INTRODUCTION

L-carnitine plays an important role in enrichment of energy by using long-chain fatty acids into mitochondria for β-oxidation. Ninety eight percent of L-carnitine is remained in skeletal muscle, and plasma. Also, L-carnitine plays a major role in exercise among athletes. Athletes use it to increase the oxidation of fat during exercise and spare muscle glycogen. One of the main rules is to enhance red blood cells’ oxygen carrying capability especially in stressful situations [1].

Supplementation of L-carnitine is beneficial to increase energy used by muscles and enhance exercise performance. In contrary, in other study of healthy persons, no exercise performance’s enhancement was recorded, despite reported increase in skeletal muscle L-carnitine concentration[2].

There aren’t many studies on how oral L-carnitine supplements affect athletes. Oral L-carnitine was not listed as a helpful therapy in a recent significant statement from the Mitochondrial Medicine Society due to a lack of evidence for its efficacy. Nonetheless, in reality, a lot of athletes continue to take oral L-carnitine supplements [3].

An important component of red meat, L-carnitine, is broken down by intestinal bacteria to create trimethylamine (TMA), which is then broken down further by the hepatic enzyme flavin monooxygenase 3 to produce trimethylamine N-oxide (TMAO) (FMO3). Many studies have recently concentrated on a potential adverse effect of TMAO on cardiovascular effectiveness. Studies in 2011 found a link between TMAO and...
cardiovascular disease in people, and other research found that TMAO can hasten atherosclerosis in animals [4]. Moreover, a significant number of clinical research discovered a connection between plasma L-carnitine levels and myocardial infarction (MI), stroke, and death [5].

Myoglobin, creatine kinase-MB, cardiac troponins I and T (cTn I, -T), and heart-type fatty acid-binding protein 3 (H-FABP3) are indicators of cardiac tissue injury. H-FABP3 is a quick and accurate indicator of myocardial infarction and acute ischemia [6,7].

Up to our knowledge, few previous studies investigated the relationship between cardiac damage biomarkers and L-carnitine metabolite in L-carnitine supplemented individuals. So, we aimed here to study the cardiac effects of L-carnitine in L-carnitine supplemented students through assessment of TMAO and cardiac damage biomarkers H-FABP3 and the relationship between them.

**SUBJECTS AND METHODS**

**Study population**

Girls from the Faculty of Physical Education at Zagazig University who had been registered in the Tennis department with L-carnitine supplementation were the subjects of this cross-sectional comparative study. At the time of study recruitment, students aged 19 to 22 who had been taking L-carnitine supplements orally for at least three months were enrolled in our study. Students who used multiple supplements, were treated for chronic drug use, or had taken L-carnitine for less than three months were not included. In accordance with these exclusion criteria, our study only included 49 pupils, informed consent from every participant was signed. The Zagazig University Faculty of Medicine's Ethics Committee for Research (Institutional Review Board, or "IRB") provided an approval letter (ZU-IRB # 10378-24-1-2023).

**METHODS**

To evaluate the improvement in physical performance, all pupils must engage in physical activities’ exercises. L-carnitine, TMAO, H-FABP3, and circulating troponin I (cTn I) levels were measured in blood samples that were taken from the subjects. In Zagazig University Hospitals, electrocardiograms were performed. Every student had her 12-lead ECG analysis collected at a pace of 25 millimeters per second in Zagazig hospital's cardiology departments. According to Apple et al., VIDAS1 Troponin I Ultra (TNU, BioMerieux Inc., France) used the enzyme linked fluorescence assay technology using immunopassay sandwich technique to measure the levels of circulating cTn-I in serum. [8]. Myocardial injury was identified as occurring at levels higher than 1.3 ng/mL [9]. According to Wodzig et al., the serum concentration of H-FABP3 was measured using the ELISA method [10], kits, (Oxis International Inc., Foster City, California, USA). 1.6–19 ng/mL is its normal range [11] Using a triple quadrupole mass spectrometer and the stable isotope dilution tandem mass spectrometry approach, plasma TMAO was analyzed by ELISA [12]. Whole blood was used for the sandwich enzyme-linked immunosorbent assay (ELISA) method to analyze L-carnitine [13], kits (Bioassay Technology Laboratory, China).

