EFFECTS OF COMBINED L-CARNITINE SUPPLEMENTATION AND MODERATE-INTENSITY EXERCISE ON BODY COMPOSITION, METABOLIC AND OXIDATIVE STRESS MARKERS, BONE PARAMETERS AND MUSCULAR PERFORMANCE IN OVERWEIGHT AND OBESE INDIVIDUALS

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by

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LIST OF ABBREVIATIONS

1CTP	– Cross-linked carboxyterminal telopeptide of type I collagen
ALP	– Alkaline phosphatase
CAT	– Catalase
SOD	– Superoxide Dismutase
BMD	– Body mass density
MDA	– Malondialdehyde
ROS	– Reactive Oxygen Species
H_2O_2	– Hydrogen Peroxide
HDL	− High-Density Lipoprotein
LDL	– Low-density lipoprotein
IL-6	– Interleukin 6
AST	– Aspartate Aminotransferase
ALT	– Alanine Aminotransferase
RHR	– Resting Heart Rate
TC	– Total Cholesterol
NF-κB	– Nuclear factor-κB
TNF-α	− Tumour necrosis factor alpha

LIST OF SYMBOLS

^{a}	Sedentary control group
^{b}	L-carnitine supplement group
^{c}	Exercise group
^{d}	Combine group

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KESAN GABUNGAN SUPLEMEN L-CARNITINE DAN SENAMAN INTENSITI SEDERHANA PADA KOMPOSISI BADAN, PENANDA TEKANAN METABOLIK DAN OKSIDATIF, PARAMETER TULANG DAN PRESTASI OTOT DALAM INDIVIDU YANG BERLEBIHAN BERAT BADAN DAN OBES

ABSTRAK

Kesan 12 minggu gabungan suplemen L-carnitine dan senaman intensiti sederhana pada komposisi badan, penanda tekanan metabolik dan oksidatif, parameter tulang dan prestasi otot dalam individu yang berlebihan berat badan dan obes telah ditentukan. Enam puluh lapan peserta berumur 29.03 ± 6.02 tahun dibahagikan kepada kumpulan kawalan (C), suplemen L-carnitine (S), senaman (E), gabungan L-carnitine dan senaman (SE). Peserta dalam kumpulan L-carnitine mengambil 1 tablet 1000mg L-carnitine setiap hari. Peserta dalam kumpulan senaman melakukan 30 minit berjalan pantas (50% HRmax) dan diteruskan dengan 10 hingga 20 minit senaman interval setiap sesi, 3 kali seminggu. Purata pengambilan tenaga adalah lebih tinggi daripada purata penggunaan tenaga mereka. Peratusan pengambilan karbohidrat, protein dan lemak adalah lebih tinggi daripada nilai pengambilan nutrien yang disyorkan (RNI). Selepas 12 minggu intervensi, terdapat pengurangan ketara (p<0.05) dalam berat badan, indeks jisim badan, peratusan lemak, jisim lemak, dan peningkatan (p<0.05) dalam jisim bebas lemak (FFM) dalam kumpulan S, E dan SE. Mengenai penanda metabolik, nisbah pinggang ke pinggul, glukosa darah, trigliserida, lipoprotein berketumpatan rendah, dan jumlah kolesterol, kumpulan SE menyatakan pengurangan tertinggi (p<0.05) berbanding kumpulan C. Semua kumpulan menunjukkan peningkatan (p< 0.05) dalam 'speed of sound' kedua-dua lengan dan kaki yang

dominan dan tidak dominan dalam kumpulan kecuali kumpulan C. Terdapat lebih besar ($p < 0.05$) 'extension' dan 'flexion' bagi tork puncak dan kuasa purata pada kedua-dua kaki dan lengan pada $60^{o}.^{s-1}$ dan $300^{o}.^{s-1}$ dalam kumpulan S, E dan SE berbanding kumpulan C dan kumpulan SE telah dinyatakan nilai yang paling besar. Berbanding dengan kumpulan C, semua kumpulan menunjukkan nilai serum ALP yang lebih besar ($p < 0.05$) dan jumlah kalsium ($p < 0.05$), tetapi nilai serum 1CTP yang lebih rendah ($p < 0.05$). Nilai 'malondialdehyde (MDA)' and 'reactive oxygen species (ROS)' berkurangan dengan ketara dalam semua kumpulan ($p < 0.05$) kecuali untuk kumpulan C. Manakala bagi penanda antioksidan, serum 'catalase (CAT)' dan 'superoxide dismustase (SOD)', semua kumpulan menunjukkan kenaikan yang lebih besar ($p < 0.05$) berbanding kumpulan C, dan kumpulan SE menunjukkan kenaikan yang paling tinggi. Dari segi interleukin-6, kumpulan S dan SE menunjukkan penurunan yang lebih tinggi ($p < 0.05$) manakala kumpulan E menunjukkan peningkatan yang lebih tinggi berbanding kumpulan C dan penurunan terbesar ($p < 0.05$) diperhatikan dalam kumpulan SE. Ujian ketoksikan menjelaskan bahawa suplemen L-carnitine selamat untuk dimakan dalam 12 minggu. Secara amnya, gabungan suplemen L-carnitine dengan berjalan pantas dan senaman interval selama 12 minggu mendatangkan kesan berfaedah untuk memperbaiki parameter keseluruhan bagi individu yang berlebihan berat badan dan obes.

