The role of serum monounsaturated fatty acids (MUFAs) and polyunsaturated fatty acids (PUFAs) in cardiovascular disease risk

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Abstract. Free fatty acids (FFA) observed as independent risk factors of cardiovascular diseases (CVD). In this study we investigated FFA levels in patients with CVD, and its risk factors. In this case-control study, 214 patients experienced coronary angiography and 222 healthy subjects were enrolled. Participants were categorized into two groups: who had >50% and <30% stenosis were assigned to the angiogram positive (N=90) and negative (N=124) group, respectively. Several risk factors were assessed and the levels of FFAs were determined using gas chromatography. Serum FFA concentrations were compared between healthy and patients with positive and negative angiograms. The association of serum FFA levels with four major risk factors (hypertension, FBG level, high BMI and WHR) were also assessed. Our data showed that median of FFAs was higher in patients than healthy subjects (p<0.0001), such as SFA and n6-FFAs (in patients; 1.59 (1.27) and 1.22 (1.06) and in healthy subjects 0.33 (0.38) and 0.36 (0.35), respectively). According to anthropometric and biochemical data, there were not statistical differences between the groups, except FBG, SBP and hs-CRP that showed significantly higher levels in patients than controls (p<0.0001, p=0.001). Also, lower median levels of total cholesterol, LDL-C, HDL-C and DBP were observed in patients which can due to lipid-lowering medication use like Statins. High serum levels of FFAs are considered as an independent risk factor for CVDs, while various types of FFAs can have different influences on CVD risk factors. Therefore, longitudinal studies are needed to clarify the association between FFAs and CVD risk factors. (www.actabiomedica.it)

Keywords: Free fatty acids, cardiovascular diseases, Risk factors
Abbreviations

High sensitivity C reactive protein: Hs-CRP; High-density lipoprotein: HDL; Low-density; apo-protein: LDL; Triglycerides: TG; Waist circumference: WC; Diastolic/systolic blood pressure: DBP/SBP; Fasting blood glucose: FBG; Mashhad University of Medical Science: MUMS; Body mass index: BMI; Standard deviation: SD; Interquartile range: IQR; Cardiovascular disease: CAD; Free fatty acid: FFA; Waist to hip ratio: WHR; Reaction species oxygen: ROS; Nuclear factor-kB: NF-kB; National Institutes for Medical Research Development: NIMAD; Acute coronary syndrome: ACS; Enzyme-linked immunosorbent assay: ELISA; Protein kinas C: PKC; Mono-unsaturated fatty acids: MUFAs; Polyunsaturated fatty acids: PUFAs; Saturated fatty acids: SFAs

Introduction

Cardiovascular disease (CVD) is reported as a major cause of mortality in the world and is considered responsible for one-third of all deaths in over 35 year-olds in west countries (1, 2). Some risk factors including high blood pressure, increased level of lipid profiles (total cholesterol, TG, LDL-C, HDL-C) and also diabetes have considerable attention because of their important role in the occurrence and development of CVD (3-5). In addition, some previous studies have suggested that a high level of fatty acids can be related to the incidence of cardiovascular disease (6-8) plasma free fatty acids (FFAs) are mainly generated during lipolysis in adipose tissues and provide a source of energy to the heart cells (9, 10). Oxidation of FFAs can lead to the accumulation of toxic intermediates, inflammatory responses and results in abnormalities known as heart diseases (11-13). The increased concentration of FFAs in plasma leads to enhance the binding activity of intranuclear NF-kB that acts to increase ROS generation and is an important step in the induction of inflammation (7, 14, 15).

Plasma FFA can negatively affect insulin activity and partly results in insulin resistance in type 2 diabetic subjects (16). Induced oxidative stress by FFA oxidation leads to endothelial apoptosis and increases insulin resistance (9, 17). Also, in diabetic individuals, lipolysis regulation of circulating FFAs is disrupted which leads to increased FFA oxidation and decreasing glycogenesis in turn (18). It was reported that oxidative modification of LDL-C leads to biological conditions, from foam cells to plaques formation, which results in heart diseases (19-21). In addition, high serum FFA levels may be an independent risk factor for hypertension (22). According to these results, in patients with hypertension and insulin resistance simultaneously, the level of fatty acids significantly increased (22).

