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Effects of Vitamin C and L-Carnitine on Lipid Profile and Oxidative Stress Parameters in the Brain of Fasted and Refed Rats

Oludare G.O., Okpagu O.E, Obafemi A.A., Okoka, E.M.

Department of Physiology, College of Medicine of the University of Lagos, Lagos, Nigeria

ABSTRACT

The roles of vitamin C and L-carnitine on lipid profile and oxidative stress parameters in the brain of rats during fasting and re-feeding were investigated. Sixty male Sprague-Dawley rats (170-180 g) were divided into four groups of control, fasting, fasting + vitamin C and fasting + L-carnitine. The test groups were further divided into recovery 1 (24 hours following a fast) and recovery 2 (72 hours following a fast). Rats were fasted in individualized cages but were allowed free water intake. The fasted rats were re-fed after the fast and parameters were obtained for indices of lipid profile and oxidative stress on days one and three of recovery. Total Cholesterol levels were increased during fasting in vitamin C and L-carnitine treated rats but these increases were abolished on day three of recovery ($p < 0.05$). Triglycerides and LDL were increased in L-carnitine treated rats on day 1 of recovery while HDL levels were unchanged during fasting but decreased during recovery days 1 and 3 in both fasted and treated rats. There was an increase in MDA levels in the brain samples of fasted rats during fasting. However, no changes in the activity of the oxidative enzymes; SOD, GSH and CAT were found. The findings suggest that oxidative status was slightly perturbed during fasting in fasted rats without supplementation. Also, increased cholesterol levels during fasting in treated rats which was abolished during re-feeding portrays that the increased cholesterol during fasting might be due to the actions of vitamin C and L-carnitine on β -oxidation of fatty acids during fasting.

Keywords: Vitamin C, L-carnitine, Lipid profile, Oxidative stress, Fasting, Re-feeding,

*Author for correspondence: E-mail: gooludare@cmul.edu.ng; Tel: +234-7035363115

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INTRODUCTION

Fasting, an act of willing abstinence or reduction from certain or all food, drink (or both) for a period of time could be both beneficial or harmful (Mitsui *et al.*, 2002; Mattson and Wan 2005; Martin *et al.*, 2006). Prolonged food deprivation is a stressful physiological condition that activates the hypothalamic–pituitary–adrenal axis (Munck *et al.*, 1984), cardiorespiratory function (Sapolsky *et al.*, 2000) and prooxidant/ antioxidant systems (Mitsui *et al.*, 2002; Sorensen *et al.*, 2006; Vazquez-Medina 2010). Sorensen *et al.*, (2006) has shown that rats deprived of food for longer periods increases hepatic mitochondrial ROS production and lipid peroxidation. In another study, Wasselin *et al.*, (2014) showed that livers of rats during late fasting had

low levels of antioxidant factors with decreased activity which resulted in oxidative damage to lipids and proteins.

Dyslipidemia is a common problem with the obese, type 2 diabetic patients and patients with metabolic syndrome (Howard *et al.*, 2003; Klop *et al.*, 2013). About 1 in every 4 people within the age of 35 and 64 years dies of diabetes. 80% of these deaths are due to increased risk of cardiovascular diseases (UKPDS 1996; Diabetes atlas 2003; Chehade *et al.*, 2013). Fasting or intermittent fasting has been suggested to aid weight reduction in obese individuals (Varady and Hellerstein 2007) however some studies have shown that acute starvation in healthy, non-obese human subjects increases serum total cholesterol, LDL cholesterol and apo B concentrations (Savendahl and Underwood 1999).

In laboratory animals, fasting increases longevity, and improves health status in diseases such as cancer, neurological disorders and disorders associated with the circadian rhythm (Thomas *et al.*, 2010; Tajés *et al.*, 2010; BaHammam *et al.*, 2010). Studies has shown that alternate days of fast in humans prevents the progression of type 2 diabetes by facilitating weight loss which consequently improves cardiovascular risk (Knowler *et al.*, 2009; Brown *et al.*, 2013).

Three phases of fasting have been described which includes; a short dynamic period of adaptation called the phase 1, a metabolic steady state in which proteins are spared and lipids provide most of the energy expended (Phase 2) and the third phase which involves a situation when there is a lower threshold of body lipids which leads to a rapid breakdown of proteins (Andriamampandry *et al.*, 1996; Belkhou, *et al.*, 1999; Cherel and Groscolas, 1999, Robin *et al.*, 2008). Although lipid profile is usually assessed while fasting overnight, the demand for lipid as a source of energy during phase two of fasting might alter the lipid profile of individuals or animals undergoing prolonged fasting or food deprivation.

L-Carnitine is an essential nutrient in lipid metabolism, which promotes the transportation of long-chain-fatty acids across the mitochondrial membrane, thus allowing the cells to break down fat and release energy from reserves of stored fat (Hoppel 2003; Flanagan *et al.*, 2010; Cave *et al.*, 2008; Zhang *et al.*, 2014). All tissues that use fatty acids as an energy source require carnitine for normal function during fatty acid oxidation (Bianchi *et al.*, 1996). Beyond its actions on lipid metabolism, L-carnitine functions as an indirect antioxidant and facilitates the repair of oxidized membranes or lipid bilayers (Neri *et al.*, 2003; Rebouche, 2004). Vitamin C on the other hand is an important antioxidant in human capable of scavenging oxygen-derived free radicals. It has also been suggested that vitamin C exert cardiovascular and other health benefits in humans in part by acting as an antioxidant (Palidori *et al.*, 2004; Mazloom *et al.*, 2011). Vitamin C is present in the brain tissue at high concentrations compared to other organs (Schreiber and Trojan 1991; Kaufman 1996) with about 10-fold gradient between the concentration of ascorbic acid in brain and serum (Agus *et al.*, 1997). The protective effect of these antioxidants on the brain tissue during fasting and re-feeding is thus investigated in this study.