EFFECTS OF COMBINED L-CARNITINE SUPPLEMENTATION AND MODERATE-INTENSITY EXERCISE ON BODY COMPOSITION, METABOLIC AND OXIDATIVE STRESS MARKERS, BONE PARAMETERS AND MUSCULAR PERFORMANCE IN OVERWEIGHT AND OBESE INDIVIDUALS

ABSTRACT

The effects of 12 weeks of combined L-carnitine supplementation and moderate-intensity exercise on body composition, metabolic and oxidative stress markers, bone parameters and muscular performance in overweight and obese individuals were determined. Sixty-eight participants aged 29.03 ± 6.02 years old were divided into control (C), L-carnitine supplement (S), exercise (E), combined L-carnitine and exercise (SE) groups. Participants in supplement groups consumed 1 tablet of 1000mg L-carnitine daily. Participants in exercise groups performed 30 minutes of brisk walking (50% HRmax) and continued with 10 to 20 minutes of interval training per session, 3 times per week. Mean energy intake was higher than their mean energy expenditure. The percentages of carbohydrate, protein and fat intakes were significantly higher than recommended nutrient intake (RNI) values. After 12 weeks of intervention, there were significant reduction ($p < 0.05$) in body weight, body mass index, fat percentage, fat mass, and increase ($p < 0.05$) in fat free mass (FFM) in S, E and SE groups. Regarding metabolic markers, waist to hip ratio, blood glucose, triglycerides, low-density lipoprotein, and total cholesterol, SE group expressed the highest reduction ($p < 0.05$) compared to C group. All groups showed enhancement ($p < 0.05$) in both radial and tibial speed of sound of dominant and non-dominant arms and legs within the groups except for C group. There were significant greater ($p < 0.05$) extension and flexion peak torque and average power in both legs and arms at $60^{o}.s^{-1}$

and $300^{o}.s^{-1}$ in S, E and SE groups compared to C group and SE group was expressed the greatest value. In comparison with C groups, all groups showed greater values of serum ALP ($p < 0.05$) and total calcium ($p < 0.05$), but significantly lower value of serum 1CTP ($p < 0.05$). Malondialdehyde (MDA) and reactive oxygen species (ROS) values significantly reduced in all groups ($p < 0.05$) except for C group. Meanwhile for antioxidant markers, serum catalase (CAT) and superoxide dismutase (SOD), all groups displayed greater increment ($p < 0.05$) compared to C group, and SE group demonstrated the highest increment. In terms of interleukin-6, S and SE groups also showed higher decrement ($p < 0.05$), meanwhile E group showed higher increment compared to C group, and the greatest decrement ($p < 0.05$) was observed in SE group. The toxicity test explained that L-carnitine supplement are safe to be consumed in 12 weeks. Generally, combinations of L-carnitine supplement with brisk walking and interval exercise for 12 weeks elicit beneficial effects to improve overall parameters in overweight and obese individuals.

CHAPTER 1

INTRODUCTION

1.0 Background

Obesity is a global pandemic, with a prevalence of more than 20% worldwide (Blüher, 2012), and one billion people are expected to be obese by 2030 (Kelly et al., 2008). The definitions of overweight and obesity include abnormal or excessive fat accumulation that could harm one's health (WHO, 2021). Body mass index (BMI) is a basic measure of weight in relation to height that is frequently used to categorise overweight and obesity in adults (WHO, 2021). BMI serves as the most useful population-level indicator of overweight and obesity due to its universal applicability to adults of all sexes and ages (WHO, 2021). However, because it might not equate to the same level of fatness in various people, it should only be used as a general reference.

Obesity management can take a variety of forms, including lifestyle changes and pharmaceutical interventions (Jackson et al., 2015). Dietary modifications, exercise, and behavioural treatment are all part of a more holistic approach to lifestyle changes (Wadden et al., 2012). In addition, poor participation in physical activities has been reported to be the main contributing factor related to overweight and obesity (Peltzer et al., 2014).

In this present study, moderate intensity training such as brisk walking and interval training were proposed for vulnerable group. Regarding brisk walking, it is an aerobic exercise that attempts to promote overall health and fitness, particularly to improve the function and efficiency of the body's metabolism, is the form of physical activity recommended for those overweight and obese (Watson et al., 2015). Brisk walking is one of the sports that lasts 30 to 60 minutes and is performed three to five times per week. Brisk walking is a very simple activity to participate in since it can be done anywhere, especially outside the home, and it is also completely free. Walking at a pace of 4 mph is referred to as brisk walking, which is a well-known and effective type exercise that is crucial for weight management. Because it boosts energy expenditure, it is frequently advised for obese people (Browning & Kram, 2005). In a previous study, it was discovered that combining a moderate-intensity walking programme to a weight-maintenance regimen improved weight loss and reduced waist circumference (Fogelholm et al., 2000). Exercise that increases the effectiveness of the aerobic energy-producing systems, such as walking and step aerobics, can increase cardiorespiratory endurance.

Regarding interval training, it can be simply defined as intermittent periods of intense exercise separated by periods of recovery (Fox et al., 1973). Moderate intensity exercise between 47% and 52% of maximum oxygen consumption is known to enhance fat oxidation activity in general population (Achten et al., 2002). Duration of exercise more than 30-minutes induces a multitude of adaptations that result in increased fat oxidation (Achten & Jeukendrup, 2004). This project is worth to be carried out to provide information and guideline on how interval training adapted from Olson (2013) study could improve body composition, metabolic markers and oxidative stress blood markers, bone parameters and muscular performance in overweight and obesity groups. Multiple rounds of interval session were performed by the participants of this present study as it meets American College of Sports Medicine (ACSM) guideline, which could increase caloric expenditure around 240 to 360kcals compared to 54kcals during one session (4-minutes).

In this present study, body composition was measured. Body composition is a term used to describe the proportions of fat, bone, and muscle in human bodies regarding health and fitness. The body fat percentage is the one that interests people the most because it is very useful for determining health. In the previous study by Miller et al. (2014) and Paoli et al. (2013) following four weeks and twelve weeks of high intensity circuit training (each session lasting 30 minutes and 50 minutes, respectively), the body composition of obese and overweight males improved. In another study by Safarzade et al. (2020), body weight, percentage of body fat, body mass index and waist-hip ratio were significantly reduced in the training group compared to control group of 8 weeks of circuit resistance training.