A study on Chinese individuals with stable coronary artery disease suggested that increased plasma level of FFAs is an independent risk factor of CVD (23). Also, it was reported that higher levels of FFAs persist and may reflect the severity of ischemia and necrosis during the subacute phase of ACS (acute coronary syndrome) attack (24). According to findings, increased FFA level can be valuable as well as high TG levels and WC measurements for the prediction of CVD and even can be applied as a clinical guide for the early treatment of heart diseases (25). Although elevated plasma FFA concentrations are considered related to CVD, a single fasting measurement of plasma FFA levels does not appear to be an absolute risk factor when the information of other risk factors are considered (8). In addition, one study has investigated the association of FFAs as a risk factor with several cardiovascular diseases in subjects who underwent coronary angiography (26). According to these results, FFA levels are not related to the presence of angiographic CVD (26). In the present study, we aimed to assess the FFA levels in patients with CVD, and its risk factors.

**Figure 1.** Levels of FFAs in subjects with and without the main risk fators of CVD. * significant difference
Materials and methods

Study population

A total of 437 subjects mean aged 59-61 years old were recruited. All subjects were classified into one of three categories: 1) control group were those with no evidence of coronary artery disease, 2) who had >50% stenosis were assigned to the angiogram positive group 3) those with <30% stenosis were assigned to the angiogram negative group. In this study, patients were under treatments with lipid-lowering drugs such as Statin. In addition, subjects were divided into two groups based on the presence of four major CVD risk factors, including hypertension (SBP >140 mm Hg and DBP >90 mmHg), fasting serum glucose (>120 mg/d L) and obesity as mean WHR (>0.94) and BMI (>30 Kg/m²). Exclusion criteria include: pregnancy, and metabolic diseases for CVD group and subjects with confirmed data about taking medicinal drugs, or definite diagnosis of diabetes mellitus, hypertension, osteoporosis, autoimmune diseases, cancers, hemato-logic disorders, cardiovascular diseases, and hepatitis were excluded from control group. Informed consent was obtained from all participants using protocols approved by the National Institutes for Medical Research Development (NIMAD), Tehran, Iran.

Anthropometric and blood pressure measurements

Height was measured to the nearest 0.1 cm, and weight was measured to the nearest 0.1 Kg without shoes. BMI (body mass index) was calculated as weight (Kg)/height (m²). Blood pressure (mmHg.) was measured three times using a DONG BANG ACUPRIME device as each subject was requested to sit for approximately 30 minutes before every measurement. Three systolic and diastolic blood pressures (SBP and DBP respectively) were recorded and the average used for the analysis.

Biochemical analysis

All subjects were asked to fast for 12 to 14 hours before the blood sampling. The serum of blood was used to determine the levels triglycerides (TG), total cholesterol (TC), fasting blood glucose (FBG), low-density lipoprotein cholesterol (LDL-C) and high-density lipoprotein cholesterol (HDL-C) as lipid profiles using commercial kits and an Alycon autoanalyzer (ABBOTT, Chicago, IL, USA). Also, serum hs-CRP levels were estimated using the enzyme-linked immunosorbent assay (ELISA) kits (27).

