28)	**Pre** (n = 39)	**Post** (n = 42)	Time effect	Supp effect	Time×Supp effect
Carbohydrate metabolism							
Acetic Acid	$3.71 \times 10^4 \pm 4.19 \times 10^3$	$3.39 \times 10^4 \pm 5.29 \times 10^{3\,\#}$	$3.70 \times 10^4 \pm 4.52 \times 10^3$	$3.58 \times 10^4 \pm 6.69 \times 10^3$	0.018**	0.379	0.270
Glyceric acid	$1.98 \times 10^6 \pm 5.92 \times 10^5$	$1.59 \times 10^6 \pm 3.82 \times 10^{5\,\#\#\#}$	$2.00 \times 10^6 \pm 7.36 \times 10^5$	$1.70 \times 10^6 \pm 5.36 \times 10^{5\,\#\#}$	<0.0001***	0.472	0.237
Lactose	$8.74 \times 10^4 \pm 7.85 \times 10^4$	$5.65 \times 10^4 \pm 1.75 \times 10^{4\,\#\#}$	$6.34 \times 10^4 \pm 3.64 \times 10^4$	$5.55 \times 10^4 \pm 2.49 \times 10^4$	0.013*	0.089*	0.150
Ribose and Ribulose	$1.76 \times 10^5 \pm 2.42 \times 10^5$	$9.45 \times 10^4 \pm 4.30 \times 10^{4\,\#}$	$1.88 \times 10^5 \pm 1.19 \times 10^5$	$1.54 \times 10^5 \pm 1.08 \times 10^5$	0.038*	0.197	0.327
Xylitol	$2.81 \times 10^4 \pm 1.19 \times 10^4$	$2.99 \times 10^4 \pm 6.62 \times 10^3$	$2.58 \times 10^4 \pm 1.05 \times 10^4$	$3.10 \times 10^4 \pm 1.54 \times 10^{4\,\#\#\#}$	0.002**	0.636	0.067
Pyruvate / Alanine	$1.22 \times 10^{-2} \pm 1.13 \times 10^{-2}$	$1.63 \times 10^{-2} \pm 7.87 \times 10^{-3}$	$1.30 \times 10^{-2} \pm 8.41 \times 10^{-3}$	$1.46 \times 10^{-2} \pm 7.91 \times 10^{-3}$	0.034*	0.877	0.586
Pyruvate / Lactate	$3.09 \times 10^{-3} \pm 2.07 \times 10^{-3}$	$4.71 \times 10^{-3} \pm 2.49 \times 10^{-3\#\#}$	$3.15 \times 10^{-3} \pm 1.93 \times 10^{-3}$	$4.22 \times 10^{-3} \pm 2.20 \times 10^{-3\,\#}$	<0.001***	0.601	0.437
Pyruvate / Malate	$3.50 \times 10^0 \pm 2.61 \times 10^0$	$4.96 \times 10^0 \pm 2.60 \times 10^{0\,\#}$	$3.89 \times 10^0 \pm 2.80 \times 10^0$	$4.57 \times 10^0 \pm 2.47 \times 10^0$	0.006**	0.926	0.473
Pyruvate / Serine	$4.05 \times 10^{-1} \pm 0.55 \times 10^{-1}$	$5.65 \times 10^{-1} \pm 5.31 \times 10^{-1}$	$2.73 \times 10^{-1} \pm 2.04 \times 10^{-1}$	$3.87 \times 10^{-1} \pm 2.85 \times 10^{-1}$	0.05*	0.037*	0.792
Urea metabolism							
Urea	$5.93 \times 10^8 \pm 1.40 \times 10^8$	$5.90 \times 10^8 \pm 1.43 \times 10^8$	$5.22 \times 10^8 \pm 1.55 \times 10^8$	$5.96 \times 10^8 \pm 1.65 \times 10^{8\,\#}$	0.116	0.229	0.071
Cadaverine	$3.11 \times 10^4 \pm 1.96 \times 10^4$	$2.77 \times 10^4 \pm 2.11 \times 10^4$	$3.34 \times 10^4 \pm 2.01 \times 10^4$	$2.19 \times 10^4 \pm 1.55 \times 10^{4\,\#\#}$	0.012*	0.675	0.204
Inosine	$1.87 \times 10^4 \pm 6.07 \times 10^3$	$1.50 \times 10^4 \pm 3.94 \times 10^{3\,\#\#\#}$	$1.55 \times 10^4 \pm 5.23 \times 10^3$	$1.35 \times 10^4 \pm 2.90 \times 10^{3\,\#}$	<0.001***	<0.01**	0.246
Ornithine	$7.27 \times 10^6 \pm 1.84 \times 10^6$	$8.01 \times 10^6 \pm 2.42 \times 10^6$	$7.52 \times 10^6 \pm 2.26 \times 10^6$	$9.31 \times 10^6 \pm 2.66 \times 10^{6\,\#\#\#}$	<0.001***	0.115	0.097
Arginine	$9.05 \times 10^7 \pm 2.07 \times 10^7$	$8.38 \times 10^7 \pm 2.23 \times 10^7$	$8.75 \times 10^7 \pm 2.31 \times 10^7$	$1.09 \times 10^8 \pm 4.15 \times 10^{7\,\#\#\#}$	0.016*	0.100*	<0.001***
Citrulline	$4.08 \times 10^5 \pm 2.70 \times 10^5$	$4.36 \times 10^5 \pm 2.20 \times 10^5$	$3.87 \times 10^5 \pm 2.25 \times 10^5$	$5.96 \times 10^5 \pm 3.89 \times 10^{5\,\#\#\#}$	0.004**	0.196	0.032*
Asparagine / Aspartate	$1.72 \times 10^{-2} \pm 1.58 \times 10^{-2}$	$2.27 \times 10^{-2} \pm 2.86 \times 10^{-2}$	$9.34 \times 10^{-3} \pm 8.49 \times 10^{-3}$	$1.59 \times 10^{-2} \pm 1.53 \times 10^{-2}$	0.023*	0.032*	0.786
Citrulline / Arginine	$4.66 \times 10^{-3} \pm 2.94 \times 10^{-3}$	$5.99 \times 10^{-3} \pm 4.38 \times 10^{-3}$	$4.54 \times 10^{-3} \pm 2.36 \times 10^{-3}$	$6.22 \times 10^{-3} \pm 4.75 \times 10^{-3\,\#}$	0.011**	0.852	0.755
Citrulline / Ornithine	$5.66 \times 10^{-2} \pm 3.49 \times 10^{-2}$	$6.22 \times 10^{-2} \pm 4.21 \times 10^{-2}$	$5.43 \times 10^{-2} \pm 3.25 \times 10^{-2}$	$7.13 \times 10^{-2} \pm 5.70 \times 10^{-2\,\#}$	0.058*	0.605	0.293
Putrescine / Ornithine	$2.77 \times 10^{-2} \pm 1.10 \times 10^{-2}$	$2.67 \times 10^{-2} \pm 1.18 \times 10^{-2}$	$2.95 \times 10^{-2} \pm 1.21 \times 10^{-2}$	$2.24 \times 10^{-2} \pm 1.11 \times 10^{-2\,\#\#\#}$	0.003**	0.465	0.026*
TCA cycle							
Fumaric acid	$2.14 \times 10^5 \pm 3.66 \times 10^4$	$2.39 \times 10^5 \pm 2.64 \times 10^{4\,\#\#}$	$1.96 \times 10^5 \pm 3.66 \times 10^4$	$2.29 \times 10^5 \pm 4.64 \times 10^{4\,\#\#\#}$	<0.001***	0.029*	0.495
Aspartic acid	$3.25 \times 10^5 \pm 1.89 \times 10^5$	$2.59 \times 10^5 \pm 8.25 \times 10^{4\,\#}$	$4.37 \times 10^5 \pm 1.96 \times 10^5$	$2.80 \times 10^5 \pm 1.31 \times 10^{5\,\#\#\#}$	<0.0001***	0.011*	0.132
Pyruvic acid and Oxaloacetic acid	$3.17 \times 10^5 \pm 2.65 \times 10^5$	$4.92 \times 10^5 \pm 3.07 \times 10^{5\,\#\#}$	$3.20 \times 10^5 \pm 2.37 \times 10^5$	$3.97 \times 10^5 \pm 2.29 \times 10^5$	0.001**	0.377	0.348
2-oxoglutaric acid	$5.12 \times 10^4 \pm 2.00 \times 10^4$	$6.89 \times 10^4 \pm 2.87 \times 10^{4\,\#\#}$	$5.01 \times 10^4 \pm 2.47 \times 10^4$	$6.04 \times 10^4 \pm 2.88 \times 10^{4\,\#}$	<0.001**	0.291	0.469
3-methylhistidine	$1.40 \times 10^4 \pm 3.72 \times 10^3$	$1.18 \times 10^4 \pm 3.47 \times 10^{3\,\#}$	$1.31 \times 10^4 \pm 4.25 \times 10^3$	$1.16 \times 10^4 \pm 2.89 \times 10^3$	0.002**	0.318	0.570
Fumarate / Malate	$2.42 \times 10^0 \pm 3.64 \times 10^{-1}$	$2.57 \times 10^0 \pm 4.13 \times 10^{-1}$	$2.43 \times 10^0 \pm 4.13 \times 10^{-1}$	$2.74 \times 10^0 \pm 5.12 \times 10^{-1\,\#\#}$	<0.001***	0.269	0.321
Oxaloacetic acid / Malate	$3.50 \times 10^0 \pm 2.61 \times 10^0$	$4.96 \times 10^0 \pm 2.60 \times 10^{0\,\#}$	$3.89 \times 10^0 \pm 2.80 \times 10^0$	$4.57 \times 10^0 \pm 2.47 \times 10^0$	0.006**	0.926	0.473
Citrate / Oxaloacetic acid	$3.67 \times 10^1 \pm 2.47 \times 10^1$	$2.08 \times 10^1 \pm 1.07 \times 10^{1\,\#\#}$	$3.04 \times 10^1 \pm 2.10 \times 10^1$	$3.07 \times 10^1 \pm 2.81 \times 10^1$	0.023*	0.749	0.035*
Oxaloacetic acid / Aspartate	$1.35 \times 10^0 \pm 1.65 \times 10^0$	$2.22 \times 10^0 \pm 1.70 \times 10^{0\,\#\#}$	$8.43 \times 10^{-1} \pm 6.57 \times 10^{-1}$	$1.60 \times 10^0 \pm 1.12 \times 10^{0\,\#\#}$	<0.001***	0.017*	0.872
Aspartate / Malate	$3.45 \times 10^0 \pm 1.03 \times 10^0$	$2.77 \times 10^0 \pm 1.05 \times 10^{0\,\#}$	$5.26 \times 10^0 \pm 2.23 \times 10^0$	$3.27 \times 10^0 \pm 1.44 \times 10^{0\,\#\#\#}$	<0.001***	<0.001***	0.018*
Fat metabolism							
Acetylcarnitine	$4.76 \times 10^8 \pm 1.55 \times 10^8$	$3.91 \times 10^8 \pm 1.48 \times 10^{8\,\#}$	$4.52 \times 10^8 \pm 1.85 \times 10^8$	$3.88 \times 10^8 \pm 1.61 \times 10^8$	0.005**	0.607	0.597
Margaric acid	$3.58 \times 10^5 \pm 8.11 \times 10^5$	$4.09 \times 10^5 \pm 8.14 \times 10^{4\,\#\#}$	$3.53 \times 10^5 \pm 8.25 \times 10^5$	$3.71 \times 10^5 \pm 7.56 \times 10^5$	0.021*	0.140	0.212
Glycerophosphorylcholine	$4.91 \times 10^6 \pm 1.71 \times 10^6$	$5.41 \times 10^6 \pm 1.99 \times 10^6$	$4.90 \times 10^6 \pm 1.63 \times 10^6$	$7.04 \times 10^6 \pm 8.09 \times 10^{6\,\#}$	0.032*	0.390	0.236
TG (12:0/12:0/16:1)	$3.10 \times 10^3 \pm 3.81 \times 10^3$	$1.64 \times 10^3 \pm 1.46 \times 10^3$	$1.69 \times 10^3 \pm 1.26 \times 10^3$	$8.37 \times 10^3 \pm 3.50 \times 10^4$	0.702	0.375	0.025*
TG (14:0/16:0/16:0)	$6.97 \times 10^5 \pm 3.85 \times 10^5$	$5.18 \times 10^5 \pm 2.55 \times 10^5$	$4.79 \times 10^5 \pm 2.77 \times 10^5$	$7.22 \times 10^5 \pm 1.05 \times 10^6$	0.813	0.943	0.030*
TG (16:0/16:1/18:2)	$4.99 \times 10^6 \pm 4.14 \times 10^6$	$3.35 \times 10^6 \pm 2.06 \times 10^{6\,\#}$	$3.46 \times 10^6 \pm 2.20 \times 10^6$	$3.10 \times 10^6 \pm 1.95 \times 10^6$	0.014*	0.056*	0.148
TG (16:1/18:1/16:0)	$1.04 \times 10^7 \pm 6.49 \times 10^6$	$7.50 \times 10^6 \pm 4.22 \times 10^{6\,\#\#}$	$7.26 \times 10^6 \pm 4.48 \times 10^6$	$7.05 \times 10^6 \pm 3.58 \times 10^6$	0.018*	0.041*	0.043*
TG (18:2/18:2/22:0)	$3.00 \times 10^4 \pm 1.79 \times 10^4$	$3.02 \times 10^4 \pm 2.55 \times 10^4$	$3.57 \times 10^4 \pm 2.83 \times 10^4$	$6.26 \times 10^4 \pm 2.43 \times 10^{5\,\#}$	0.044*	0.301	0.135
Undecanoic acid	$1.24 \times 10^5 \pm 5.32 \times 10^4$	$9.59 \times 10^4 \pm 3.39 \times 10^{4\,\#}$	$1.13 \times 10^5 \pm 4.97 \times 10^4$	$1.31 \times 10^5 \pm 7.56 \times 10^4$	0.831	0.298	0.015*

