Effects of Combined L-Carnitine Supplementation and Moderate-Intensity Exercises on Oxidative Stress, Antioxidant, and Anti-Inflammatory Responses in Overweight and Obese Individuals: A Randomized Controlled Trial

Samsudin Na, Ahmad NS, Ooi FK, Abdul Kadir Ab, Kassim NK

Exercise and Sport Science Programme, School of Health Sciences, Universiti Sains Malaysia, Kubang Kerian, Kelantan
Department of Family Medicine, School of Medical Sciences, Health Campus, Universiti Sains Malaysia, Kubang Kerian, Kelantan
Basic and Medical Sciences Unit, School of Dental Sciences, Universiti Sains Malaysia, Health Campus, Kubang Kerian, Kelantan

ABSTRACT

INTRODUCTION: Obesity has been associated with persistent oxidative stress and inflammation that could lead to chronic diseases. MATERIALS AND METHODS: Sixty-eight overweight and obese participants aged 29.03±6.02 years old were randomly assigned, with 17 participants per group, into sedentary control (C), L-carnitine supplement alone (S), exercise alone (E), and combined L-carnitine supplementation and exercise (SE) groups. The participants in S and SE groups took one tablet of 1000 mg of L-carnitine every day. The E and SE groups performed brisk walking exercise for 30 minutes at 50% HRmax followed by Tabata exercise for 10 to 20 minutes per session, 3 sessions per week. During pre- and post-tests, blood markers of participants were assessed to determine their reactive oxygen species (ROS), malondialdehyde (MDA), catalase (CAT), superoxide dismutase (SOD), and interleukin-6 (IL-6) levels. All the observed parameters were analysed using two-way mixed ANOVA for repeated measures to determine significant differences within and between the groups and comparisons of mean differences were performed using one way ANOVA. RESULTS: Results showed that there were significant decreases in ROS and MDA in the S, E, and SE groups at post-test after 12 weeks of intervention. The greatest reduction of these parameters were observed in SE group among all groups. At the post-test, both CAT and SOD levels increased in the S, E, and SE groups. Regarding IL-6, it was found to be increased in C and E groups. In contrast, there were significant decreases in S and SE groups following the intervention. CONCLUSION: Twelve weeks of L-carnitine supplementation and engagement in brisk walking and Tabata exercise had significant improvement on oxidative stress, antioxidant, and anti-inflammatory responses in overweight and obese individuals.

KEYWORDS: exercise, free radicals, overweight, catalase, obese

Corresponding Author
Dr. Nur Syamsina Ahmad
Exercise and Sport Science Programme, School of Health Sciences, Universiti Sains Malaysia, Health Campus, 16150 Kubang Kerian, Kelantan
Email: nursyamsina@usm.my

Received: 14th June 2022; Accepted: 28th February 2023

Doi: https://doi.org/10.31436/imjm.v22i2

INTRODUCTION

Generally, obesity is related with oxidative stress and chronic diseases which are related to inflammation and inflammatory process. Inflammation is a complex oxidative stress.3 Metabolic rate of skeletal muscle biological response to infection, irritation, and other increases up to 100 times over resting values during conditions.1 Higher amounts of free radicals and reactive oxygen species (ROS) are produced during chronic inflammation and oxidative stress, which can cause structural damage to cells.2 Atherosclerosis, diabetes mellitus (DM), neurological disorders, lung diseases, the effect of the proper intensity and duration of aerobic cancers, and rheumatoid arthritis (RA) are example of or anaerobic exercise.6,7 Physical activities increases the
production of ROS in the skeletal muscles, and it has been postulated that exercise training can help in the upregulation of enzyme and non-enzymatic antioxidant defence systems. According to Rada’k et al. endurance training has a beneficial protective effect against oxidative damage regardless of age. It has been reported that moderate exercise may lead to decreased levels of pro-inflammatory cytokines and reactive oxygen species which subsequently decrease nuclear factor κB (NF-κB) activation and inflammation. L-carnitine can effectively prevent lipid peroxidation end products formation due to its anti-inflammatory and antioxidant effects. Its physiologic role is to transport long-chain fatty acids across the mitochondrial membrane, which helps in oxidative energy release. It also assists in eliminating short- and medium-chain fatty acids from mitochondria.

It has been reported that in patients with end-stage renal failure, supplementing with L-carnitine at 20 mg.kg⁻¹ of body weight for eight weeks was associated with a significant reduction in oxidative stress biomarkers. Furthermore, two-month of L-carnitine treatment for individuals with maple syrup urine disease (MSUD) at a dose of 1.5 g/day has been shown to have antioxidant and anti-inflammatory effects. L-carnitine supplementation for three months has been reported could reduce oxidative damage in patients with age-related macular degeneration (AMD) by lowering the lipid peroxidation of marker malondialdehyde (MDA) and increasing glutathione (GSH) levels.