About 25% of total body glucose consumption occurs in the brain (Clarcke and Scoloff 1996; Raichle and Gusnard 2002; Magistretti, 2008). After the exhaustion of the glycogen reserve, and for the next 2-3 days of fasting, fatty acids are the principal metabolic

fuels. Once other tissues start producing metabolic fuel from fatty acids, all the remaining glucose is made available to the brain for use. The liver then begins to synthesize ketone bodies from precursors obtained from fatty acid breakdown which the brain uses as fuel, thus cutting its requirement for glucose. After fasting for 3 days, the brain gets 30% of its energy from ketone bodies and this rises to about 75% after 4 days of fasting (Coffee, 2004). Thus, the production of ketone bodies cuts the brain's glucose requirement from 80 g per day to about 30 g per day. Of the remaining 30 g requirement, 20 g per day can be produced by the liver from glycerol. But this still leaves a deficit of about 10 g of glucose per day that must be supplied from some other source. This other source will be the body's own proteins. Due to this critical energy requirement of the brain, this study was set out to determine the effects of vitamin C and L-Carnitine on lipid profile and brain oxidative stress status in rats during fasting and re-feeding.

MATERIALS AND METHODS

Animals

Sixty male Sprague-Dawley rats used in this study were obtained from the Animal facility of the College of Medicine, University of Lagos. The rats were kept under standard conditions of 12-hours light and dark cycles. They were acclimatized for 2weeks, kept at room temperature and were allowed to feed and drink water *ad libitum* prior to the experimental stage. The experiment was conducted in accordance to the U.S. National Institute of Health on the care and use of laboratory animals. The rats were divided into ten (10) groups of rats as described below:

- Group I: Control (CN)
- Group II: Fasting alone (F)
- Group III: Fasting + Vitamin C (VC + F)
- Group IV: Fasting + L-Carnitine (LC + F)
- Group V: Fast alone + 24 hrs recovery (FR-1)
- Group VI: Fasting + Vitamin C + 24 hrs recovery (VCFR-1)
- Group VII: Fasting + L-Carnitine + 24hrs recovery (LCFR-1)
- Group VIII: Fasting + 72 hrs recovery (FR-3)
- Group IX: Fasting + Vitamin C + 72 hrs recovery (VCFR-3)
- Group X: Fasting + L-Carnitine + 72 hrs recovery (LCFR-3)

Rats were placed in special individualized cages and were allowed to drink water during the food deprivation period. Vitamin C treated rats in group III, VI, and IX were administered 200mg/kg body weight of vitamin C orally (Eteng *et al.*, 2006). Groups IV, VII and X also received an oral dose of L-Carnitine (200mg/kg body weight) (Kopple *et al.*, 2002). Animals on 24 hours recovery were given food and water for 24hrs while

those on 72 hours recovery were allowed to eat and drink water for 72 hours.

Sample collection

Animals were sacrificed by cervical dislocation and blood samples were obtained via cardiac puncture for lipid profile assay. The skull was cut open and the brain of the rats were removed, weighed and then used to assess the levels of lipid peroxidation and antioxidant enzymes level.

Lipid profile analysis

The lipid profile assay for total cholesterol, triglycerides, HDL and LDL were carried out on a fully automated analyzer based on spectrophotometric principle using kits obtained from ERBA diagnostics (Transasia Bio-Medicals Ltd, Germany) as described by Singh *et al.*, (2014). The system employs the wet chemistry and photometric technology to perform absorbance readings from the colour of the sample. The absorbance is then converted automatically into concentrations based on the standard calibration curves stored by the instruments microprocessor.

Oxidative stress analysis

Lipid Peroxidation: Malondialdehyde (MDA) an index of lipid peroxidation was determined using the method of Sharma *et al.*, (2013). 1.0 ml of the supernatant was added to 2 ml of (1:1:1 ratio) TCA-TBA-HCl reagent (thiobarbituric acid 0.37%, 0.24N HCL and 15% TCA) tricarboxylic acid thiobarbituric acid-hydrochloric acid reagent boiled at 100°C for 15 min, and allowed to cool. Flocculent materials were removed by centrifuging at 3000rpm for 10 min. The supernatant was removed and the absorbance read at 535 nm against a blank. MDA was calculated using the molar extinction coefficient for MDATBA-complex of $1.56 \times 10^5 \text{ M}^{-1} \text{ CM}^{-1}$.