Amino acids metabolisms							
Homoserine	$2.22 \times 10^7 \pm 4.63 \times 10^6$	$2.11 \times 10^7 \pm 4.54 \times 10^6$	$2.19 \times 10^7 \pm 4.53 \times 10^6$	$1.93 \times 10^7 \pm 5.33 \times 10^6$ ##	0.005**	0.253	0.276
2-hydroxybutyric acid	$5.32 \times 10^4 \pm 1.66 \times 10^4$	$4.31 \times 10^4 \pm 1.45 \times 10^4$ #	$5.07 \times 10^4 \pm 2.93 \times 10^4$	$4.22 \times 10^4 \pm 1.92 \times 10^4$	0.004**	0.583	0.507
2-aminoadipic acid	$2.04 \times 10^5 \pm 1.14 \times 10^5$	$2.41 \times 10^5 \pm 8.07 \times 10^4$	$2.17 \times 10^5 \pm 1.17 \times 10^5$	$3.04 \times 10^5 \pm 1.55 \times 10^5$ ###	<0.001**	0.088*	0.154
Nucleic acid metabolism							
Guanosine	$1.67 \times 10^6 \pm 2.30 \times 10^6$	$2.76 \times 10^6 \pm 3.45 \times 10^6$	$9.54 \times 10^5 \pm 1.57 \times 10^6$	$3.39 \times 10^6 \pm 5.52 \times 10^6$ ##	<0.001**	0.771	0.189
Leucine metabolism							
2-oxovaleric acid	$6.41 \times 10^3 \pm 1.51 \times 10^3$	$5.40 \times 10^3 \pm 1.53 \times 10^3$ #	$6.14 \times 10^3 \pm 1.68 \times 10^3$	$5.25 \times 10^3 \pm 1.74 \times 10^3$ #	<0.001**	0.418	0.822
BCAA metabolism							
Ketoisovaleric acid	$9.91 \times 10^4 \pm 3.14 \times 10^4$	$1.13 \times 10^5 \pm 2.92 \times 10^4$	$9.32 \times 10^4 \pm 3.36 \times 10^4$	$9.79 \times 10^4 \pm 3.15 \times 10^4$	0.033*	0.102	0.527