It was mentioned by Fisher et al. (2015) that total training intensity (i.e., level of resistance) and volumes (i.e., total repetitions times) are required to be taken into consideration especially for whole body exercises (e.g., squats, lunges). Fisher et al. (2015) found that all the participants from obese population can complete a training program which is suitable to reduce body weight, lose fat and improve their cardiovascular risk profiles. The authors showed that the prescribed 20 minutes of interval training for 18 sessions in 6 weeks significantly decreased total cholesterol, triglycerides, very-low-density lipoprotein (VLDL) cholesterol and increased high-density lipoprotein (HDL) in overweight and obese males. Similarly, the metabolic syndrome risk factors such as triglyceride, and total cholesterol were significantly decreased following 12-week circuit training program in obese female college students (Kim et al., 2018).

Ho et al. (2012) also reported that 12-week training program of resistance exercise alone and combination exercise of resistance and aerobic at moderate-intensity for 30 min, five days per week, resulted in improvements in the cardiovascular risk profile, body weight and fat loss in overweight and obese participants compared to no exercise group. Kolahdouzi et al. (2019) found that insulin, insulin resistance index, total cholesterol, triglyceride, and low-density lipoprotein, were significantly mitigated in the circuit resistance training group, but high-density lipoprotein plasma levels was increased compared to that of the control group after circuit resistance training. Kong et al. (2016) found that the interval training protocol implemented in their study is enjoyable and time-efficient in improving cardiorespiratory fitness in obese young women.

Regarding oxidative stress, it is an imbalance between antioxidants [e.g., superoxide dismutase (SOD) and glutathione peroxidase (GPX)] and reactive oxygen species [e.g., superoxide (O_2^-), hydrogen peroxide (H_2O_2), and hydroxyl radical (OH^-) (Sies, 1997). When oxygen is chemically reduced, unstable free radicals with an unpaired electron are created, which are oxidising agents produced during cellular metabolism (Montezano & Touyz, 2012). However, antioxidants are required to keep the level of ROS in cells at a physiologically advantageous level. Antioxidants are enzymatic and nonenzymatic substances that greatly reduce or cease ROS from doing their oxidative harm by either inhibiting ROS synthesis and action or by mending ROS-damaged cells (Kunwar & Priyadarsini, 2011). Exercise-induced oxidative stress has been shown to depend on a number of variables, including the mode (the form or type of exercise being used, such as cycling, jogging, or swimming), intensity (the percentage of maximum oxygen uptake, or VO_{2max}), and duration (the amount of time spent exercising at a given VO_{2max}) (Bloomer et al., 2007; Bloomer & Goldfarb, 2004; Bloomer et al., 2005; Goto et al., 2003; Goto et al., 2007). Oh et al. (2013) showed that obese persons' thiobarbituric acid reactive substances (TBARS) and body weight reduced after 12 weeks of moderate- to high-intensity aerobic exercise while baseline levels of the antioxidant glutathione peroxidase (GPX) increased after 6 months of aerobic exercise in obese women (Shin et al., 2008). More significantly, compared to acute exercise-induced reactions pre-training, exercise-induced elevations in the oxidative stress marker malondialdehyde (MDA), superoxide dismutase (SOD), and GPX levels were decreased after exercise intervention (Shin et al., 2008). Similarly, after 24 weeks of resistance-type circuit training, overall oxidative stress, as measured by TBARS and total lipid hydroperoxide (PEROX), was decreased in healthy obese adults (Phillips et al., 2012).

In addition, the relationship between inflammation, oxidative stress, and obesity may be explained by this physiological connection (Korda et al., 2008). Furthermore, obesity-induced inflammation is frequently associated with increased oxidative stress. Interestingly, antioxidant treatment has been demonstrated to inhibit TNF-α- induced oxidative stress in skeletal muscle, indicating that TNF-α may offer a crucial target for treating oxidative stress caused by obesity (Li & Reid, 2000).

The effects of loading on skeleton produced by exercises are an important factor affecting bone density and its architecture. To date, the extent to which bony stresses from exercises affect bone health status in overweight and obese individuals is uncertain. Low bone mineral density (BMD) and micro-architectural deterioration of bone tissue, leading to decreased bone strength and increased evidence of fragility fractures (Rizzoli et al., 2001). A study conducted by Alghadir et al. (2014) assessed the osteogenic effect (T-score) and changes in bone markers in healthy subjects by conducting 12 weeks of aerobic training. Their results showed that the 12 weeks of moderate aerobic training produced significant improvement in all bone metabolism indices including serum bone-specific alkaline phosphatase, serum osteocalcin, and serum free calcium, as well as bone mineral density among all subjects. These findings implying that moderate intensity of aerobic training exerts significant positive effects on bone formation that could assist in preventing or decelerating osteoporosis. Thus, in this present study, moderate intensity exercise was proposed by modifying interval training to reduce its intensity and combined with brisk walking.

Muscular contractions induce extracellular fluid shear stress within the bone matrix, producing deformations in bone which subsequently stimulating bone formation. In previous study by Abdelmoula et al. (2012), knee extension strength was compared between individuals who were obese and non-obese. Dual-energy X-ray absorptiometry was used to determine the composition of the entire body and individual regions' soft tissues (DXA). An isometric dynamometer was used to measure the maximal voluntary contraction (MVC) torque of the knee extensors at a knee angle of 60° (0° is full extension). In comparison to controls, obese adolescents had considerably higher absolute MVC torque. Although obese adolescent boys had significantly lower MVC torque expressed per unit of body mass, there was no discernible difference in MVC torque between groups when MVC torque was normalised to fat-free mass. On the other hand, MVC torque was significantly higher in the obese group (17.9% and 22.2%, respectively; $P< 0.05$) after adjusting for thigh lean mass and estimated thigh muscle mass. Finally, compared to our non-obese controls, our sample of obese adolescent boys had greater absolute and relative knee extension strength. However, further research is needed to determine whether or if relative strength is greater in obese adolescents than in non-obese controls when tested using more precise in vivo techniques like magnetic resonance imaging.