Activity of antioxidant enzymes

Superoxide Dismutase (SOD) Activity: Superoxide dismutase activity was determined by its ability to inhibit the auto-oxidation of epinephrine determined by the increase in absorbance at 480 nm as described by Doherty *et al.*, (2010). The reaction was carried out in 0.05M sodium carbonate buffer pH 10.2 and was initiated by the addition of 3×10^{-4} epinephrine in 0.005N HCl. Enzyme activity was calculated by measuring the change in absorbance at 480 nm for 5 mins. SOD activity was then expressed as (U/mg protein)

Catalase activity: Catalase activity was determined according to the method of Beers and Sizer as described by Usoh *et al.* (2005) by measuring the decrease in absorbance at 240 nm due to the decomposition of H_2O_2 in a UV recording spectrophotometer. The reaction mixture (3 ml) contained 0.1 ml of serum in phosphate buffer (50 mM, pH 7.0) and 2.9 ml of 30 Mm H_2O_2 in phosphate buffer pH 7.0. An extinction coefficient for H_2O_2 at 240 Nm of $40.0 \text{ M}^{-1} \text{ cm}^{-1}$ was used for the calculation. The specific activity of catalase was expressed as moles of H_2O_2 reduced per minute per mg protein.

Glutathione peroxidase (GPx) Activity: The activity of glutathione peroxidase (GPx) was measured as described by Pari and Latha (2004). To the homogenate 10% TCA was added, centrifuged. 1.0 ml of supernatant was treated with 0.5ml of Ellmans reagent (19.8 mg of 5,5-dithiobisnitro benzoic acid (DTNB) in 100 ml of 0.1% sodium nitrate) and 3.0 ml of phosphate buffer (0.2M, pH 8.0) The absorbance was then read at 412 nm spectrophotometrically and GPx activity was expressed as U/mg protein.

Protein content: This was determined using Biuret method as described by Tietz (1995). 5.0 ml of blank Biuret reagent prepared by dissolving CuSO_4 crystal in 500 ml of distilled water was added to sample blank. These were mixed well and allowed to stand for 20 min at room temperature 25-27°C. Absorbance was read for test and standard against a blank at 540 nm. The concentration of protein was calculated using the absorbance of the standard concentration.

Statistical analysis

All the values are expressed as mean \pm standard error of mean (SEM). The values were analysed by one-way ANOVA followed by Student's Newman-Keuls post-hoc test using the Graph Pad software. Differences were considered significant when $p < 0.05$.

RESULTS

Total Cholesterol and Triglyceride: Figure 1 shows the total cholesterol of rats administered vitamin C and L-carnitine during fasting and re-feeding. Total cholesterol level was significantly increased in the fasted group supplemented with vitamin C and L-carnitine when compared to control and the fasted group ($p < 0.05$). On day one of recovery, both vitamin C and L-carnitine treated rats had increase in cholesterol levels compared

with FR-1 group ($p < 0.05$). The cholesterol level of the FR-1 group was also significantly decreased when compared with both control and fasting alone group. Day three of recovery showed similar pattern with day 1 of recovery with vitamin C and L-carnitine cholesterol levels higher than that of FR-3 alone group ($p < 0.05$), with cholesterol levels in the fasting recovery 3 group still decreased when compared with both control and fasting alone group ($p < 0.05$).

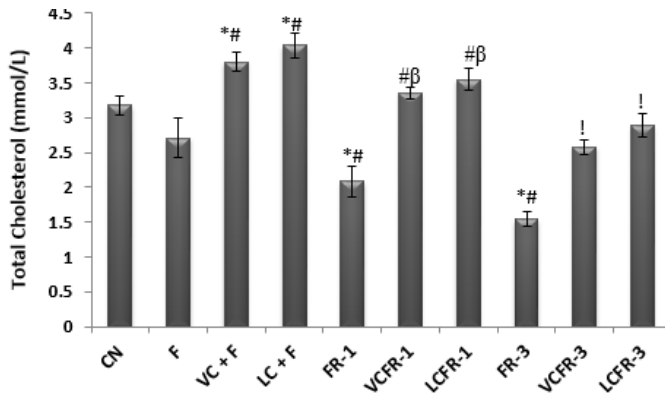


Figure 1: Total cholesterol levels in rats administered vitamin C and L-carnitine during fasting and re-feeding. * = significant difference from control ($p < 0.05$), # = significant difference from fasting group ($p < 0.05$), β = significant difference from FR-1 ($p < 0.05$) ! = significant difference from FR-3 ($p < 0.05$). CN= control, F= fasting, VC+F = vitamin C supplementation (200mg/kg b.w) and fasting, LC + F = L-carnitine supplementation (200mg/kg b.w) and fasting, FR-1 = 72 hours fasting + 24 hrs recovery, VC + FR-1 = vitamin C supplementation (200mg/kg b.w) + 72 hours fasting and 24 hours recovery, LC + FR-1 = L-carnitine supplementation (200mg/kg. b.w) + 72 hours fasting and 24 hours recovery, FR-3 = 72 hours fasting + 72 hours recovery, VC +FR-3 = vitamin C supplementation (200mg/kg b.w) + 72 hours fasting and 72 hours recovery, LC + FR-3 = L-carnitine supplementation (200mg/kg. b.w) + 72 hours fasting and 72 hours recovery.

No difference was found in the TG levels during fasting. However, on day one of recovery, triglyceride level was increased in L-carnitine treated rats when compared with FR-1 group, fasting alone group and control ($p < 0.05$). No difference was found in the levels of triglyceride on day three of recovery (figure 2).