Data are presented as: mean of abundance unit ± SD. a.u: abundance unit. HIIT = High-Intensity Interval Training; PLA = Placebo supplementation; CIT = Citrulline supplementation; Pre = before the 12-week intervention; Post = after the 12-week intervention; Supp = Supplement; TCA = Tricarboxylic acid cycle; BCAA = Branched chain amino acids; TG = Triglyceride; / = ratio. *: $p < 0.05$, **: $p < 0.01$, ***: $p < 0.001$ = Time, Supp, as well as Time×Supp effects (analyzed using two-ways repeated measures ANOVA). #: $p < 0.05$; ##: $p < 0.01$; ###: $p < 0.001$ = Significant intra-group differences between pre and post intervention using two-ways repeated measures ANOVA followed by post-hoc analyses done with simultaneous tests for general linear hypotheses

Table S1-a: False Discovery Rate Analysis (FDR) on the 44 significant metabolites obtained following a 12-week High-Intensity Interval Training (HIIT) combined to a placebo (PLA) or citrulline (CIT) supplementation in obese older adults

Metabolites	ANOVA		FDR (BH)	
	Time effect	Time×Supp effect	Time effect	Time×Supp effect
Carbohydrate metabolism				
Acetic Acid	0.016**	0.263	0.214	0.847
Glyceric acid	<0.001***	0.249	0.007**	0.847
Lactose	0.023*	0.150	0.265	0.825
Ribose and Ribulose	0.026*	0.349	0.282	0.847
Xylitol	0.002**	0.065	0.048*	0.954
Pyruvate / Alanine	0.034*	0.584	0.327	0.919
Pyruvate / Lactate	<0.001***	0.480	0.018*	0.886
Pyruvate / Malate	0.007**	0.473	0.116	0.886
Pyruvate / Serine	0.030*	0.782	0.3	0.964
Urea metabolism				
Urea	0.071	0.057*	0.545	0.825
Cadaverine	0.012*	0.193	0.181	0.825
Inosine	<0.001***	0.236	0.015*	0.847
Ornithine	<0.001***	0.121	0.012*	0.825
Arginine	0.022*	<0.001***	0.265	0.18
Citrulline	0.007**	0.031*	0.116	0.825
Asparagine / Aspartate	0.023*	0.719	0.265	0.954
Citrulline / Arginine	0.013**	0.777	0.191	0.963
Citrulline / Ornithine	0.057*	0.261	0.512	0.847
Putrescine/ Ornithine	0.004*	0.026*	0.084	0.825

FDR = False Discovery Rate, BH = Benjamini-Hochberg, / = ratio, *: < 0.05, **: < 0.01, ***: < 0.001.

Table S1-b: False Discovery Rate Analysis (FDR) on the 44 significant metabolites obtained following a 12-week High-Intensity Interval Training (HIIT) combined to a placebo (PLA) or citrulline (CIT) supplementation in obese older adults

Metabolites	ANOVA		FDR (BH)	
	Time effect	Time×Supp effect	Time effect	Time×Supp effect
TCA cycle				
Fumaric acid	<0.001***	0.527	0.005**	0.985
Aspartic acid	<0.001***	0.114	0.007**	0.825
Pyruvic acid and Oxaloacetic acid	<0.001***	0.325	0.038*	0.847
2-oxoglutaric acid	<0.001***	0.458	0.018*	0.886
3-methylhistidine	0.003**	0.526	0.081	0.890
Fumarate / Malate	<0.001***	0.286	0.018*	0.847
Oxaloacetic acid / Malate	0.007**	0.473	0.116	0.886
Citrate / Oxaloacetic acid	0.024*	0.034*	0.273	0.825
Oxaloacetic acid/ Aspartate	<0.001***	0.992	0.007**	1.00
Aspartate / Malate	<0.001***	0.014*	0.007**	0.825
Fat metabolism				
Acetylcarnitine	0.008**	0.568	0.129	0.915
Margaric acid	0.015*	0.195	0.206	0.825
Glycerophosphoryl-choline	0.045*	0.248	0.412	0.847
TG(12:0/12:0/16:1)	0.723	0.027*	0.889	0.825
TG(14:0/16:0/16:0)	0.818	0.032*	0.898	0.825
TG(16:0/16:1/18:2)	0.023*	0.127	0.265	0.825
TG(16:1/18:1/16:0)	0.028*	0.037*	0.291	0.825
TG(18:2/18:2/22:0)	0.044*	0.122	0.412	0.825
Undecanoic acid	0.726	0.014*	0.889	0.825
Other metabolisms				
Homoserine	0.008**	0.279	0.130	0.847
2-hydroxybutyrice acid	0.004**	0.550	0.084	0.905
2-aminoadipic acid	<0.001**	0.161	0.036*	0.825
Guanosine	<0.001**	0.189	0.038*	0.825
2-oxovaleric acid	<0.001**	0.726	0.036*	0.954
Ketosiovaleric acid	0.033*	0.544	0.327	0.904

FDR = False Discovery Rate, BH = Benjamini-Hochberg, TCA = Tricarboxylic acid, / = ratio, *: < 0.05, **: < 0.01, ***: < 0.001

Table S2-a: Values of the measured clinical parameters significantly correlated to the significant serum metabolites.