**Statistics**

SPSS (Statistical Program for the Social Sciences) version 26 was used for data analysis. When necessary, the chi square test was used to compare categorical variables and describe them using their absolute frequencies. To validate the assumptions employed in parametric tests, the Shapiro-Wilk test was applied. Depending on the type of data, the means, standard deviations, or median and interquartile range of quantitative variables were used to characterize them. Independent sample t tests (for normally distributed data) and Mann Whitney tests (for not normally distributed data) were used to compare quantitative data between two groups. For properly distributed data, the Pearson correlation coefficients were utilized to determine the degree and direction of the link between two continuous variables. The beast cutoff of a specific quantitative parameter in diagnosis was determined using ROC curve. The best cutoff value for a particular quantitative measure was determined using the ROC curve to diagnose a particular medical condition. Using binary logistic regression, it was possible to pinpoint independent risk variables linked to certain health issues. To quantify the associated independent components for the dependent factor, linear regression analysis was done. The level statistical significance was set at \( P < 0.05 \). Highly significant difference was present if \( P < 0.001 \).

**RESULTS**

Regarding the age, there was no significant difference between the L-carnitine supplemented pupils and the controls (Table 1).

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Laboratory findings of L-carnitine, TMAO, H-FABP3, and cTn-I in group II compared to controls are presented in (Table 2). There is statistically significant difference between two groups (Table 2).

Between the tested groups, there is a statistically significant difference in terms of cardiovascular symptoms and ECG abnormalities. Heart rate was
the most frequent cardiovascular symptom (significantly higher in group II). In group II, ST segment depressions were found in 24 students (49%) and inverted T waves in 19 individuals (38.8%), as well as short PR intervals in 38 students (77.6% of whom were in group II) (Figure 2). For the control group, there were no ECG alterations and a normal ECG (Figure 1). Based on sinus tachycardia and a short PR interval, early clinical indicators of cardiotoxicity, 38 students (77.6%) had sinus tachycardia and a short PR interval, early clinical indicators of cardiotoxicity, 38 students (77.6%) had sinus tachycardia and a short PR interval, early clinical indicators of cardiotoxicity, 38 students (77.6%) had sinus tachycardia and a short PR interval, early clinical indicators of cardiotoxicity, 38 students (77.6%) had sinus tachycardia and a short PR interval, early clinical indicators of cardiotoxicity, 38 students (77.6%) had sinus tachycardia and a short PR interval, early clinical indicators of cardiotoxicity, 38 students (77.6%) had sinus tachycardia and a short PR interval, early clinical indicators of cardiotoxicity, 38 students (77.6%) had sinus tachycardia and a short PR interval. The relationship between cTnI and T wave inversion is significantly correlated with T wave inversion and L carnitine and H-FABP3, and cTnI laboratory results in group II compared to controls are shown in (Table 2). Two groups differ significantly from one another (Table 2).

Higher levels of L carnitine and HFABP3 independently enhance cardiotoxicity by 1.344 and 1.617 times, respectively, according to multivariate regression analysis of predictors of cardiotoxicity (Table 3).

Every parameter was noticeably greater than what the control group had. L carnitine positively correlates with all other aberrant measures, including heart rate, TMAO, CNT-1, and H-FABP3, in a statistically significant manner (Table 4). L carnitine and TMAO both independently correlated with H-FABP3 (unstandardized \( p=0.011 \) and \( p=0.001 \), respectively) (Table 5). L carnitine level was independently linked with TMAO (unstandardized =1.276, \( p=0.001 \)) (Table 6).

L-carnitine, TMAO, and H-FABP3 are statistically significantly correlated with T wave inversion and are statistically considerably higher in individuals with T wave inversion. While the relationship between cTn-I and T wave inversion is insignificant (Table 7). A statistically significant correlation exists between ST depression and TMAO, as well as H-FABP3, which is statistically significantly greater in ST segment depression patients. Although there is a statistically significant correlation exists between ST depression and TMAO, as well as H-FABP3, which is statistically significantly greater in ST segment depression patients. Although L carnitine or cTn-I have no statistically significant relationship with ST segment depression (Table 7).

The optimal L-carnitine cutoff for detecting L-carnitine-induced cardiotoxicity is 99.43 with an area under the curve of 0.952, a sensitivity of 94.7%, and a specificity of 81.8%. The optimal TMAO cutoff for detecting L-carnitine-induced cardiotoxicity is 95.9, with an area under the curve of 1, a sensitivity of 78.9%, and a specificity of 90.9%. With an area under the curve of 1, sensitivity of 78.9%, and specificity of 72.7%; 0.315 is the optimal CNT-1 cutoff for the diagnosis of L-carnitine-induced cardiotoxicity. The area under curve cutoff for H-FABP3 in the diagnosis of L-carnitine-induced cardiotoxicity is 43.645. The best cutoff of L carnitine in diagnosis of L-carnitine induced cardiotoxicity is ≥99.43 with area under curve 0.952, sensitivity 94.7% and specificity 81.8%. The best cutoff of TMAO in diagnosis of L-carnitine induced cardiotoxicity is ≥95.9 with area under curve 1, sensitivity 78.9% and specificity 90.9%. The best cutoff of CNT-1 in diagnosis of L-carnitine induced cardiotoxicity is ≥0.315 with area under curve 1, sensitivity 78.9% and specificity 72.7%. The best cutoff of H-FABP3 in diagnosis of L-carnitine induced cardiotoxicity is ≥ 43.645 with area under curve 1, sensitivity 89.5% and specificity 72.7% (Table 8) (Figure 3).