LDL-cholesterol and HDL-cholesterol: Figure 3 shows the LDL-cholesterol levels in the rats administered vitamin C and L-carnitine during fasting and re-feeding. No difference was found in LDL levels during fasting but on day one of re-feeding, LDL level was reduced in FR-1 group when compared with control while L-carnitine treated rats had a higher LDL when compared with FR-1 group ($p < 0.05$). Day three of re-feeding showed no difference in the LDL levels.

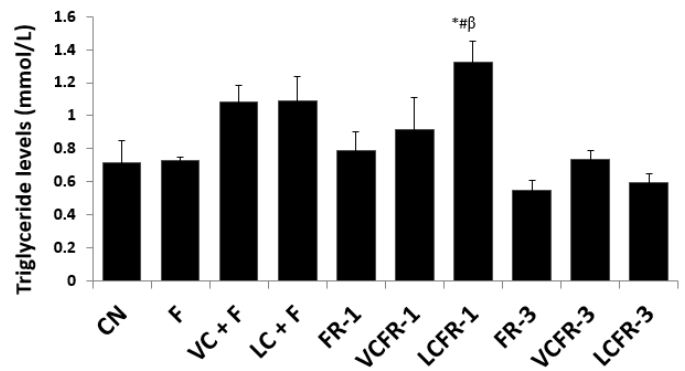


Figure 2: Triglyceride levels in rats administered vitamin C and L-carnitine during fasting and re-feeding. * = significant difference from control ($p < 0.05$), # = significant difference from fasting group ($p < 0.05$), β = significant difference from FR-1 ($p < 0.05$). CN= control, F= fasting, VC+F = vitamin C supplementation (200mg/kg b.w) and fasting, LC + F = L-carnitine supplementation (200mg/kg b.w) and fasting, FR-1 = 72 hours fasting + 24 hrs recovery, VC + FR-1 = vitamin C supplementation (200mg/kg b.w) + 72 hours fasting and 24 hours recovery, LC + FR-1 = L-carnitine supplementation (200mg/kg. b.w) + 72 hours fasting and 24 hours recovery, FR-3 = 72 hours fasting + 72 hours recovery, VC +FR-3 = vitamin C supplementation (200mg/kg b.w) + 72 hours fasting and 72 hours recovery, LC + FR-3 = L-carnitine supplementation (200mg/kg. b.w) + 72 hours fasting and 72 hours recovery.

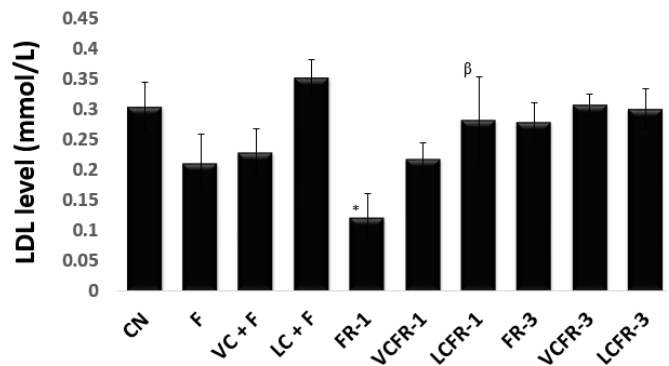


Figure 3: Low density lipoprotein-cholesterol levels in rats administered vitamin C and L-carnitine during fasting and re-feeding. * = significant difference from control ($p < 0.05$), β = significant difference from FR-1 group ($p < 0.05$), CN= control, F= fasting, VC+F = vitamin C supplementation (200mg/kg b.w) and fasting, LC + F = L-carnitine supplementation (200mg/kg b.w) and fasting, FR-1 = 72 hours fasting + 24 hrs recovery, VC + FR-1 = vitamin C supplementation (200mg/kg b.w) + 72 hours fasting and 24 hours recovery, LC + FR-1 = L-carnitine supplementation (200mg/kg. b.w) + 72 hours fasting and 24 hours recovery, FR-3 = 72 hours fasting + 72 hours recovery, VC +FR-3 = vitamin C supplementation (200mg/kg b.w) + 72 hours fasting and 72 hours recovery, LC + FR-3 = L-carnitine supplementation (200mg/kg. b.w) + 72 hours fasting and 72 hours recovery.

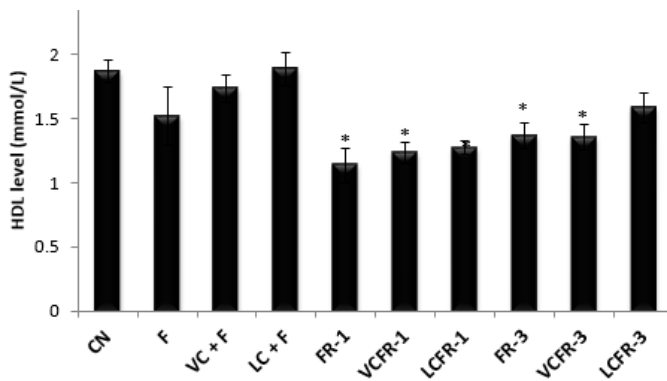


Figure 4: High density lipoprotein-cholesterol level in rats administered vitamin C and L-carnitine during fasting and re-feeding. * = significant difference from control ($p < 0.05$). CN= control, F= fasting, VC+F = vitamin C supplementation (200mg/kg b.w) and fasting, LC + F = L-carnitine supplementation (200mg/kg b.w) and fasting, FR-1= 72 hours fasting + 24 hrs recovery, VC + FR-1 = vitamin C supplementation (200mg/kg b.w) + 72 hours fasting and 24 hours recovery, LC + FR-1 = L-carnitine supplementation (200mg/kg. b.w) + 72 hours fasting and 24 hours recovery, FR-3 = 72 hours fasting + 72 hours recovery, VC +FR-3 = vitamin C supplementation (200mg/kg b.w) + 72 hours fasting and 72 hours recovery, LC + FR-3 = L-carnitine supplementation (200mg/kg. b.w) + 72 hours fasting and 72 hours recovery.