Meanwhile, Maffiuletti et al. (2008) who reported significantly higher absolute voluntary isometric strength in the obese adolescent at short muscle lengths (+25% at 40 ^{o} extension) and during isokinetic efforts (+16 %). The authors hypothesised that the obese teenage cohort's preferring working at shorter muscle lengths to avoid excessive stress during an activity or sport or to prevent injury could be the explanation for their significantly better strength at short muscle lengths. Obese teenagers would be at a disadvantage in daily activities requiring a larger range of mobility, such as deep squatting, getting up from a chair, walking quickly, and bending, because of such a chronic loading procedure that would shift the length-tension relationship to the left.

L-carnitine is present in numerous plants based and animal-based diets and is abundant in dairy products, red meats and green leafy vegetables (Figure 1.1). Carnitine can also be found as a supplement in various forms, including acetyl L-carnitine, propionyl-L-carnitine, and the most widely available, L-carnitine. L-carnitine is well-known as a cofactor of carnitine acyltransferases, which transport long-chain fatty acids through the mitochondrial inner membrane (McGarry & Brown, 1997). The inner mitochondrial membrane would be impermeable to long-chain fatty acids and fatty acyl-CoA esters without L-carnitine. These molecules can be reduced to acetyl-CoA inside the mitochondria by a process known as beta-oxidation. Carnitine is also important for maintaining the cell's acetyl CoA/CoA ratio.

L-carnitine is the bioactive form of carnitine, an endogenous branched of essential amino acid derivative such as lysine and methionine **(Figure 1.1)**. Then, dietary carnitine is absorbed in the small intestine and then enters the bloodstream (Flanagan et al., 2010). L-carnitine can be generated in the liver or kidneys or taken from diets. It has been reported that overweight and obese individuals, recreationally active, competitive, and highly trained athletes consume L-carnitine as a dietary supplement (Sawicka et al., 2020). The concept behind carnitine supplementation as a weight-loss aid is that regular oral consumption increases the substance's intracellular concentration. Because of its involvement in the conversion of fat into energy, the L-carnitine has become increasingly attractive as a potential ergogenic supplement. L-carnitine is an amino acid that aids in fat metabolism. Meanwhile, the effect of L-carnitine on blood glucose control and lipid-lowering actions is thought to play a major role in the management of obesity (Jeukendrup & Randell, 2011; Kim et al., 2015). This statement was in a line with Hakimi et al. (2015a) found that although the implementation of a combination of resistance and endurance training for 8 weeks improves body composition and lipid profile in obese subjects, but the rate of progress was significantly improved with supplementation of L-carnitine. In another study, Stephens et al. (2013) examined the effect of twelve weeks of L-carnitine feeding on energy expenditure and body fat accumulation in recreationally active males. This study has shown the potential of L-carnitine supplementation to prevent increases in fat accumulation.

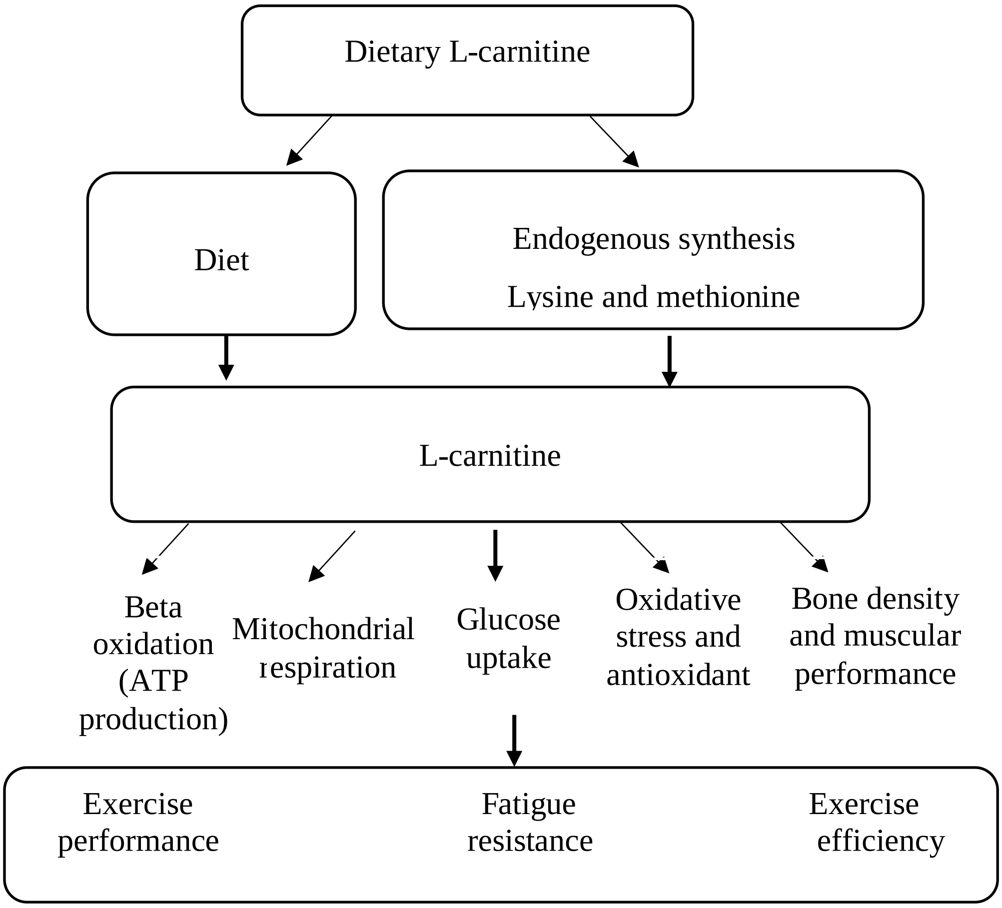

Figure 1.1 Schematic representation of proposed the effects of L-carnitine supplementation in human body metabolism.