Table (1) Age among the studied groups.

<table>
<thead>
<tr>
<th></th>
<th>Group I (n=49)</th>
<th>Group II (n=49)</th>
<th>T</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>19.5 ± 2.2</td>
<td>20.02 ± 1.14</td>
<td>0.63</td>
<td>NS</td>
</tr>
</tbody>
</table>

Table (2) Comparison between the studied groups regarding the studied parameters.

<table>
<thead>
<tr>
<th></th>
<th>Group I (n=49)</th>
<th>Group II (n=49)</th>
<th>( \chi^2 )</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>ST depression</td>
<td>0 (0%)</td>
<td>24 (49%)</td>
<td>31.784</td>
<td>&lt;0.001**</td>
</tr>
<tr>
<td>T wave inversion</td>
<td>0 (0%)</td>
<td>19 (38.8%)</td>
<td>23.57</td>
<td>&lt;0.001**</td>
</tr>
<tr>
<td>Short PR interval</td>
<td>0 (0%)</td>
<td>38 (77.6%)</td>
<td>12.391</td>
<td>&lt;0.001**</td>
</tr>
<tr>
<td>Heart rate</td>
<td>78.86 ± 7.06</td>
<td>111.37 ± 15.75</td>
<td>-23.411</td>
<td>&lt;0.001**</td>
</tr>
<tr>
<td>L carnitine</td>
<td>44.95 ± 5.27</td>
<td>112.85 ± 15.14</td>
<td>-29.638</td>
<td>&lt;0.001**</td>
</tr>
<tr>
<td>TMAO</td>
<td>4.92 ± 0.82</td>
<td>99.72 ± 9.52</td>
<td>-69.471</td>
<td>&lt;0.001**</td>
</tr>
<tr>
<td>H-FABP3</td>
<td>1.71 ± 0.28</td>
<td>48.24 ± 2.87</td>
<td>-112.823</td>
<td>&lt;0.001**</td>
</tr>
</tbody>
</table>
Group I (n=49) | Group II (n=49) | \(\chi^2\) | P
---|---|---|---
Median (IQR) | Median (IQR) | Z | P
CNT-1 | 0.01 (0.01 – 0.02) | 0.39 (0.27 – 0.52) | -8.663 | <0.001**

**p≤0.001 is statistically highly significant**

**Table (3) Multivariate analysis of factors**

<table>
<thead>
<tr>
<th>B</th>
<th>P</th>
<th>AOR</th>
<th>95% C.I.</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Lower</td>
<td>Upper</td>
</tr>
<tr>
<td>L- carnitine</td>
<td>0.295</td>
<td>0.026*</td>
<td>1.344</td>
</tr>
<tr>
<td>H-FABP3 (ng/mL)</td>
<td>0.481</td>
<td>0.099</td>
<td>1.617</td>
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</table>

AOR adjusted odds ratio  CI Confidence interval *p<0.05 is statistically significant

**Table (4) Correlation between L-carnitine and the studied parameters:**

<table>
<thead>
<tr>
<th>R</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>TMAO</td>
<td>0.956</td>
</tr>
<tr>
<td>H-FABP3</td>
<td>0.943</td>
</tr>
<tr>
<td>CNT-1</td>
<td>0.904</td>
</tr>
</tbody>
</table>

**p≤0.001 is statistically highly significant**

**r Pearson correlation coefficient**

**Table (5) Linear stepwise regression analysis of factors associated with H-FABP3**

<table>
<thead>
<tr>
<th>Unstandardized Coefficients</th>
<th>Standardized Coefficients</th>
<th>t</th>
<th>P</th>
<th>95.0% Confidence Interval</th>
</tr>
</thead>
<tbody>
<tr>
<td>B</td>
<td>Std. Error</td>
<td>Beta</td>
<td>95.0% Confidence Interval</td>
<td></td>
</tr>
<tr>
<td>(Constant)</td>
<td>-0.740</td>
<td>1.732</td>
<td>-.427</td>
<td>0.670</td>
</tr>
<tr>
<td>TMAO</td>
<td>.469</td>
<td>.030</td>
<td>0.968</td>
<td>15.777</td>
</tr>
<tr>
<td>L-Carnitine</td>
<td>.011</td>
<td>.040</td>
<td>0.017</td>
<td>.280</td>
</tr>
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</table>

**p≤0.001 is statistically highly significant**

**Figure 1.** Normal ECG in control students. ECG: electrocardiography.
Figure 2. Some examples of ECG changes (short PR interval, ST segment depression) in L-carnitine supplemented students. ECG: electrocardiography.