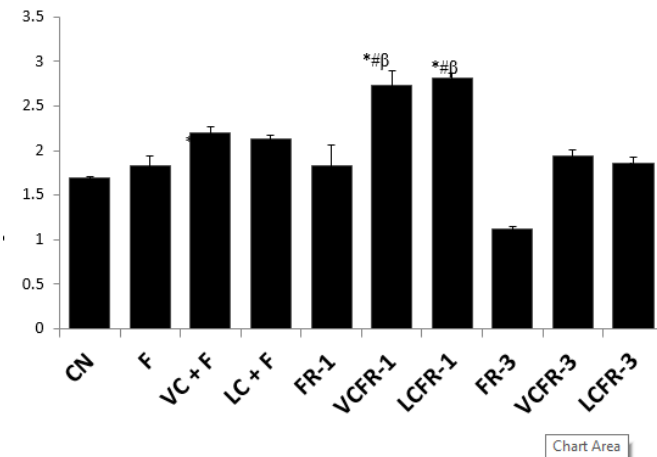


Figure 5: Total cholesterol: High density lipoprotein-cholesterol ratio in rats administered vitamin C and L-carnitine during fasting and re-feeding. * = significant difference from control ($p < 0.05$), # = significant difference from fasting group ($p < 0.05$), β = significant difference from FR-1 ($p < 0.05$) != significant difference from FR-3 ($p < 0.05$). CN= control, F= fasting, VC+F = vitamin C supplementation (200mg/kg b.w) and fasting, LC + F = L-carnitine supplementation (200mg/kg b.w) and fasting, FR-1= 72 hours fasting + 24 hrs recovery, VC + FR-1 = vitamin C supplementation (200mg/kg b.w) + 72 hours fasting and 24 hours recovery, LC + FR-1 = L-carnitine supplementation (200mg/kg. b.w) + 72 hours fasting and 24 hours recovery, FR-3 = 72 hours fasting + 72 hours recovery, VC +FR-3 = vitamin C supplementation (200mg/kg b.w) + 72 hours fasting and 72 hours recovery, LC + FR-3 = L-carnitine supplementation (200mg/kg. b.w) + 72 hours fasting and 72 hours recovery

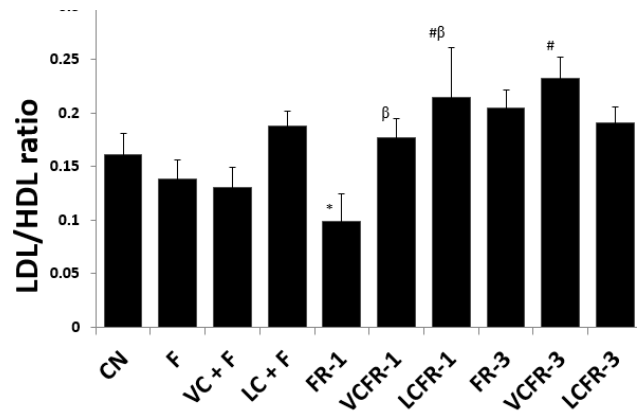


Figure 6: Low density lipoprotein-cholesterol: High density lipoprotein-cholesterol ratio in rats administered vitamin C and L-carnitine during fasting and re-feeding. * = significant difference from control ($p < 0.05$), # = significant difference from fasting group ($p < 0.05$), β = significant difference from FR-1 ($p < 0.05$). CN= control, F= fasting, VC+F = vitamin C supplementation (200mg/kg b.w) and fasting, LC + F = L-carnitine supplementation (200mg/kg b.w) and fasting, FR-1= 72 hours fasting + 24 hrs recovery, VC + FR-1 = vitamin C supplementation (200mg/kg b.w) + 72 hours fasting and 24 hours recovery, LC + FR-1 = L-carnitine supplementation (200mg/kg. b.w) + 72 hours fasting and 24 hours recovery, FR-3 = 72 hours fasting + 72 hours recovery, VC +FR-3 = vitamin C supplementation (200mg/kg b.w) + 72 hours fasting and 72 hours recovery, LC + FR-3 = L-carnitine supplementation (200mg/kg. b.w) + 72 hours fasting and 72 hours recovery