Moreover, long-chain fatty acids account for up to 80% of the energy sources in the overnight fasted state, during rest, and during low to moderate intensity activity. During exercise, the formation of acetyl carnitine is essential for the maintenance of a viable pool of free co-enzyme. An oral L-carnitine feeding can help for fatty acids across the inner mitochondrial membrane for subsequent β-oxidation and convert fat to energy (Rebouche, 2004). Increasing muscle carnitine content will increase muscle fat oxidation and delay muscle glycogen depletion (Wall et al., 2011). For instance, Wall et al. (2011) studied moderately trained male athletes to show whether elevated serum insulin levels and hyperargininaemia could cause an increase in muscle carnitine concentrations. According to the study's findings, adding dietary carnitine to a diet boosted the amount of carnitine in muscle by 21%. Additionally, an 11% increase in work output over baseline data allowed the researchers to prove that carnitine had an ergogenic effect on the participants. These results indicate that carnitine supplementation may have some effects on athletic performance and that those without deficits may be able to enhance their muscle carnitine concentrations. Furthermore, it is also involved in the energy synthesis, acetyl group trapping, and glucose metabolism (Ribas et al., 2014; Takenaka et al., 2007).

Studies have reported that L-carnitine have an effective of 1,1-diphenyl-2-picryl-hydrazyl (DPPH) acts as free radical scavenging, superoxide anion radical scavenging, hydrogen peroxide scavenging, and total reducing power of free radical substances which can reduces metabolic stress in the cells (Gülçin, 2006). In addition, carnitine supplementation may contribute to the modulation of oxidative stress due to its effects on the increasing of antioxidant system components like glutathione peroxidase, chelating of ferrous ions (Türker et al., 2011), interfering with the ROS formation (Molfino et al., 2010), and the stabilization of free radicals (Molfino et al., 2010; Ruggenenti et al., 2009). A study by Parandak et al. (2014b) that examined at the impact of acute carnitine administration on exercise-induced oxidative stress and muscle injury in healthy and active men is another example of acute carnitine feeding. Participants took 2 capsules of L-carnitine (2,000 mg) or 2 capsules of lactose (2,000 mg) as a placebo every day for a total of 14 days. The study's findings showed that both groups reported significantly higher levels of markers of muscle damage (such as lactate dehydrogenase activity, creatine kinase activity, and total antioxidant activity) immediately following exercise as well as 2 and 24 hours later. Overall, this study was able to demonstrate that acute L-carnitine supplementation may help to alleviate some of the side effects of short bouts of exercise, but more research is needed. Furthermore, 9 weeks of LC supplementation in conjunction with resistance training revealed a significant increase of circulating total antioxidant capacity and glutathione peroxidase activity and decrease in malondialdehyde concentration (Koozehchian et al., 2018).

Meanwhile, L-carnitine may be capable of reducing inflammation by regulating the activity of inflammatory cells (Koc et al., 2011). It is means that carnitine could exert a significant anti-inflammatory role through downregulation of nuclear factor kappa B pathway which leads to decrease in the expression of pro-inflammatory cytokines (Koc et al., 2011). This meta-analysis by Haghighatdoost et al. (2019) measured the effect of L-carnitine on inflammatory mediators. The results revealed that L-carnitine decreased interleukin-6 (IL-6), TNF-α, and C-reactive protein (CRP). According to this subgroup analysis, L-carnitine was more effective in reducing inflammation in studies with a duration of more than 12 weeks and L-carnitine supplemented with a dose greater than 2000 mg/day was more effective in TNF-α reduction.

L-carnitine, a nutritional supplement, is still novelty with possible bone-protective properties. Carnitine has been shown to have anabolic effects in osteoblasts and bone marrow cells (Benvenga et al., 2004; Colucci et al., 2005). Carnitine is vital for tissues with high energy demands because it increases energy consumption (Chiu et al., 1999). Carnitine levels have been shown to decrease with age, and its deficit affects energy utilisation as well as physical performance (Chiu et al., 1999). Its shortage has been linked to cardiac and skeletal muscle weakness (Costell et al., 1989; Maccari et al., 1990), therefore it is possible that supplementing with carnitine can help these tissues perform better. Unfortunately, there is limited number of research studies (Benvenga et al., 2004; Colucci et al., 2005) indicate the positive on bone. Previously, carnitine was found to have favourable effects on bone mineral density (BMD) in patients with hyperthyroidism in one human research study (Benvenga et al., 2004), but not in overweight and obese people. Importantly, lowering intracellular ROS levels is the major defence against cell harm caused by oxidative stress and the imbalance between the synthesis and breakdown of superoxide radicals surpasses the detoxification capacity of osteoblasts (Munro & Treberg, 2017). As a result of high oxidative stress, osteoblast is reduced, whereas osteoclast activities are increased due to unbalanced of bone coupling (Wauquier et al., 2009). Therefore, maintaining balance synthesis and breakdown of ROS is crucial important for bone formation.

Progressive decline in skeletal muscle mass and function are growing concerns in an aging population. Diet and physical activity are important for muscle maintenance, but these requirements are not always met. This highlights the potential for nutritional supplementation. Evans et al. (2017) assessed the effect of a novel combination of L-Carnitine, creatine and leucine on muscle mass and performance in older subjects. L-carnitine combined with creatine and L-leucine significantly improved the composite score which reflects muscle mass and strength, at the end of the study compared to placebo. The combination showed an increase in mTOR protein level, a driver for increased muscle mass which translated to an improvement in muscle strength. This new combination may provide a potential nutritional intervention to promote muscle growth and improved physical functioning in older adults.