	Intervention (n=59)		HIIT-PLA (n=26)		HIIT-CIT (n=33)		*p*-value	
	Pre	Post	Pre	Post	Pre	Post	Time effect	Time×Supp effect

Clinical Parameters

Body weight (kg)	81.4 ± 14.0	80.9 ± 13.9	82.1 ± 14.3	81.9 ± 14.2	80.8 ± 13.9	80.1 ± 13.9	0.14	0.34
Hip circumference (cm)	107.3 ± 9.9	105.2 ± 9.7$^{###}$	109.6 ± 10.6	107.3 ± 10.2$^{###}$	105.5 ± 9.2	103.4 ± 9.0$^{###}$	< 0.0001***	0.98
Calf circumference (cm)	38.8 ± 3.3	38.9 ± 3.1	39.6 ± 3.6	39.4 ± 3.6	38.2 ± 2.8	38.6 ± 2.5	0.86	0.03*
Thigh circumference (cm)	53.6 ± 6.1	54.2 ± 5.4	55.0 ± 6.7	54.7 ± 6.0	52.4 ± 5.4	53.7 ± 4.9$^{##}$	0.06	0.01*
Total fat mass (kg)	30.6 ± 8.9	29.7 ± 8.7$^{##}$	31.0 ± 9.6	30.7 ± 9.3	30.2 ± 8.5	28.9 ± 8.2$^{###}$	0.03*	0.06
Relative total fat mass (%)	37.4 ± 6.9	36.4 ± 6.8$^{###}$	37.7 ± 7.9	37.2 ± 7.7	37.1 ± 6.3	35.8 ± 6.1$^{###}$	0.0005***	0.12
Arm fat mass (kg)	3.2 ± 0.9	2.9 ± 0.9$^{##}$	3.3 ± 1.1	2.9 ± 1.0$^{#}$	3.1 ± 0.7	2.8 ± 0.8$^{#}$	0.002**	0.55
Relative arms fat mass (%)	35.8 ± 9.1	34.3± 8.9	35.9 ± 10.7	33.1 ± 10.3	35.7 ± 7.7	33.6 ± 7.7$^{###}$	<0.0001***	0.04*
Leg fat mass (kg)	10.1 ± 3.7	9.7 ± 3.6$^{##}$	10.2 ± 4.2	10.1 ± 4.1	9.9 ± 3.5	9.5 ± 3.3$^{##}$	0.004**	0.22
Relative legs fat mass (%)	35.5 ± 9.9	34.5 ± 9.8$^{##}$	35.8 ± 11.1	35.1 ± 11.0	35.2 ± 9.1	33.9 ± 8.9$^{##}$	0.0008***	0.32
Android fat mass (kg)	3.3 ± 1.2	3.2 ± 1.2	3.3 ± 1.1	3.3 ± 1.1	3.3 ± 1.2	3.2 ± 1.2$^{##}$	0.06	0.05
Relative android fat mass (%)	46.7 ± 7.8	45.7 ± 7.8	47.1 ± 7.4	46.6 ± 7.5	46.3 ± 8.2	44.9 ± 7.9$^{##}$	0.01*	0.17
Gynoid fat mass (kg)	4.9 ± 1.6	4.8 ± 1.6	4.9 ± 1.7	5.0 ± 1.7	4.9 ± 1.5	4.7 ± 1.5	0.28	0.28
Relative gynoid fat mass (%)	39.7 ± 9.3	39.0 ± 9.4	39.8 ± 10.5	39.6 ± 10.3	39.6 ± 8.5	38.5 ± 8.8$^{#}$	0.04*	0.26
Relative trunk fat mass (%)	40.9 ± 7.5	40.1 ± 7.5$^{#}$	41.7 ± 7.1	41.3 ± 6.9	40.4 ± 7.9	39.1 ± 7.8$^{##}$	0.01*	0.19
Trunk fat mass (kg)	16.5 ± 5.9	16.2 ± 5.9	16.6 ± 5.8	16.8 ± 5.9	16.3 ± 6.1	15.7 ± 5.9$^{#}$	0.16	0.04*
Relative intramuscular Fat Area (%)	5.1 ± 2.6	4.3 ± 2.3	5.1 ± 2.3	4.8 ± 2.3	5.1 ± 2.8	3.9 ± 2.2	0.11	0.30
Leg lean mass (kg)	16.8 ± 3.1	17.1 ± 3.1$^{##}$	16.8 ± 3.4	17.2 ± 3.6	16.8 ± 2.8	17.0 ± 2.7$^{#}$	0.007**	0.54
Daily Energy Expenditure (kcal.d^{-1})	2235.1 ± 385.1	2213.2 ± 350.1	2308.2 ± 466.7	2213.9 ± 406.4	2171.7 ± 290.7	2212.6 ± 311.2	0.60	0.07
Resting heart rate	67.2 ± 9.9	67.9 ± 9.3	65.2 ± 11.8	65.0 ± 9.3	68.6 ± 8.2	70.3 ± 8.8	0.41	0.48
Diastolic blood pressure	75.0 ± 8.9	73.9 ± 7.1	73.4 ± 10.6	73.2 ± 8.05	76.2 ± 7.2	74.5 ± 6.3	0.30	0.46

Data are presented as: mean ± SD. HIIT = High-Intensity Interval Training; PLA = Placebo supplementation; CIT = Citrulline supplementation; Pre = before the intervention; Post = after the intervention; *: $p < 0.05$, **: $p < 0.01$, ***: $p < 0.001$ = Significant Time, as well as Time×Supp effects analyzed using two-ways repeated measures ANOVA. #: $p < 0.05$; ##: $p < 0.01$; ###: $p < 0.001$ = Significant intra-group differences between pre and post intervention using two-ways repeated measures ANOVA followed by post-hoc analyses done with simultaneous tests for general linear hypotheses.

Table S2-b: Values of the measured biological parameters significantly correlated to the significant serum metabolites**.**

Data are presented as: mean ± SD. HIIT = High-Intensity Interval Training; PLA = Placebo supplementation; CIT = Citrulline supplementation; Pre = before the intervention; Post = after the intervention; *: $p < 0.05$, **: $p < 0.01$, ***: $p < 0.001$ = Significant Time, as well as Time×Supp effects analyzed using two-ways repeated measures ANOVA. #: $p < 0.05$; ##: $p < 0.01$; ###: $p < 0.001$ = Significant intra-group differences between pre and post intervention using two-ways repeated measures ANOVA followed by post-hoc analyses done with simultaneous tests for general linear hypotheses.

	Intervention (n=59)		**HIIT-PLA (n=26)**		**HIIT-CIT (n=33)**		*p*-value	
	Pre	Post	Pre	Post	Pre	Post	Time effect	Time×Supp effect
Biological Parameters								
Adiponectin (ug.mL^{-1})	14.2 ± 8.5	14.5 ± 9.5	14.5 ± 7.9	14.2 ± 8.0	14.1 ± 8.9	14.7 ± 10.7	0.75	0.50
Leptin (ng.mL^{-1})	25.4 ± 19.7	23.4 ± 19.3	22.7 ± 18.8	26.2 ± 21.4	27.6 ± 20.4	21.0 ± 17.4$^{\#}$	0.34	0.02*
Glucose (mmol.L^{-1})	5.7 ± 1.0	5.9 ± 1.1	5.9 ± 1.3	6.0 ± 1.4	5.5 ± 0.8	5.7 ± 0.7	0.06	0.34
Insulin (pmol.L^{-1})	50.8 ± 25.7	51.3 ± 28.7	49.1 ± 23.5	49.0 ± 29.3	52.2 ± 27.6	53.1 ± 28.4	0.93	0.93
HOMA-IR	2.2 ± 1.2	2.3 ± 1.5	2.2 ± 1.2	2.2 ± 1.4	2.2 ± 1.3	2.4 ± 1.5	0.51	0.60
HDL-Cholesterol (mmol.L^{-1})	1.4 ± 0.3	1.4 ± 0.3	1.4 ± 0.4	1.4 ± 0.3	1.4 ± 0.3	1.4 ± 0.3	0.45	0.33
LDL-Cholesterol (mmol.L^{-1})	2.9 ± 0.9	2.9 ± 0.8	2.8 ± 0.9	2.9 ± 0.9	3.1 ± 0.9	2.8 ± 0.7	0.34	0.09
Triglycerides (mmol.L^{-1})	1.5 ± 0.8	1.3 ± 0.7$^{\#\#}$	1.5 ± 0.7	1.3 ± 0.7$^{\#}$	1.4 ± 0.8	1.3 ± 0.7	0.009**	0.81
IGFBP3 (µg.mL^{-1})	1.8 ± 0.4	1.9 ± 0.4	1.8 ± 0.4	1.9 ± 0.3	1.9 ± 0.5	1.9 ± 0.5	0.31	0.26
IGF-1 (ng.mL^{-1})	90.4 ± 25.2	93.6 ± 27.0	86.0 ± 19.6	90.4 ± 18.9	94.1 ± 28.9	96.3 ± 32.5	0.14	0.62
Free fatty acids (mmol.L^{-1})	0.5 ± 0.1	0.5 ± 0.16	0.5 ± 0.15	0.4 ± 0.5	0.4 ± 0.2	0.5 ± 0.1	0.49	0.08