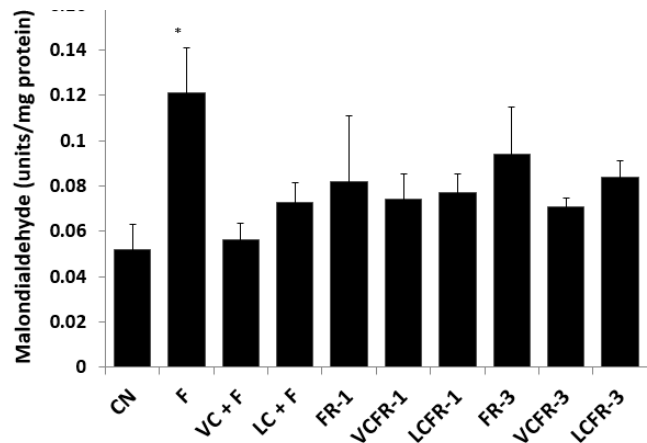


Figure 7: Malondialdehyde levels in rats administered vitamin C and L-carnitine during fasting and re-feeding. * = significant difference from control ($p < 0.05$). CN= control, F= fasting, VC+F = vitamin C supplementation (200mg/kg b.w) and fasting, LC + F = L-carnitine supplementation (200mg/kg b.w) and fasting, FR-1= 72 hours fasting + 24 hrs recovery, VC + FR-1 = vitamin C supplementation (200mg/kg b.w) + 72 hours fasting and 24 hours recovery, LC + FR-1 = L-carnitine supplementation (200mg/kg. b.w) + 72 hours fasting and 24 hours recovery, FR-3 = 72 hours fasting + 72 hours recovery, VC +FR-3 = vitamin C supplementation (200mg/kg b.w) + 72 hours fasting and 72 hours recovery, LC + FR-3 = L-carnitine supplementation (200mg/kg. b.w) + 72 hours fasting and 72 hours recovery.

HDL level was not different during fasting (figure 4). Day one of re-feeding however showed a significant decrease in HDL levels in the FR-1 group, Vitamin C group and L-carnitine supplemented group when compared with control. On day three of re-feeding, HDL level was decreased in the FR-3 group and Vitamin C group treated rats when compared with control.

TC/HDL ratio and LDL/HDL ratio: Figure 5 shows the total cholesterol: HDL ratio in rats administered vitamin C and L-carnitine during fasting and re-feeding. There was an increase in TC/HDL ratio during fasting in rats supplemented with vitamin C compared with control ($p < 0.05$). On day one of re-feeding, both vitamin C and L-carnitine treated rats had a significant increase in TC/HDL ratio when compared with control, fasting alone and FR-1 group. Day three of recovery showed that there was a significant decrease in TC/HDL ratio in FR-3 group when compared with fasting alone. Also, both vitamin C and L-carnitine supplementation rats on day 3 of recovery showed a significant increase in TC/HDL ratio when compared FR-3 group rats.

The LDL/HDL ratio showed no significant difference during fasting in fasting alone rats and in rats supplemented with vitamin C or L-carnitine when compared to control (Figure 6). Day one of re-feeding showed a significant decrease in LDL/HDL ratio in the FR-1 group compared with control. Vitamin C and L-carnitine treated rats had an increase in LDL/HDL ratio compared to FR-1 group. While there was also an increase in LDL/HDL ratio in L-carnitine treated rats when compared with fasting alone rats. Day three of re-feeding showed an increase in LDL/HDL ratio in vitamin C treated rats when compared with fasting alone rats ($p < 0.05$).

Lipid peroxidation: Figure 7 shows malondialdehyde level in rats administered vitamin C and L-carnitine during fasting and re-feeding. The result shows an increase in MDA level in the fasting alone rats when compared with control. No difference was found in the MDA levels during re-feeding day 1 and re-feeding day 3 ($p < 0.05$).

Antioxidant enzyme activity: Figure 8-10 shows the activity of SOD, GSH-px and catalase in rats administered vitamin C and L-carnitine during fasting and re-feeding. No significant difference was found in the SOD levels and catalase levels during fasting and during re-feeding on day 1 and 3 (figure 8 and 10). Glutathione peroxidase level (figure 9) was not different during fasting and day one of re-feeding. However, on day 3 of re-feeding, glutathione peroxidase levels were

increased when compared with control, fasting alone and FR-3 group alone.

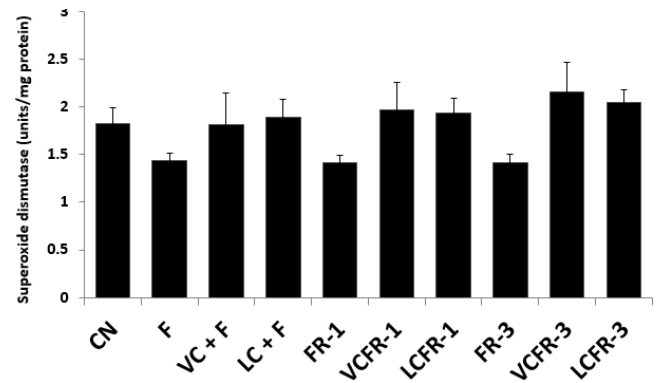


Figure 8: Superoxide dismutase activity in rats administered vitamin C and L-carnitine during fasting and re-feeding. No significant difference observed. CN= control, F= fasting, VC+F = vitamin C supplementation (200mg/kg b.w) and fasting, LC + F = L-carnitine supplementation (200mg/kg b.w) and fasting, FR-1= 72 hours fasting + 24 hrs recovery, VC + FR-1 = vitamin C supplementation (200mg/kg b.w) + 72 hours fasting and 24 hours recovery, LC + FR-1 = L-carnitine supplementation (200mg/kg. b.w) + 72 hours fasting and 24 hours recovery, FR-3 = 72 hours fasting + 72 hours recovery, VC +FR-3 = vitamin C supplementation (200mg/kg b.w) + 72 hours fasting and 72 hours recovery, LC + FR-3 = L-carnitine supplementation (200mg/kg. b.w) + 72 hours fasting and 72 hours recovery