In this present study, liver and renal markers also were assessed. The liver produces the enzymes alanine transaminase (ALT), aspartate transaminase (AST), and gamma-glutamyl transferase (GGT), which are frequently assessed as part of liver function testing (Giannini et al., 2005a). Elevated levels of these enzymes may be a sign of inflammation or damage to liver cells, or, less frequently, damage to other organs that contribute to their production, such as the heart, kidney, brain, and muscles, even though they are crucial for human health and play a significant role in amino acid metabolism (Benedict & Zhang, 2017). Since the liver enzymes are the main parameters through which the function of the liver is measured, to clarify the role of L-carnitine, its effect on these enzymes should be noted. Previous study found that supplementing with L-carnitine enhanced tissue survival duration and survival rate following hepatic cell injury (Moghaddas & Dashti-Khavidaki, 2018). In one experiment, the administration of L-carnitine preserved the levels of the liver enzymes ALT, AST, and GGT after causing hepatic cell damage (Atila et al., 2002). L-carnitine supplementation has been proven to be more successful in treating patients with liver diseases, as shown in earlier research. In these patients, who may respond better to L-carnitine administration, it may be explained by higher liver enzyme levels.

To date, the effects of L-carnitine combined with exercise on oxidative stress as well as weight management, metabolic markers, bone parameters and muscular performance are still debateable. Therefore, the present study was proposed to investigate the effects of combined L-carnitine supplementation and moderate-intensity exercise program on body composition, metabolic and oxidative stress markers, bone parameters and muscular performance in overweight and obese individuals.

1.1 Problem Statement

L-carnitine has ability inhibit the activities and expressions of superoxide dismutase (SOD) and catalase (CAT) induced by hydrogen peroxide (H_2O_2) which means that L-carnitine can protect the cells from hydrogen peroxidase (H_2O_2) induced cytotoxicity by its antioxidant property (Li et al., 2012). Hydrogen peroxidase is a major component of reactive oxygen species (ROS) produced intracellularly during many physiological and pathological processes and can cause oxidative damage. Malondialdehyde (MDA), as a product of lipid peroxidation, is usually used to estimate the extent of lipid peroxidation. Superoxide dismutase and catalase are the most relevant enzyme involved in detoxification of H_2O_2 and protection from oxidative stress (De Bleser et al., 1999). Low level of endogenous carnitine could initiate depletion of ATP in cells and attenuating cell injury due to high H_2O_2. It is likely that the enhancement of β-oxidation induced by L-carnitine would generate more ATP because ATP is a critical event in lethal cell injury produced by oxygen radicals.

Previously, moderate-intensity interval exercise has been recommended as the primary exercise method for body mass and body fat loss (Keating et al., 2014; Racil et al., 2013). Brisk walking is one of the moderate exercises that has been introduced by a few previous studies (Hardman et al., 1992; Jakicic et al., 2003; Santiago et al., 1995). However, it took a long duration to show a significant improvement, i.e., 10 months (Santiago et al., 1995), 12 months (Jakicic et al., 2003) and 13 months (Hardman et al., 1992). Thus, it was suggested that interventions should initially target the adoption and maintenance of at least 150 min per week of moderate intensity exercise, and when appropriate, eventually progress to exercise levels consistent with the Institute of Medicine's recommendation of 60 min per day.

Several studies stated that high intensity interval training potentially provides health benefits in a time-efficient manner, however, it produced a similar magnitude as those achieved with regular moderate intensity interval exercise (Gillen & Gibala, 2014). These benefits include increased cardiorespiratory fitness (Ciolac et al., 2010), work capacity (Burgomaster et al., 2005), increased muscle mitochondrial and GLUT-4 level (Little et al., 2010) and improved insulin sensitivity (Ciolac et al., 2010). Relative to the effect of moderate intensity interval exercise, it has also been shown to induce comparable improvements in fitness and insulin sensitivity in clinical populations, including those with overweight and obesity (Hood et al., 2011; Richards et al., 2010). Thus, combination of moderate intensity brisk walking and interval training would give expected positive result in overweight and obese individuals.

While the results of cross-sectional studies point to an association between step counts per day and BMD in various skeletal sites (Boyer et al., 2011), the effect of walking-only intervention on BMD is still equivocal according to skeletal sites determinant (Martyn-St James & Carroll, 2008; Palombaro, 2005). Contrary to other parts of the skeleton, the calcaneus of the foot is subjected to repeated loads and a relatively high ground-reaction force during walking, which increases with increasing speed (Barela et al., 2014; Nilsson & Thorstensson, 1989). This indicates that walking could elicit a positive effect on the bone of the legs and even intensified after the application of body weight with average speed higher than usual walking in adults and older adults such as brisk walk. Moreover, Boyer et al. (2011) claimed that the influence of walking on BMD is affected not only by speed but also by the body weight of an individual such as overweight and obese individuals.

To date, there is no published data regarding the combined effects of moderate-intensity exercise (brisk walking and interval training) and L-carnitine supplementation on body composition, metabolic markers and oxidative stress blood markers, bone parameters and muscular performance in overweight and obese individuals. Therefore, the present study was proposed as a guideline to reduce the numbers of obesity rates especially in Malaysia. In addition, health care professionals could deliver messages to related individuals for improving their motivation and understanding on healthy losing weight strategy (Bocquier et al., 2005; Darden et al., 2013). It is hoped that the findings of the present study could encourage healthy lifestyle among overweight and obese and enhance theirs quality of life.

1.2 Research Questions

1. Are there any significant differences mean of total energy intakes, macronutrients, micronutrients and its relationship with energy expenditure, energy availability and body composition.

2. Are there any significant differences of body composition (body weight, body mass index, fat free mass, and fat percentage) and waist-hip ratio among control (C), L-carnitine supplementation alone (S), exercise alone (E) and combined L-carnitine supplement and exercise (SE) groups after 12 weeks of intervention.