To date, studies related to combined effects of exercise and L-carnitine supplementation on oxidative stress, antioxidant, and anti-inflammatory responses in overweight and obese people are scarce. Therefore, this study was undergone to investigate the effects of L-carnitine supplementation combined with moderate-intensity exercises on oxidative stress, antioxidant, and anti-inflammatory responses in overweight and obese individuals.

MATERIALS AND METHODS

Research design

This study is an intervention study that involved randomization of four groups of overweight and obese participants with pre-test and post-test measurements. Randomization was done by block randomisation using computer based random number (www.randomization.com). Opportunistic sampling method was used. The manual randomization of the participants was based on gender matching to divide the 68 participants equally in each of the groups. Each group was with 5 males and 12 females. One-way ANOVA was performed for baseline values in each parameter to ensure there was no significant differences between all the groups. The study was approved by the Human Research Ethics Committee of Universiti Sains Malaysia (USM/JEPeM/19100617).

Participants

Sixty-eight Malaysian participants (age between 18-40 years old) required to fill up the study information sheet and received explanations about the study purpose, procedures such as the experimental protocol and possible risks before being given the consent form. Recruitment of the participants was based on Asian cut-off point BMI classification for overweight ≥23.00-25.00 which is further subdivided as obese class I 25.00-30.00 (WHO, 2004). Recruitment of participants were done by getting permission from the Director of Hospital Universiti Sains Malaysia (HUSM) and contact staff (nurses) at Klinik Rawatan Keluarga (KRK) in the Universiti Sains Malaysia (USM).

However, because we had difficulty to recruit participants during the COVID-19 pandemic period, a poster was advertised offline as well as posted in the social medias. The participants were required to perform exercise 3 times
per week. During Covid-19 period, the data collection was only allowed by the Dean of School of Health Science, USM to conduct exercise sessions in small groups. The participants exercised 2 times per week in the Sport Complex 2 on campus and once their own at their house. They were requested to record a video when doing exercise and later sent to researcher as a proof. Pre- and post-tests for blood measurements were conducted at the Exercise and Sport Science Laboratory, USM, Kota Bharu, Kelantan. Males (n=20; mean age: 29.6±1.4 years) and females (n=48; mean age: 28.8±0.9 years) of sixty-eight (n=16) overweight (n=16) and obese (n=52) participants (mean age: 29.0±6.0 years; mean body mass index: 27.0±0.3 kg·m−2) participated in this study. There were 17 participants in each of the four groups: sedentary control (C), L-carnitine supplementation alone (S), exercise alone (E), and combined L-carnitine supplement and exercise (SE) groups. The participants in S and SE groups consumed one tablet of 1000 mg L-carnitine daily for 12 weeks.

Participants in the E and SE groups involved in moderate-intensity exercise of brisk walking for 30 minutes and Tabata exercise for 10-20 minutes, 3 sessions per week for 12 weeks. The inclusion criteria were Malaysian males and females aged 18-40 years with a BMI (23-30.0 kg·m−2) of overweight or class 1 obesity of the participants 17, without any health problems and physically inactive and exercised less than 2 times per week. The exclusion criteria were participants with diseases such as asthma, stroke, diabetes, heart disease, hypertension, and kidney diseases or had history of upper and lower limb injuries in the past 6 months and participants who having habits of consuming any nutritional supplements on regular basis and on medication including usage of antibiotic for the past 2 weeks are excluded from this study.

Exercise program protocol

The intervention programme consisted of approximately 60 minutes of workout time. The exercise began with a 5-minute warm-up session (stretching activities), followed by brisk walking for 30 minutes at 50% HRmax and continued with 10 minutes of Tabata exercise (two segments) for the first four weeks, 15 minutes (three segments) for the subsequent four weeks, and 20 minutes (four segments) for the final four weeks. Table 1 tabulates the type of activity included in the Tabata exercise. Tabata exercise required the participants to complete two sets of exercise for 20 seconds followed by a 10-second rest period. The intensity of the exercises was determined by participants’ heart rate i.e. between 40% to 60% of heart rate reserve. The exercise activities were performed by the participants with changing between upper and lower limbs alternately.