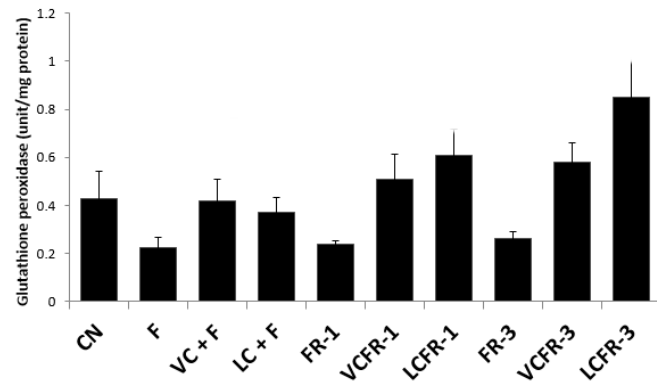


Figure 9: Glutathione peroxidase activity in rats administered vitamin C and L-carnitine during fasting and re-feeding. * = significant difference from control ($p < 0.05$), # = significant difference from fasting group ($p < 0.05$), != significant difference from FR-3 ($p < 0.05$). CN= control, F= fasting, VC+F = vitamin C supplementation (200mg/kg b.w) and fasting, LC + F = L-carnitine supplementation (200mg/kg b.w) and fasting, FR-1= 72 hours fasting + 24 hrs recovery, VC + FR-1 = vitamin C supplementation (200mg/kg b.w) + 72 hours fasting and 24 hours recovery, LC + FR-1 = L-carnitine supplementation (200mg/kg. b.w) + 72 hours fasting and 24 hours recovery, FR-3 = 72 hours fasting + 72 hours recovery, VC +FR-3 = vitamin C supplementation (200mg/kg b.w) + 72 hours fasting and 72 hours recovery, LC + FR-3 = L-carnitine supplementation (200mg/kg. b.w) + 72 hours fasting and 72 hours recovery.

DISCUSSION

Abnormalities in lipoprotein metabolism are a major cornerstone for cardiovascular disease prevention. It represents about 50% of population-attributable risk of developing cardiovascular disease (Yusuf *et al.*, 2004; Millan *et al.*, 2009). Prolonged fasting leads to break down of fat stores for energy production which results in an increase of the end products of lipids in circulation. The current studies showed an increase in total cholesterol levels in the rats supplemented with vitamin C and L-carnitine following a 72 hour fast. The increase in cholesterol levels might be due to the fact that both Vitamin C and L-carnitine are involved in the oxidation of fatty acids. While vitamin C is an essential cofactor for the biosynthesis of carnitine, a molecule required for the oxidation of fatty acids (Reyes *et al.*, 2013; Eichelberger *et al.*, 2014), L-Carnitine, itself transports long-chain acyl groups from fatty acids into the mitochondrial matrix, so they can be broken down through β -oxidation to acetyl-coA to enable us obtain energy via the Citric acid cycle (Mehta 2013; Marcovina *et al.*, 2013). This effect of L-carnitine on fat metabolism is therefore very important as fat stored in adipose tissue provides the major source of energy once the glycogen stores in the liver have been depleted during fast (Bykov *et al.*, 2003).

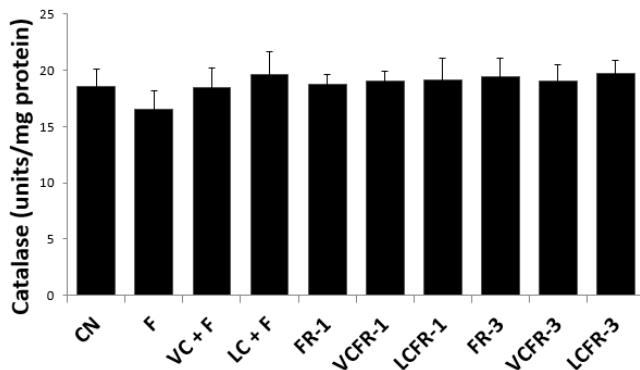


Figure 10:

Catalase activity in rats administered vitamin C and L-carnitine during fasting and re-feeding. No significant difference observed. CN= control, F= fasting, VC+F = vitamin C supplementation (200mg/kg b.w) and fasting, LC + F = L-carnitine supplementation (200mg/kg b.w) and fasting, FR-1= 72 hours fasting + 24 hrs recovery, VC + FR-1 = vitamin C supplementation (200mg/kg b.w) + 72 hours fasting and 24 hours recovery, LC + FR-1 = L-carnitine supplementation (200mg/kg. b.w) + 72 hours fasting and 24 hours recovery, FR-3 = 72 hours fasting + 72 hours recovery, VC +FR-3 = vitamin C supplementation (200mg/kg b.w) + 72 hours fasting and 72 hours recovery, LC + FR-3 = L-carnitine

supplementation (200mg/kg. b.w) + 72 hours fasting and 72 hours recovery.