3. Are there any significant differences of metabolic parameters (total cholesterol, high density lipoprotein, low density lipoprotein, triglyceride, insulin resistant, and glucose), oxidative stress markers [reactive oxygen species (ROS) and malondialdehyde (MDA)], antioxidant markers [superoxide dismutase (SOD) and catalase (CAT)] and interleukin-6 (IL-6) among control (C), L-carnitine supplementation alone (S), exercise alone (E) and combined L-carnitine supplement and exercise (SE) groups after 12 weeks of intervention.

4. Are there any significant differences of bone parameters (bone speed of sound, serum alkaline phosphate (ALP), serum C-terminal telopeptide of type I procollagen (1CTP) and total calcium) and muscular performance (isokinetic muscular strength and power of legs and arms) among control (C), L-carnitine supplementation alone (S), exercise alone (E) and combined L-carnitine supplement and exercise (SE) groups after 12 weeks of intervention.

5. Are there any significant differences of renal and liver markers for toxicity (aspartate transaminase (AST), alanine transaminase (ALT), creatinine, urea, and total bilirubin) among control (C), L-carnitine supplementation alone (S), exercise alone (E) and combined L-carnitine supplement and exercise (SE) groups after 12 weeks of intervention.

1.3 Research Objectives

1.3.1 General Objective:

To investigate the effects of 12-week combined L-carnitine supplementation and moderate-intensity exercise (brisk walking and interval training) on body composition, metabolic and oxidative stress markers, bone parameters and muscular performance in overweight and obese individuals.

1.3.2 Specific Objective:

1. To determine the mean of total energy intakes, macronutrients, micronutrients and its relationship with energy expenditure, energy availability and body composition in overweight and obese individuals.

2. To determine the effects of 12-week combined L-carnitine supplementation and moderate intensity exercise (brisk walking and interval training) on body

composition (body weight, BMI, fat free mass, fat percentage) and waist-hip ration between and within overweight and obese individuals.

3. To determine the effects of 12-week combined L-carnitine supplementation and moderate intensity exercise (brisk walking and interval training) on metabolic markers (total cholesterol, high density lipoprotein, low density lipoprotein, triglyceride, insulin resistant and glucose), oxidative stress markers [reactive oxygen species (ROS) and malondialdehyde (MDA)], antioxidant markers [superoxide dismutase (SOD) and catalase (CAT)] and interleukin 6 (IL-6) between and within overweight and obese individuals.

4. To determine the effects of combined L-carnitine supplementation and moderate intensity exercise (brisk walking and interval training) on bone parameters (bone speed of sound, serum alkaline phosphate (ALP), serum type c-terminal telopeptide of type I collagen (1CTP) and total calcium) and isokinetic muscular strength and power between and within overweight and obese individuals.

5. To determine the level of renal and liver markers for toxicity (aspartate transaminase (AST), alanine transaminase (ALT), creatinine, urea, and total bilirubin) between and within overweight and obese individuals.

1.4 Research Hypotheses

1.4.1 Null hypotheses

$H0_1$: There are no significant differences mean of total energy intakes, macronutrients, micronutrients and its relationship with energy expenditure, energy availability and body composition.

$H0_2$: There are no significant differences of body composition (body weight, body mass index, fat free mass, fat mass and fat percentage) and waist-hip ratio among control (C), L-carnitine supplementation alone (S), exercise alone (E) and combined L-carnitine supplement and exercise (SE) groups after 12 weeks of intervention.

$H0_3$: There are no significant differences of metabolic markers (total cholesterol, high density lipoprotein, low density lipoprotein, triglyceride, insulin resistant, and glucose), oxidative stress markers [reactive oxygen species (ROS) and malondialdehyde (MDA)] and antioxidant markers [superoxide dismutase (SOD) and catalase (CAT)] and interleukin-6 (IL-6) among control (C), L-carnitine supplementation alone (S), exercise alone (E) and combined L-carnitine supplement and exercise (SE) groups after 12 weeks of intervention.

$H0_4$: There are no significant differences of bone parameters (bone speed of sound, serum alkaline phosphate (ALP), serum type c-terminal telopeptide of type I collagen (1CTP) and total calcium) and isokinetic muscular strength and power among control (C), L-carnitine supplementation alone (S), exercise alone (E) and combined L-carnitine supplement and exercise (SE) groups after 12 weeks of intervention.

$H0_5$: There are no significant differences of renal and liver markers for toxicity (aspartate transaminase (AST), alanine transaminase (ALT), creatinine, urea, and total bilirubin) among control (C), L-carnitine supplementation alone (S), exercise alone (E) and combined L-carnitine supplement and exercise (SE) groups after 12 weeks of intervention.

1.4.2 Alternative hypotheses

HA_1: There are significant differences mean of total energy intakes, macronutrients, micronutrients and its relationship with energy expenditure, energy availability and body composition.

HA_2: There are significant differences of body composition (body weight, body mass index, fat free mass, fat mass and fat percentage) and waist-hip ratio among control (C), L-carnitine supplementation alone (S), exercise alone (E) and combined L-carnitine supplement and exercise (SE) groups after 12 weeks of intervention.

HA_3: There are significant differences of metabolic markers (total cholesterol, high density lipoprotein, low density lipoprotein, triglyceride, insulin resistant, and glucose), oxidative stress markers [reactive oxygen species (ROS) and malondialdehyde (MDA)] and antioxidant markers [superoxide dismutase (SOD) and catalase (CAT)] and interleukin-6 (IL-6) among control (C), L-carnitine supplementation alone (S), exercise alone (E) and combined L-carnitine supplement and exercise (SE) groups after 12 weeks of intervention.

HA_4: There are significant differences of bone parameters (bone speed of sound, serum alkaline phosphate (ALP), serum type c-terminal telopeptide of type I collagen (1CTP) and total calcium) and isokinetic muscular strength and power among control (C), L-carnitine supplementation alone (S), exercise alone (E) and combined L-carnitine supplement and exercise (SE) groups after 12 weeks of intervention.