Measurement of fatty acids levels

The first step included preparation and lipid extraction from plasma. Briefly, 500µl of the sample, 2ml chloroform, and 1ml ethanol were mixed vigorously. Then 600µl NaCl (0.9%) was added to the mixture and the reaction mixture was, vortexed uniformly and centrifuged at 2000 rpm for 3 minutes. After that, the lower phase was then collected and the solvent (chloroform) was removed by the rotary device evaporator (474 mbar, 40 °C, laborota 4003, heidolph rotary evaporator, Germany). For transesterification, the dried lipid extract was dissolved in 500 µl heptane and 100 µl of methanolic KoH (2 mol/L). Then the mixture was shaken vigorously and maintained at 78 °C for 15 minutes. Finally, the samples were centrifuged at 4000 rpm for 5 minutes and the upper phase was collected into a microtube and stored at -70 °C prior to GC injection. An amount of 1 µl was used for injection to the Gas Chromatography (GC) device (variant 450, USA) to detect the fatty acids. The device was equipped with a CP-Sill 88 coated with silicon-based polymers (polysiloxanes), polyethylene glycols and solid adsorbent (EU) fused silica capillary column (100 × 0.25 mm ID × 0.2 mm film thickness). The conditions were as followed: initial temperature 120 °C (holding for 5 minutes), temperature ramp 3 °C per minutes, 240 °C (holding for 10 minutes), and injector temperature at 260 °C. The detector was FID model, carrier gas was nitrogen, and split ratio was 1:20. The fatty acids were detected by comparing of retention time of samples with a synthetic standard (37 component FAME mix, SUPELCO, USA) with known FA composition, Then, a standard curve was prepared using the 3-point linear plot of the different dilutions of the standard, and the concentrations of fatty acids of each sample was reported as mg/ml.

Statistical analysis

Statistical analysis was performed using version 18 of SPSS. Continuous and categorical variables are reported as median with IQR and frequency percent-
age, respectively. A Student’s T-test was used for variables with a normal distribution. The Mann–Whitney U and Kruskal–Wallis tests were used not normally distributed data. For categorical variables a chi-square test was applied. P-value less than 0.05 was considered significant.

Results

All participants were 437 including control group (n=222) and patients (n=215). We compared baseline characteristics between healthy individuals and patients with CVD (angiography and non-angiography) in Table 1. The mean age of including subjects in both groups was in a similar range from 59 to 63 years old. Data showed the patients had a higher FBG level (106.66±52.63 mg/d Lit) than the control group (90.81±29.88 mg/d Lit) (p<0.0001). Although the ratio of waist to hip (WHR) and BMI revealed no statistical difference between the two groups, the blood pressure in patients differed significantly from the healthy group (p<0.0001). The mean of systolic blood pressure in patients was higher than controls (130.01±26.42 mm HG and 122.34±17.90 mm Hg, respectively), while it has not been shown for diastolic blood pressure. Interestingly, the level of lipid profiles had inconsistent results including higher levels of total cholesterol, HDL-C and LDL-C in control groups (190.83±41.28, 42.29±9.53 and 121.50±36.66 mg/d Lit respectively) and had no significant difference in TG level between controls and patients (p=0.21) did not show. Also, the higher level of hs-CRP was observed in patients (median 1.62 (2.83) mg/d Lit) in compared with a healthy group (median 1.33 (1.97) mg/d Lit) (p<0.0001).

The plasma levels of FFAs in the control group and patients were shown in Table 2. We also compared the level of various types of FFAs into separate groups of patients including angiography (Angio +) and non-angiography (Angio -) (n= 91 and n=124 respectively). Data indicated the higher FFAs levels in patients as mean significant differences in C17:0 (p<0.01), C18:3n3 (p=0.013), C14:0, C18:0, C20:3n6

Table 1. Baseline characteristics of the study population.

|                      | Control n=222 | Total n=215 | P-value |
|----------------------|---------------|-------------|---------|
| Age (year)           | 63.49±95.84   | 59.75±69.80 | 0.51    |
| FBG (mg/d Lit)       | 90.81±29.88   | 106.66±52.63| >0.0001 |
| Total cholesterol (mg/d Lit) | 190.83±41.28 | 183.53±48.22| >0.0001 |
| HDL (mg/d Lit)       | 42.29±9.53    | 42.00±10.94 | 0.02    |
| LDL (mg/d Lit)       | 121.50±36.66  | 114.46±39.42| >0.0001 |
| WHR                  | 0.93±0.05     | 0.94±0.07   | 0.29    |
| SBP (mm Hg)          | 122.34±17.90  | 130.01±26.42| >0.0001 |
| DBP (mm Hg)          | 80.31±10.46   | 77.27±12.68 | >0.0001 |
| BMI (Kg/m²)          | 27.90±4.72    | 27.63±5.31  | 0.20    |