This pattern of increase in total cholesterol continued during recovery 1 (24hours post fasting) and recovery 2 of fasting (72 hours post fasting) when compared with the rats fasted without supplementation. However, the cholesterol levels in the treated rats were not different from the control levels during recovery. This portrays that the increase in cholesterol levels by the treated rats might be an attempt to increase energy levels/production during fasting. The oxidation of long-chain fatty acids to acetyl-CoA is a central energy-yielding pathway in many organisms and tissues. The heart and Liver for example receives as much as 80% of the energetic needs from this process under physiological circumstances (Nelson and Micheal 2005; Lopaschuk *et al.*, 2010). In a fasted state, when glycogen reserves are used up, these percentages might increase tremendously. Since vitamin C and L-carnitine has to do with β -oxidation of fatty acid to enable us obtain energy via the Citric acid cycle it is likely that this surge in cholesterol levels during this stage could be linked to the energy needs.

LDL levels reduced on day 1 of recovery in fasted rats but recovered on day 3 of recovery to the levels of the control values. This indicates that fasting reduces LDL-cholesterol levels. On the other hand, HDL levels were unchanged during fasting in both fasted and treated rats but HDL levels reduced during re-feeding in both fasted and treated rats on Days 1 and 3 of recovery compared with control values. This portrays that, during fasting, LDL levels declines but begins to increase back during re-feeding. The opposite is true for HDL-cholesterol in which its levels were increased during fasting and reduced during re-feeding. This pattern is true for both fasted and supplemented rat. Studies by Adlouni *et al.*, (1997) and Qujeq *et al.*, (2002) has shown that human LDL-cholesterol were reduced during Ramadan fasting and HDL-cholesterol levels increased during fasting. This suggests that fasting may prevent the risk of atherosclerosis and coronary heart disease. This is because increased LDL-cholesterol (bad cholesterol) is implicated in atherosclerosis in human and animal models (Haberland *et al.*, 1988; Palinski *et al.*, 1989).

Triglycerides serve as storage for metabolic fuels. Its energy source is preferred to those of polysaccharides such as glycogen and starch, because the carbon atoms of fatty acids are more reduced than those of sugars (Nelson and Micheal 2005). Oxidation of triacylglycerols yields more than twice as much energy, gram for gram yield, as the oxidation of carbohydrates (Nelson and Micheal 2005). This study showed that

triglyceride was increased in the L-carnitine treated rats on day one of recovery when compared with control and fasted rats. This might be associated with the demand for energy during fasting and the role of L-carnitine during beta-oxidation of fatty acid.

The total/high-density lipoprotein (HDL) cholesterol ratio, known as the atherogenic or Castelli index and the LDL/HDL cholesterol ratio are two important components and indicators of vascular risk, the predictive value of which is greater than the isolated parameters (Millan *et al.*, 2009). No difference was observed in these ratios during fasting except for the increased TC/HDL ratio in vitamin C treated rats during fasting. During re-feeding on day one these ratios were increased in vitamin C and L-carnitine treated rats but returned towards control values after 72 hours of re-feeding. This trend shows that the initial increase in these index was as a result of the contributions of vitamin C and L-carnitine to β -oxidation of fatty acids during fasting (Reyes *et al.*, 2013; Eichelberger *et al.*, 2014). Portraying that these supplements would not create a vascular risk normally but tends to increase the use of fatty acids for energy during fasting.

Oxidative stress, caused by the imbalance between the generation and detoxification of reactive oxygen and nitrogen species (ROS/RNS), plays an important role in brain aging, neurodegenerative diseases, and other related adverse conditions, such as ischemia (Wang and Michealis 2010). The brain is one of the most sensitive organs to ROS-mediated damage due to its high oxygen consumption rate, high content of polyunsaturated fatty acids that oxidizes easily in the presence of redox-active metals (Valko *et al.*, 2007; Santos *et al.*, 2009). The change in the source of fuel to the brain from glucose to ketone bodies during prolonged fasting could pose some stressful conditions to the brain. Previous studies using 50% food deprivation showed that oxidative status in the brain is affected which could predispose brain cells to degeneration and death (Santos *et al.*, 2009). Results from this study showed an increase in MDA levels in the fasted rats. Though the MDA level in the brain was increased, there were no differences in the antioxidant enzyme activities of SOD, GSH and CAT. When the antioxidant defense system is unable to neutralize ROS production, an imbalance favoring oxidative stress occurs. In this study, since the activities of the enzymes were not reduced, it is plausible to say that mopping up actions by the endogenous antioxidants was going on. Although defenses against damage produced by ROS are extensive, including enzymatic and small molecule antioxidants as well as repair enzymes, an increased production of ROS or a poor antioxidant defense

network can lead to a progressive damage in the cell with a decline in physiological function (Mariani *et al.*, 2005).

It is also of noteworthy to state that the supplementation of the conventional antioxidant vitamin C prevented oxidative stress during fasting and re-feeding. This was also observed in the L-carnitine treated rats. L-carnitine has previously been reported to possess antioxidant capacity (Neri *et al.*, 2003; Rebouche, 2004). In conclusion, brain oxidative status was slightly perturbed during fasting, while the increase in cholesterol levels in vitamin C and L-carnitine treated rats was abolished during re-feeding. This portrays that the increased in cholesterol in the treated rats might be due to their actions on β -oxidation of fatty acids during fasting.

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