HA_5: There are significant differences of renal and liver markers for toxicity (aspartate transaminase (AST), alanine transaminase (ALT), creatinine, urea, and total bilirubin) among control (C), L-carnitine supplementation alone (S), exercise alone (E) and combined L-carnitine supplement and exercise (SE) groups after 12 weeks of intervention.

1.5 Operational definitions

L-carnitine supplementation: L-carnitine supplementation used in this study are manufactured by Nutra Manufacturing INC which has registered with the Malaysian Ministry of Health (MAL20040677X) and was certified Halal (00048107150655). The participants from the L-carnitine (S) and combine L-carnitine with exercise (SE) groups consumed one tablet of 1000 mg L-carnitine between 8.00am to 9.00am daily for 12 weeks.

Moderate intensity exercise: The moderate-intensity exercises prescribed in the present study based on exercise intensity controlled by heart rate (40-60% heart rate reserve).

Brisk walking: A walking activity or action is done quickly and in an energetic way that may increase heart rate.

Interval training programme: An exercise and training program in which each session consists of periods of intense exertion alternating with periods of rest or lighter exertion.

Body composition: Measurement of percentage of fat, fat mass and fat free mass.

Metabolic markers: Measurement of total cholesterol, high density lipoprotein, low density lipoprotein, triglyceride, insulin resistant, and glucose.

Oxidative stress markers: Measurement of reactive oxygen species (ROS) and malondialdehyde (MDA). In addition, antioxidant markers of superoxide dismutase (SOD) and catalase (CAT) were measured.

Bone parameters: Measurement of bone speed of sound, blood bone metabolism markers of serum alkaline phosphate (ALP), serum type c-terminal telopeptide of type I collagen (1CTP) and total calcium concentration.

Muscular strength and power: Measurements of both knee and shoulder peak torque (muscular strength) and average power at $60°.s^{-1}$ and average power at $300°.s^{-1}$ of both dominant and non-dominant arms and legs.

Overweight and obese individuals: Both males and females of overweight and class 1 obesity (BMI between 23-30 $kg.\ m^{-2}$) between the ages of 18 and 40.

1.6 Significance of the study

It is hoped that the present study can find that there are potential of L-carnitine supplement for improving overall body composition, metabolic and oxidative stress markers, bone parameters and muscular performance following moderate exercise training session. This study will establish new knowledge regarding the potential metabolism action in fat oxidation in human body after consumption of L-carnitine as a supplement during exercise among overweight and obese individuals. Following positive finding in this study, it will provide scientific evidence for society to promote active lifestyle in our community especially among overweight and obese individuals because they are high risk for developing non-communicable diseases. Subsequently, this may improve quality of life and reduce the negative effect of obesity in society.

CHAPTER 2

LITERATURE REVIEW

2.1 Prevalence and associated risk factors of overweight and obesity groups

Overweight and obesity are major issues that have been a worldwide epidemic since the 1960s and have not abated until now (WHO, 2004). Earlier, these conditions were prevalent in developed countries. Over 1.9 billion adults aged 18 and older were overweight in 2016 and over 650 million of these people were obese (WHO, 2021). In 2016, 13% of adults aged 18 and older were obese and 39% were overweight as well as most people on world live in countries where being overweight or obese kills more people than being underweight (WHO, 2021). However, in recent years, overweight issues have expanded to most regions in developing countries in Southeast Asia (Kaur et al., 2018), including Malaysia (Boo et al., 2010; Gopalakrishnan et al., 2012; Ismail et al., 2002b). In adults, body mass index (BMI) is commonly used to classify overweight and obese individuals. It is calculated by dividing a person's weight in kilogrammes by the square of his height in metres (kg/m^2). According to the WHO (2004), a BMI greater than or equal to 25 is classified as overweight, whereas obesity is when the BMI is greater than or equal to 30. However, according to WHO, Asian populations with a BMI greater than or equal to 23 are classified as overweight, mainly associated with diseases. BMI has been widely used to measure obesity in humans and predict the risk of metabolic diseases such as diabetes (Molarius & Seidell, 1998; Vazquez et al., 2007).

Meanwhile, the National Health and Morbidity Survey (NHMS) results in 2019 revealed that 50.1% of Malaysian adults were either overweight or obese (NHMS, 2019). 19.7% of the population was obese, while 30.4% were overweight. The NHMS

2019 data also showed that women (54.7%), ethnic Indians (63.9%), and people in the 55 to 59 ages ranged had relatively high rates of overweight and obesity (60.9%) (NHMS, 2019).

Other researchers found that overweight and obesity in Malaysia have increased from 20.7% to 29.1% and 5.5% to 14.0%, respectively (Khambalia & Seen, 2010). Another study found that obesity was higher among boys than girls and in urban areas than rural areas (Ismail et al., 2002b). From 1979 to 1991, a study in Japanese universities discovered a progressive and significant increase in obesity among participants (Ohe et al., 1992). A study conducted at the University of Crete, Greece, found that around 40% of young male participants and 23% of young female participants were overweight (Bertsias et al., 2003). Among the government workers in Sicily, Italy, 33.2% of males and 16% of females were overweight (Abbate et al., 2006). A study done among 240 participants found that 30.1% were overweight based on the BMI of the WHO for the Asian population (Boo et al., 2010). In 2012, a study conducted among 290 participants from AIMST University students found that 13.7% of males and 15.7% of females were overweight, while 5.2% of total males and females were obese with a BMI of more than 30 kg/m^2 (Gopalakrishnan et al., 2012).

A study conducted by the Research and Development (RAND) organisation found that obesity is more damaging to health compared to smoking, alcohol, and poverty (Shashikiran et al., 2004). Overweight and obesity contributed to 44% of patients with diabetes, 23% of ischemic heart disease, and 7% to 41% of some cancers. In Malaysia, the prevalence of diabetes from (Organization, 2016) data found that 37.3% of the population is overweight, and 12.9% is obese.