<table>
<thead>
<tr>
<th>Table 1 Types of activity included in the 20-minute Tabata exercise which were modified from Emberts et al. 18 study.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Segment 1</td>
</tr>
<tr>
<td>---------</td>
</tr>
<tr>
<td>Donkey kick</td>
</tr>
<tr>
<td>Jump rope</td>
</tr>
<tr>
<td>Burpees</td>
</tr>
<tr>
<td>Mountain Climbers</td>
</tr>
</tbody>
</table>

Blood sampling

Blood samples of participants were collected at pre- and post-tests to determine the levels of oxidative stress, antioxidant, and anti-inflammatory markers. After a 10-hour fasting, five millilitres of blood samples were drawn from the medical cubical vein of participants by venepuncture with 23-gauge needle, and a tourniquet were used on the upper selected arms to make vein become prominent and released as soon as blood flowed into the syringe. Then, blood samples were added into SST (gel) tubes for measuring all blood parameters. Next, the samples were then centrifuged by using Hettich Rotina RS Centrifuge (Hettich Zentrifugen-Rotina 46RS, Germany) at 4000 revolutions per minutes for 10 minutes at 4°C. The serum obtained after centrifuge was divided into equal portions in 1.5 mL Eppendorf bullet tube using a disposable plastic Pasteur pipette and then being stored at -20°C in freezer for subsequent analysis.

Biochemical test

Serum Reactive Oxidative Stress (ROS), Malondialdehyde (MDA), Catalase (CAT), Superoxide Dismutase (SOD), and Interleukin-6 (IL-6)

A double-antibody sandwich enzyme-linked immunosorbent one-step technique test was used to determine the
concentrations of reactive oxidative stress (ROS), malondialdehyde (MDA), catalase (CAT), superoxide dismutase (SOD), and interleukin-6 (IL-6) in samples (Human ELISA kit, Shanghai). The plate was run by a photometric microplate reader (Thermo Scientific Varioskan Flash, US).

**STATISTICAL ANALYSIS**

Statistical Package for Social Science (SPSS) Version 26.0 was used to perform statistical analysis. The normality of the data distribution was determined using the Shapiro-Wilk test. All the observed parameters were analysed using two-way mixed ANOVA for repeated measures to determine significant differences within and between the groups. Significant differences in two-way mixed ANOVA were followed up by post-hoc Bonferroni’ test. In addition, comparisons of mean differences between pre- and post-tests were performed among all the groups by using one way ANOVA. The data are presented as mean and standard error (mean±SE). A *p*-value<0.05 indicates statistical significance.

**RESULTS**

**Serum Reactive Oxygen Species (ROS)**

Table 2 shows the means serum reactive oxygen species of all the groups. There were significant mean effect of time (*p*<0.05) and mean effect of group (*p*<0.05) and mean effect of time (p<0.05) and mean effect of group (p<0.05), as well as interaction between time and group (p<0.05) on mean serum MDA concentration. According to within-group analysis, MDA levels were significantly lower at post-test than pre-test in the S, E, and SE groups (p<0.05). However, after 12 weeks of intervention, C group exhibited a modest increase (Table 2). Fig. 1B shows the mean difference of serum malondialdehyde (MDA) between pre- and post-tests of all the groups. Analyses between groups showed that the S, E and SE groups (p<0.05) showed the greater reduction in serum MDA compared to C group. Overall, SE group (p<0.05) elicited greatest decrement (-150.8±5.2 ng. ml⁻¹) compared to other groups.

Table 2: Means of oxidative stress, antioxidant, and inflammatory markers in all the groups