	Intervention (n=59)	**HIIT-PLA (n=26)**	**HIIT-CIT (n=33)**
Clinical Parameters			

Body weight	Aspartic acid:0.37**	-	-
Hip circumference	Guanosine: - 0.49***	-	Guanosine: -0.64***
Calf circumference	Fumaric acid: 0.48*** Lactose: 0.74*** Xylitol: 0.41** 3-methylhistidine: 0.35**	Homoserine: 0.58** Lactose: 0.82***	Fumaric acid: 0.60*** Lactose: 0.46** 2-aminoadipic acid: 0.48** Citrulline: 0.45**
Thigh circumference	Lactose: 0.39** Putrescine/Ornithine: -0.35**	Lactose: 0.51**	Aspartic acid: 0.50** Cadaverine: 0.48** TG (14:0/16:0/16:0): 0.51**
Total fat mass	-	Guanosine: 0.55** Pyruvate/Serine: 0.51** Oxaloacetic acid/Aspartate: 0.56**	-
Relative total fat mass	-	Guanosine: 0.55** Pyruvate/Serine: 0.53** Oxaloacetic acid/Aspartate: 0.57**	-
Arm fat mass	2-hydroxybutyric acid: 0.41**	-	Aspartic acid: 0.47** Fumaric acid: 0.47** 2-oxovaleric acid: 0.5** TG (16:1/18:1/16:0): 0.49**
Relative arms fat mass	Inosine: 0.36** 2-oxovaleric acid: 0.36**	Pyruvate/Serine: 0.55** Oxaloacetic acid/Aspartate: 0.56**	Aspartic acid: 0.48** 2-oxovaleric acid: 0.6***
Leg fat mass	Inosine: 0.34** Lactose: 0.41**	Inosine: 0.62*** Lactose: 0.65***	-
Relative leg fat mass	Inosine: 0.36** Lactose: 0.40**	Inosine: 0.67*** Lactose: 0.66***	Fumarate/malate: -0.44**
Android fat mass	Pyruvate/Serine: 0.35**	Guanosine: 0.56** Pyruvate/Serine: 0.59** Oxaloacetic acid/Aspartate: 0.62***	Aspartic acid: 0.45** TG (12:0/12:0/16:1): 0.5** TG (16:1/18:1/16:0): 0.52**

Table S3-a: Significant correlations between clinical parameters delta changes and significant metabolites delta changes.
Delta changes (%) are estimated as: [(post-pre)/pre] ×100. HIIT = High-Intensity Interval Training, PLA = Placebo supplementation; CIT = Citrulline supplementation; / = ratio. Relative data are expressed in % at pre and post. Correlation analysis was performed using parametric Pearson's test. Significance: $^{**}p < 0.01$; $^{***}p < 0.001$; -: no significant correlations.

Table S3-b: Significant correlations between clinical parameters delta changes and significant metabolites delta changes
Delta changes (%) are estimated as: [(post-pre)/pre×100. HIIT = High-Intensity Interval Training, PLA = Placebo supplementation; CIT = Citrulline supplementation. Relative data are expressed in % at pre and post. Correlation analysis was performed using parametric Pearson's test.
Significance: $^{**}p < 0.01$; $^{***}p < 0.001$; -: no significant correlations.

	Intervention (n=59)	HIIT-PLA (n=26)	HIIT-CIT (n=33)
		Clinical Parameters	
Relative android fat mass	-	Acetic acid: -0.53**	TG (12:0/12:0/16:1): 0.49** TG (16:1/18:1/16:0): 0.50**
Gynoid fat mass	-	Inosine: 0.57** Pyruvate/Serine: 0.55** Oxaloacetic acid/Aspartate: 0.62***	-
Relative gynoid fat mass	-	Inosine: 0.6** Pyruvate/Serine: 0.53** Oxaloacetic acid/Aspartate: 0.59**	2-oxovaleric acid: 0.45**
Relative trunk fat mass	-	-	TG (12:0/12:0/16:1): 0.46** TG (16:1/18:1/16:0): 0.50**
Trunk fat mass	-	-	Aspartic acid: 0.45** TG (16:1/18:1/16:0): 0.46**
Relative intramuscular Fat Area	TG (18:2/18:2/22:0): 0.4**	-	TG (18:2/18:2/22:0): 0.66***
Leg lean mass	2-oxovaleric acid: -0.35**	TG (16:1/18:1/16:0): -0.61***	2-oxovaleric acid: -0.47**
Daily Energy Expenditure	-	-	Acetic acid: 0.53**
Resting heart rate	-	-	TG (16:1/18:1/16:0): 0.46**
Diastolic blood pressure	-	TG (12:0/12:0/16:1): 0.53**	-

Table S3-c: Significant correlations between biological parameters delta changes and significant metabolites delta changes

	Intervention (n=59)	HIIT-PLA (n=26)	HIIT-CIT (n=33)
		Biological Parameters	
Adiponectin	-	-	TG (14:0/16:0/16:0): 0.62*** Pyruvate/Alanine: -0.47**
Leptin	Aspartic acid: 0.39** Glycerin acid: 0.35** TG (14:0/16:0/16:0): 0.41** TG (16:1/18:1/16:0): 0.37** TG (18:2/18:2/22:0): 0.39**	TG (14:0/16:0/16:0): 0.59**	Aspartic acid: 0.63*** Glycerin acid: 0.59*** TG (14:0/16:0/16:0): 0.61*** TG (16:1/18:1/16:0): 0.67*** TG (18:2/18:2/22:0): 0.48**
Glucose	Arginine: 0.41** Urea: 0.34**	Arginine: 0.5**	-
Insulin	TG (14:0/16:0/16:0): 0.35**	Pyruvic acid and Oxaloacetic acid: -0.52** Citrate / Oxaloacetic acid: 0.55**	TG (14:0/16:0/16:0): 0.47**
HOMA-IR	TG (14:0/16:0/16:0): 0.37**	Pyruvic acid and Oxaloacetic acid: -0.52** Citrate / Oxaloacetic acid: 0.51**	TG (14:0/16:0/16:0): 0.52**
HDL-Cholesterol	Xylitol: - 0.39**	Ribose and Ribulose: 0.58**	-
LDL-Cholesterol	Inosine: 0.36** Ketoisovaleric acid: 0.40**	-	Inosine: 0.48** Aspartic acid: 0.51** TG (14:0/16:0/16:0): 0.48**
Triglycerides	TG (16:1/18:1/16:0): 0.46***	-	TG (16:1/18:1/16:0): 0.49**
IGFBP3	Lactose: 0.59***	Lactose: -0.71***	-
IGF-1	Inosine: -0.38** Lactose: -0.40**	Lactose: -0.57**	-
Free fatty acids	Ketoisovaleric acid: 0.40**	3-methylhistidine: 0.68***	Ketoisovaleric acid: 0.55** TG (16:0/16:1/18:2): 0.57*** 2-oxoglutaric acid: 0.51**

Delta changes (%) are estimated as: [(post-pre)/pre]×100. HIIT = High-Intensity Interval Training, PLA = Placebo supplementation; CIT = Citrulline supplementation. Relative data are expressed in % at pre and post. Correlation analysis was performed using parametric Pearson's test.