Figure (3) ROC curve showing Performance of L carnitine, TMAO, CNT-1 and H-FABP3 in diagnosis of cardiotoxicity among students receiving L carnitine

**DISCUSSION**

This study’s major goal was to analyze the available data and assess the effects of L-carnitine supplementation on cardiac performance while assessing its impacts on exercise performance. The initial oral L-carnitine supplementation studies were unable to increase performance during moderate-intensity exercise. Studies on the long-term effectiveness and safety of L-carnitine supplementation have not been conducted but have recently been advised [14].

In our research, an aberrant ECG analysis revealed short PR intervals, ST segment depressions, and T wave inversions. No ECG alterations were seen in the control group. These results are consistent with those reported by Skagen et al. [15], who claimed that L-carnitine supplemented individuals had diagnostic ischemic changes recorded in the ECG that were in the form of T-wave changes (a “flat or inverted T-wave”) and non-specific ST-changes, either elevation or depression of the segment.

In our investigation, group II had significantly higher blood levels of L-carnitine than the control group, and this finding was consistent with those of Arazzi and Mehrtash [16].

Electrocardiographic evaluation in our work showed abnormal ECG findings including short PR interval, ST segment depression, and T wave inversion. In the control group, no ECG changes were found. These findings agree with Skagen et al. [17] who reported that L-carnitine supplemented individual had diagnostic ischemic changes recorded in the ECG which were in the form of T-wave changes ‘flat or inverted T-wave, and non-specific ST-changes, either elevation or depression of the segment.

In our study, blood L-carnitine was highly increased in group II compared to the control group, and this result was in agreement with Arazzi and Mehrtash [18].

In our investigation, there was no correlation between T wave inversion and other ECG
The smallest molecule among the cardiac injury biomarkers, heart-type fatty acid-binding protein 3 is able to leave injured cardiomyocytes earlier [25]. Serum H-FABP3 levels were measured in a rat model research by Sayed-Ahmed et al. [26] following a month of L-carnitine administration. Rats treated with L-carnitine had higher levels than the untreated rats. According to their findings, rats treated with L-carnitine may exhibit early cardiotoxicity depending on H-FABP3 levels. The findings of Valle et al. [27], who said that H-FABP3 is a helpful and sensitive sign in the early assessment of acute coronary syndrome, validated our findings. Due to its strong affinity for and ability to interact with long-chain fatty acid oxidative stress products, FABP is thought to be a potent antioxidant. The amount of total methionine residues in FABP is high. Amino acids methionine and cysteine are thought to be scavengers of cellular oxidative damage brought on by xenobiotics [28].

In our investigation, group II’s cTn-I level was significantly higher than that of the control group. This outcome is consistent with Emran et al’s findings [29].

Not just in acute myocardial infarction, cardiac troponins are sensitive indicators of heart muscle injury (AMI). Troponin levels can also rise under a variety of circumstances that either directly or indirectly cause heart muscle dysfunction. They start to show up in the serum four to eight hours after the commencement of the symptoms, reach their peak concentration, and continue to do so for seven to ten days following myocardial infarction [30].

Under non-pathological conditions, H-FABP3 serum concentration in the blood is very low (5 mg/L)[31]. H-FABP3 secretion, however, is brought on by cardiac injury, which is connected to a rise in myocardial cell membrane permeability. Several reports have been made. showing H-FABP3 monitoring can accurately and without false-negative results predict myocardial infarction within 1 hour [32].

CONCLUSION

The focus of this investigation is on the relation between long-term L-carnitine supplementation and its metabolites, TMAO, and the novel heart injury biomarker H-FABP3. H-FABP3 appears to be a marker for cardiovascular harm brought on by L-carnitine early on. To further understand the involvement of H-FABP3 and TMAO in L-carnitine-induced cardiotoxicity, larger clinical trials should be done in the future as this study only used a small sample size. Moreover, a thorough
trans-thoracic ECG examination of heart function would be beneficial.

Acknowledgement
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Conflict of Interests Statement
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REFERENCES


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