<table>
<thead>
<tr>
<th>Variables</th>
<th>C</th>
<th>S</th>
<th>E</th>
<th>SE</th>
</tr>
</thead>
<tbody>
<tr>
<td>Oxidative stress markers ROS</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pre-test</td>
<td>192.8±6.7</td>
<td>216.1±7.6</td>
<td>212.7±6.5</td>
<td>207.2±7.8</td>
</tr>
<tr>
<td>Post-test</td>
<td>223.3±5.5</td>
<td>180.9±6.0</td>
<td>181.5±6.0</td>
<td>149.0±4.7</td>
</tr>
<tr>
<td><em>p</em>-value</td>
<td>&lt;0.05</td>
<td>&lt;0.05</td>
<td>&lt;0.05</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>MDA (ng.mL⁻¹)</td>
<td>175.6±5.8</td>
<td>286.0±8.1</td>
<td>306.4±6.9</td>
<td>320.0±6.6</td>
</tr>
<tr>
<td>Pre-test</td>
<td>281.8±7.5</td>
<td>169.1±6.2</td>
<td>184.8±7.2</td>
<td>169.1±4.4</td>
</tr>
<tr>
<td>Post-test</td>
<td>&lt;0.05</td>
<td>&lt;0.05</td>
<td>0.001</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>CAT (ng.mL⁻¹)</td>
<td>165.7±4.5</td>
<td>176.5±4.3</td>
<td>184.1±5.5</td>
<td>193.0±4.7</td>
</tr>
<tr>
<td>Pre-test</td>
<td>138.2±6.3</td>
<td>200.6±3.5</td>
<td>205.4±5.8</td>
<td>256.0±5.2</td>
</tr>
<tr>
<td>Post-test</td>
<td>&lt;0.05</td>
<td>&lt;0.05</td>
<td>&lt;0.05</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>SOD (ng.mL⁻¹)</td>
<td>171.9±4.2</td>
<td>216.1±6.5</td>
<td>212.7±6.5</td>
<td>207.2±7.8</td>
</tr>
<tr>
<td>Pre-test</td>
<td>146.8±2.9</td>
<td>243.4±9.1</td>
<td>250.7±6.3</td>
<td>310.8±8.9</td>
</tr>
<tr>
<td>Post-test</td>
<td>&lt;0.05</td>
<td>0.003</td>
<td>&lt;0.05</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>IL-6 (pg.mL⁻¹)</td>
<td>7.2±0.2</td>
<td>6.4±0.2</td>
<td>6.6±0.2</td>
<td>6.3±0.2</td>
</tr>
<tr>
<td>Pre-test</td>
<td>7.4±0.2</td>
<td>5.9±0.1</td>
<td>7.0±0.2</td>
<td>5.6±0.2</td>
</tr>
<tr>
<td>_post-test</td>
<td>&lt;0.05</td>
<td>&lt;0.05</td>
<td>0.002</td>
<td>&lt;0.05</td>
</tr>
</tbody>
</table>

Values are expressed as means ± standard deviations (SD); ROS, reactive oxygen species; MDA, malondialdehyde; CAT, Catalase; SOD, superoxide.
Superoxide Dismutase (SOD)

The result of serum superoxide dismutase (SOD) concentration for the all groups after 12-week intervention is shown in Table 2. There were significant mean effect of time \((p<0.05)\) and mean effect of group \((p<0.05)\). In addition, there was significant interaction between time and group \((p<0.05)\) on mean serum SOD concentration. Within-group analysis revealed that S, E, and SE groups had significantly increments \((p<0.05)\) of SOD at the post-test. However, after 12 weeks of intervention, C group exhibited a modest decline. The mean difference of serum superoxide dismutase (SOD) between pre- and post-tests of all the groups in Fig. 2B. Compared to C group, the S, E, and SE groups showed greater increment in serum SOD levels \((p<0.05)\). Meanwhile, SE group showed the largest increase \((103.6\pm7.3 \text{ ng.ml}^{-1})\) \((p<0.05)\) in SOD \((p<0.05)\) compared to S and E groups.

Interleukin-6 (IL-6)

The result of interleukin-6 (IL-6) is shown in Table 2. There were significant mean effect of time \((p<0.05)\) and mean effect of group \((p<0.05)\). There was significant interaction between time and group \((p<0.05)\). IL-6 levels
The main finding of the present study is that S, E, and SE groups showed significant decreases levels of serum ROS and MDA concentrations, a measure of oxidative stress, and significant increased levels of antioxidant levels, i.e. SOD and CAT. We also observed that SE group exhibited the greatest decrease in serum ROS and MDA levels, as well as greatest increase in serum SOD and CAT levels among the groups. These findings suggest that combining brisk walking and Tabata exercise with L-carnitine supplementation has potential to lower oxidative damage and increase antioxidant levels.

According to Deminice et al.\textsuperscript{22} circuit training reduces oxidative damage. Bogdanis et al.\textsuperscript{23} also reported that interval exercise attenuates oxidative stress and while boosting antioxidants. In addition, Lima et al.\textsuperscript{24} reported that a single session of combined exercise session reduces oxidative stress. The findings of the previous studies are in agreement with our findings that decreased levels of serum ROS and MDA concentrations after 12 weeks of exercise. It is speculated that our present observation could be due to engagement in exercise caused an increase in antioxidant activity or inhibiting free radicals were produced, resulting in a reduction in oxidative stress. Antioxidant defences such as SOD and CAT have been observed as a response to oxidative stress. The increase in CAT activity observed after 12 weeks of brisk walking and Tabata exercise of the present study could be attributed to the rapid mobilisation of tissue antioxidant in the circulation, most likely as a result of CAT leakage from muscle fibres or erythrocytes.\textsuperscript{25} In a short period of 2 weeks L-carnitine supplementation previous study by Parandak et al.\textsuperscript{26}, found that after 24 hours of exercise, the active and healthy males in carnitine group had significantly lower serum MDA levels, TBARS, creatine kinase, and lactate dehydrogenase than those in the placebo group after two weeks of intervention. Their study showed that supplementing with L-carnitine for 2 weeks seems to have a significant effect on reducing oxidative stress. They also found that antioxidant capacity increased significantly after 14 days of treatment in the L-carnitine group. Similarly, in the present longer-term study,