Significance: **$p < 0.01$; ***$p < 0.001$; -: no significant correlations.

Table S4-a: Significant correlations between clinical parameters delta changes and significant serum metabolites delta changes without outliers

	Intervention (n=59)	HIIT-PLA (n=26)	HIIT-CIT (n=33)
	Clinical Parameters		
Body weight	Aspartic acid : 0.37**	-	-
Hip circumference	-	-	Guanosine : -0.64***
Calf circumference	Fumaric acid: 0.48*** Lactose: 0.44*** Xylitol: 0.41** 3-methylhistidine: 0.35** Inosine: 0.46***	Homoserine: 0.58** Inosine: 0.67***	Lactose: 0.46** Citrulline: 0.45**
Thigh circumference	2-aminoadipic acid: 0.35** Putrescine/Ornithine: -0.35**	-	Aspartic acid: 0.50** Cadaverine: 0.48** TG (14:0/16:0/16:0): 0.51**
Total fat mass	-	Guanosine: 0.55**	-
Relative total fat mass	-	Guanosine: 0.55**	-
Arm fat mass	-	Guanosine: 0.55**	Aspartic acid: 0.47** Fumaric acid: 0.47** 2-oxovaleric acid: 0.50** TG (16:1/18:1/16:0): 0.49**
Relative arms fat mass	-	-	-
Leg fat mass	-	Inosine: 0.52**	-
Relative legs fat mass	-	Inosine: 0.52**	-
Android fat mass	-	Guanosine: 0.56**	Aspartic acid: 0.45** TG (12:0/12:0/16:1): 0.50** TG (16:1/18:1/16:0): 0.52**

Delta change (%) are estimated as: [(post-pre)/pre]×100. HIIT = High Intensity Interval Training, PLA: placebo, CIT: citrulline. Relative data are expressed in % at pre and post. Correlation analysis was performed using parametric Pearson's test; significance: $**p < 0.01$; $***p < 0.001$; -: no significant correlations

Table S4-b: Significant correlations between clinical parameters delta changes and significant serum metabolites delta changes without outliers

	Intervention (n=59)	HIIT-PLA (n=26)	HIIT-CIT (n=33)
	Clinical Parameters		
Relative android fat mass	-	-	TG (12:0/12:0/16:1): 0.49** TG (16:1/18:1/16:0): 0.50**
Gynoid fat mass	-	-	2-oxovaleric acid: 0.45**
Relative gynoid fat mass	-	-	-
Relative trunk fat mass	-	-	TG (12:0/12:0/16:1): 0.46** TG (16:1/18:1/16:0): 0.5**
Trunk fat mass	-	-	TG (12:0/12:0/16:1): 0.46** TG (16:1/18:1/16:0): 0.50**
Relative intramus-cular Fat Area	TG (18:2/18:2/22:0): 0.4**	-	-
Leg lean mass	2-oxovaleric acid: -0.35**	TG (16:1/18:1/16:0): -0.61***	2-oxovaleric acid : -0.47**
Daily Energy Ex-penditure	-	-	Acetic acid : 0.53**
Resting heart rate	-	-	-
Diastolic blood pressure	-	-	-

Delta change (%) are estimated as: [(post-pre)/pre]×100. HIIT = High Intensity Interval Training, PLA: placebo, CIT: citrulline. Relative data are expressed in % at pre and post. Correlation analysis was performed using parametric Pearson's test; significance: $**p < 0.01$; $***p < 0.001$; -: no significant correlations.

Table S4-c: Significant correlations between blood parameters delta changes and significant serum metabolites delta changes without outliers.

	Intervention (n=59)	HIIT-PLA(n=26)	HIIT-CIT(n=33)
		Biological Parameters	
Adiponectin	-	-	TG (14:0/16:0/16:0): 0.62*** Pyruvate/Alanine: -0.47**
Leptin	-	Lactose: 0.55** TG (14:0/16:0/16:0): 0.59**	Homoserine: 0.48**
Glucose	Arginine: 0.41** Urea: 0.34**	Arginine: 0.50**	-
Insulin	TG (14:0/16:0/16:0): 0.35**	Pyruvic and Oxaloacetic acid: -0.52** Citrate / Oxaloacetic acid: 0.55**	TG (14:0/16:0/16:0): 0.35**
HOMA-IR	TG (14:0/16:0/16:0): 0.37**	Pyruvic and Oxaloacetic acid: -0.52** Citrate / Oxaloacetic acid: 0.51**	TG (14:0/16:0/16:0): 0.37**
HDL-Cholesterol	Xylitol: -0.39**	-	
LDL-Cholesterol	Inosine: 0.38** Ketoisovaleric acid: 0.4**	Inosine: 0.59**	Ketoisovaleric acid: 0.48**
Triglycerides	-	-	-
IGFBP3	-	Inosine: -0.54**	-
IGF-1	-	Inosine: -0.53**	-
Free fatty acids	Ketoisovaleric acid: 0.4**	3-methlhistidine: 0.68***	2-oxoglutaric acid: 0.51** Ketoisovaleric acid: 0.55**

Delta change (%) are estimated as: [(post-pre)/pre]×100. HIIT = High Intensity Interval Training, PLA: placebo, CIT: citrulline. Relative data are expressed in % at pre and post. Correlation analysis was performed using parametric Pearson's test; significance: **$p < 0.01$; ***$p < 0.001$; -: no significant correlations.

Table S5: Significant correlations between clinical parameters and significant serum metabolites.

Metabolites	Measured Parameters	r	*p*-value
Xylitol	Insulin	0.38	***
Xylitol	HOMA	0.48	***
Inosine	Total Cholesterol	0.38	***
TG(14:0/16:0/16:0)	Triglycerides	0.41	***
TG(16:1/18:1/16:0)	Triglycerides	0.54	***
Ribose and Ribulose	Adiponectin / Leptin	0.36	***
Pyruvic acid and Oxaloacetic acid	IGFBP3	0.39	***
Pyruvate/Malate	IGFBP3	0.35	***
Oxaloacetic acid/Malate	IGFBP3	0.35	***
Pyruvate/Alanine	IGFBP3	0.5	***
Pyruvate/Serine	IGFBP3	0.49	***
Oxaloacetic acid/Aspartate	IGFBP3	0.4	***

Correlation analysis was performed using parametric Pearson's test; significance: ***p < 0.001; r = correlation coefficient; / = ratio

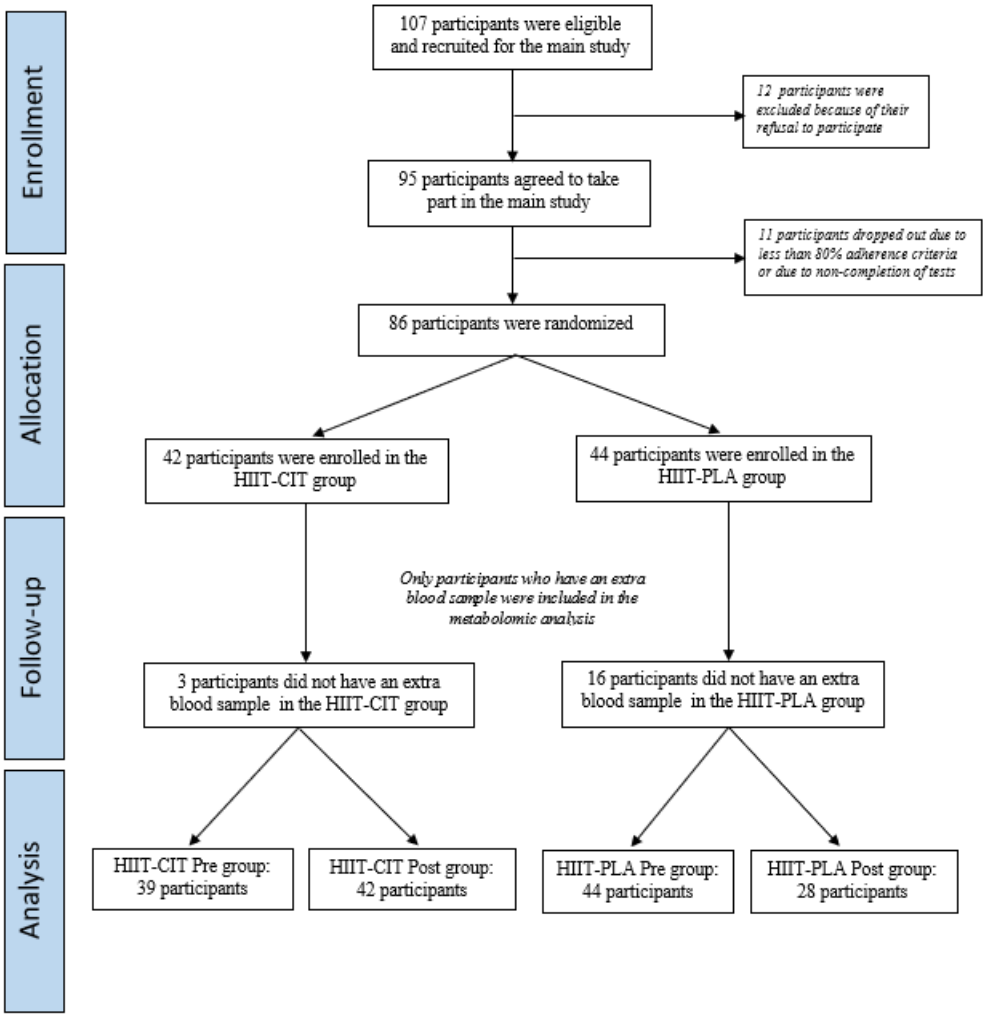

Figure S1: Flow chart of the study.
HIIT = High-Intensity Interval Training; CIT = Citrulline supplementation; PLA = Placebo supplementation; Pre = Before the 12-week intervention; Post = After the 12-week intervention

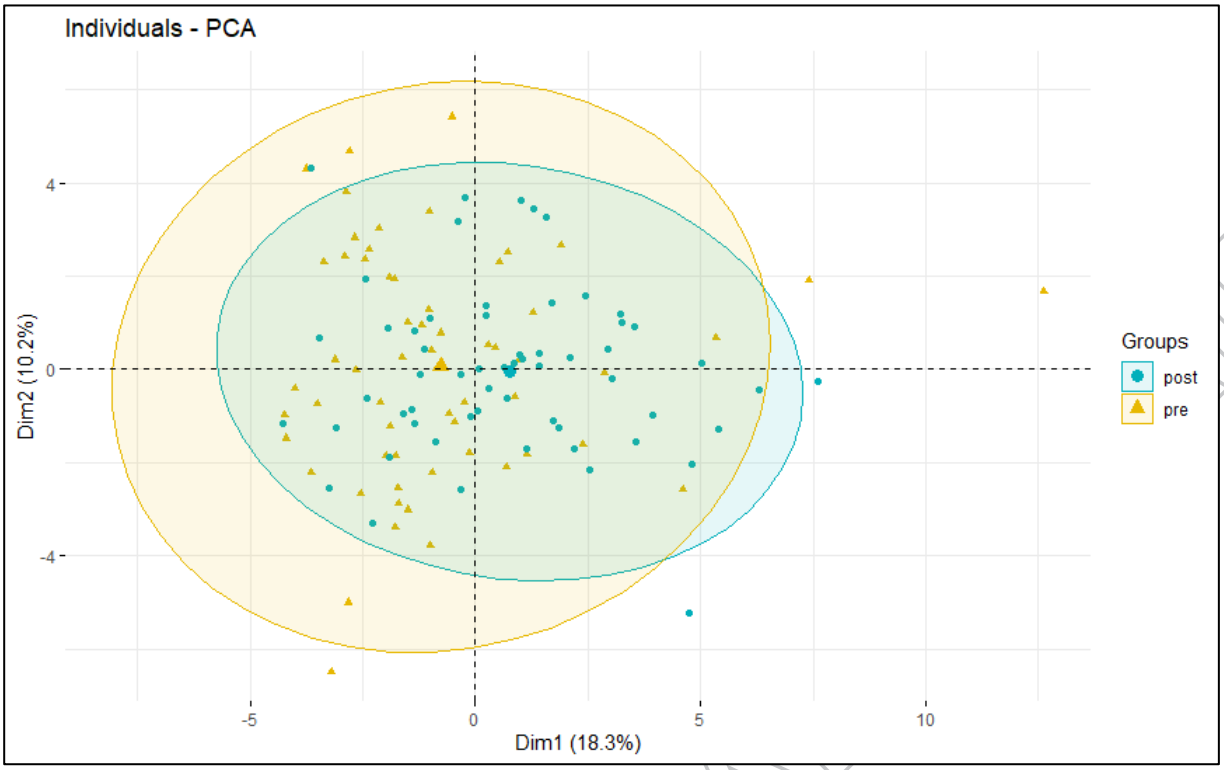

Figure S2: Principal Component Analysis (PCA) performed on all identified metabolites following the 12-week intervention in obese older adults.