**DISCUSSION**

Antioxidants aid in the removal of ROS by scavenging them, by limiting their synthesis or neutralising their effects. The natural antioxidants like SOD, CAT, and GSH-Px are the three enzyme defences that remove harmful oxygen products in the human body.\textsuperscript{19} Some significant antioxidants in the human body include GSH, GSH-Px, SOD, and CAT.\textsuperscript{20} Maintaining the pro-oxidant and antioxidant balance in a normal cell is crucial. When ROS and antioxidant defence mechanisms are out of balance, oxidative alteration of the cellular membrane or intracellular molecules occurs and as a result, when oxygen species production rises and antioxidant levels fall, the balance may shift in favour of the pro-oxidant.

This condition is known as oxidative stress, and if it is severe or long-term, it can cause significant cell damage.\textsuperscript{19} It was reported that moderate exercise and a healthy lifestyle can help to avoid oxidative stress. In addition, whether reactive species are beneficial or harmful is depending on the duration and intensity of exercise, as well as fitness level of the individuals, and their nutritional status.\textsuperscript{21}
12 weeks of combination of L-carnitine supplementation and exercise has shown its potential to decrease oxidative stress and enhance antioxidant levels. Volek et al. found a reduction in plasma MDA after three weeks of 2000 mg L-carnitine in recreationally weight-trained males who were prescribed with squat exercise. Koozehchian et al. reported that resistance exercise combined with 2000 mg of L-carnitine supplementation for nine weeks could increase total antioxidant capacity, GPx indicators, and lower MDA levels in untrained men. Similarly, our study found that walking exercise and Tabata exercise when combined with L-carnitine consumption as a dietary supplement could reduce oxidative stress in overweight and obese participants, implying that this combination has protective antioxidant effects in overweight and obese individuals. It is generally known that intensity, duration and type of exercise can affect plasma levels of pro-inflammatory tumour necrosis factor (TNF)-α, interleukin (IL)-1, IL-1 receptor antagonist (IL-1ra), TNF-receptors (TNF-R), interleukin-6 (IL-6) and macrophage inflammatory protein (MIP-1). The present study found that IL-6 was found to be increased in the S and SE groups.

Nielsen et al. reported that exercise intensity affects IL-6 response, IL-6 response can also be influenced by type of activity involved with skeletal muscle recruitment. The author also stated that rowing is an exercise that stimulates major muscle groups from the upper and lower limbs which may cause muscle damage. Similarly, Tabata exercise prescribed in our study also involved major muscle groups from both the upper and lower limbs, which may cause muscle damage. The present observation of increase IL-6 level in exercise alone (E) group could be associated with muscle damage is followed by healing mechanisms such as macrophage entrance into the muscle, which leads to the production of IL-6. Nielsen et al. reported that the IL-6 response to exercise is long considered a response to exercise-induced muscle damage. Findings of the current study indicate that blood IL-6 level increased after exercising three times per week for 12 weeks. However, when exercise was combined with L-carnitine supplement, the blood IL-6 level decreased. This finding reflects that supplementing with L-carnitine may help to maximise anti-inflammatory effects. L-carnitine might serve as an antioxidant to reduce ROS generation in the human body.

CONCLUSION

In conclusion, consumption of L-carnitine supplementation and engagement in brisk walking and Tabata exercise for 12 weeks has beneficial effects on oxidative stress, antioxidant, and anti-inflammatory responses in overweight and obese individuals. Therefore, this combination can be recommended to reduce oxidative stress and improve antioxidant and anti-inflammatory responses in this population.

ACKNOWLEDGEMENTS

The authors wish to thank all the participants for their commitment and cooperation in this study. Special gratitude to all the staff from Exercise and Sports Science
lecturers and Laboratory, School of Health Sciences for their guidance and technical support.

FUNDING
This study was financially supported by Fundamental Research Grant Scheme provided by Ministry of Higher Education Malaysia with Project Code: FRGS/1/2019/STG04/USM/03/3.

CONFLICT OF INTEREST
The authors declare that there are no conflicts of interest.

REFERENCES


1) Mawicka J, Zieba A. Antioxidants as a defence