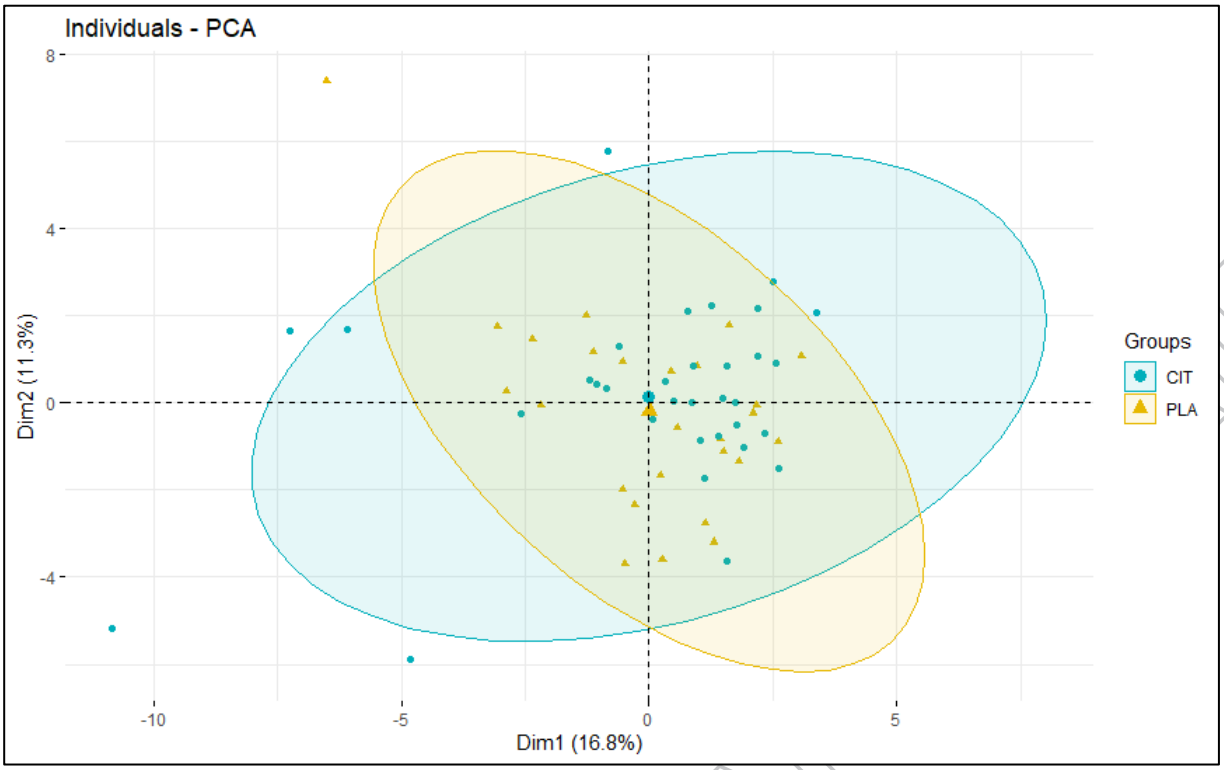

Figure S3: Principal Component Analysis (PCA) performed on the significant metabolites (obtained from the ANOVA analysis) following the 12-week intervention in obese older adults.

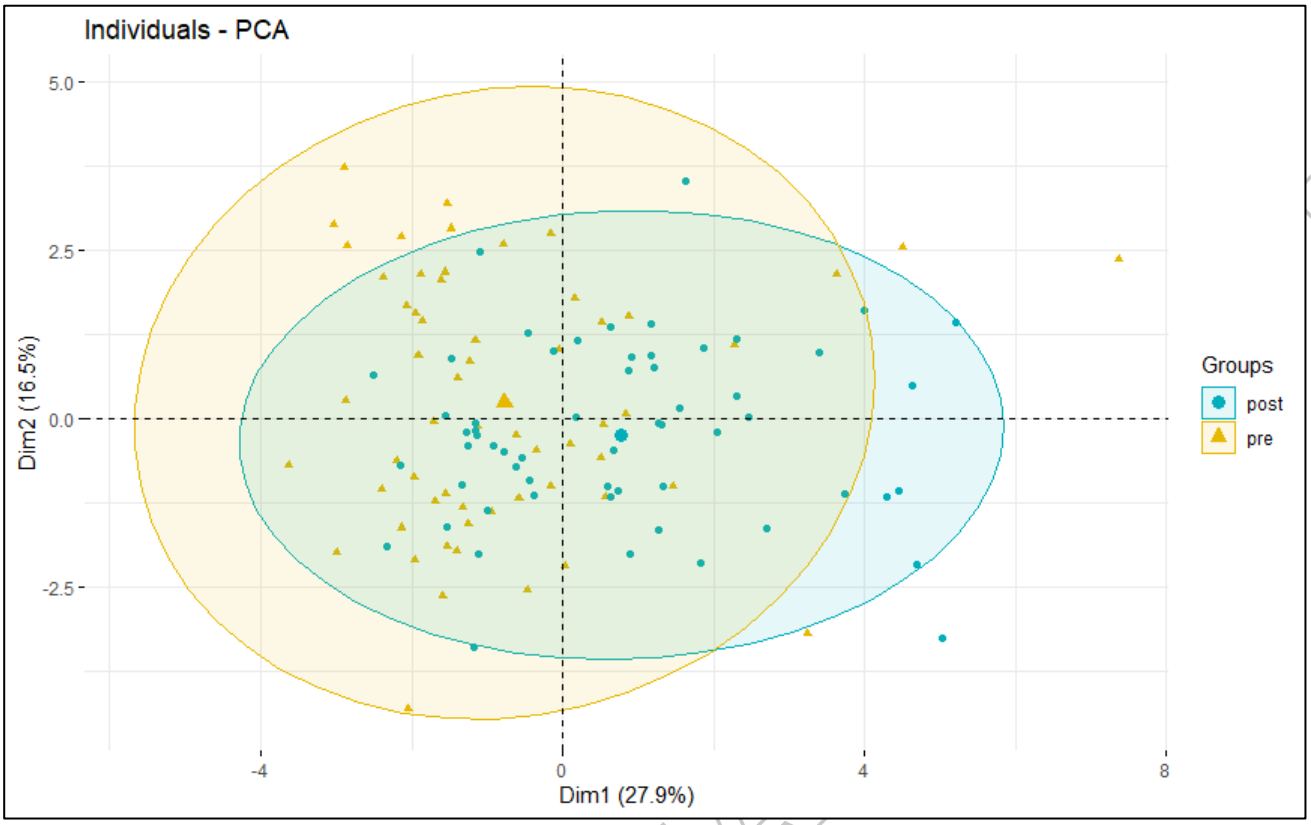

Figure S4: Principal Component Analysis (PCA) performed on the significant metabolites (obtained from the FDR analysis) following the 12-week intervention in obese older adults.

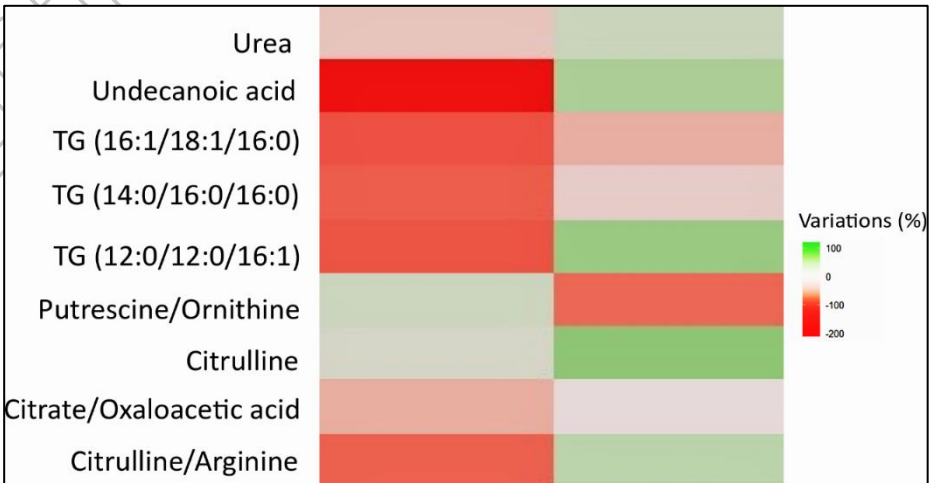

Figure S5: Heatmap showing the delta change (%) of the metabolites abundance and metabolites ratio with significant interaction effect (Time×Supplement effect) in both groups. PLA = Placebo supplementation group. CIT = Citrulline Supplementation group.