|                      | Control Median (IQR) | Total Median (IQR) | P-value |
|----------------------|----------------------|-------------------|---------|
| TG (mg/d Lit)        | 125.00 (95.00)       | 127 (100)         | 0.21    |
| Hs-CRP (mg/d Lit)    | 1.33 (1.97)          | 1.62 (2.83)       | 0.001   |
| N (%)                |                      |                   |         |
| Hypertension (>140/90) | 40 (20.1)           | 97 (52.4)         | < 0.001 |
| WHR (>0.94)          | 104 (51.7)           | 93 (50.8)         | 0.85    |
| Obesity (BMI > 30)   | 62 (30.8)            | 31 (25.2)         | 0.27    |
| FBG (>120 mg/d Lit)  | 15 (7.4)             | 57 (35)           | < 0.001 |

Abbreviations: FBG = fast blood sugar, HDL = high density lipoprotein, LDL = low density lipoprotein, WHR = waist/hip ratio, SBP = systolic blood pressure, DBP = diastolic blood pressure, BMI = Body Mass Index, TG = triglyceride, Hs-CRP = high sensitivity C-reactive protein. *The data reported as median. *t-test, Mann–Whitney test was used.
(p<0.0001). Particularly, a dramatic increase has detected in C16:0, C16:1, C18:2n6c, and C18:1 oleic level (p<0.0001) in patients. Also, lower levels of SFA, MUFA, and PUFA were observed in the control group (p<0.0001) and according to data, the median level of SFA in patients (1.59 (1.27)) had much more difference in comparison to median of MUFA and PUFA levels (1.59 (1.27) and 0.69 (0.43)) respectively. The median levels of n6 and n3 fatty acids in patients (1.22 (1.06) and 0.48 (0.33) respectively) were higher than healthy group (0.36 (0.35) and 0.00 (0.08) respectively) (p<0.0001). The level of N6-FFAs was nearly three times less in controls and almost no n3-FFAs were observed in the healthy group. Notably, in patients, the median level of n6-FFA was dramatically higher in comparison to the n3-FFA level. In addition, Levels of FFAs in subjects with and without the main risk factors of CVD were investigated (Graph 1). According to this date, the level of FFAs had no significant difference in BMI and WHR categorized (p=0.51 and p=0.14), while showed revealed a significant difference in blood pressure and FBG categorized (p<0.0001).

| Fatty acid Type | Sample Size | Control Median (IQR) | Angio - Median (IQR) | Sample Size | Angio + Median (IQR) | P value | Sample Size | All patients Median (IQR) |
|----------------|-------------|----------------------|----------------------|-------------|----------------------|---------|-------------|-------------------------|
| SFA            | 222         | 0.33 (0.38)          | 124                  | 1.36 (1.39) | 90                   | 1.76 (1.39) | 0.0001      | 214                    | 1.59 (1.27)*            |
| C12:0          | 17          | 0.05 (0.00)          | 35                   | 0.57 (0.00) | 8                    | 0.05 (0.00) | 0.56         | 43                     | 0.058 (0.00)            |
| C14:0          | 81          | 0.10 (0.01)          | 96                   | 0.12 (0.02) | 47                   | 0.11 (0.04) | 0.0001      | 143                    | 0.124 (0.034)*          |
| C15:0          | 21          | 0.07 (0.00)          | 67                   | 0.072 (0.00) | 17                  | 0.077 (0.01) | 0.05         | 84                     | 0.073 (0.00)            |
| C16:0          | 221         | 0.14 (0.10)          | 124                  | 0.45 (0.33) | 90                   | 0.51 (0.38) | 0.0001      | 214                    | 0.48 (0.36)*            |
| C17:0          | 32          | 0.08 (0.022)         | 79                   | 0.092 (0.03) | 35                  | 0.096 (0.04) | 0.018        | 114                    | 0.094 (0.038)*          |
| C18:0          | 217         | 0.11 (0.06)          | 124                  | 0.26 (0.13) | 90                   | 0.27 (0.14) | 0.0001      | 214                    | 0.26 (0.148)*           |
| C20:0          | 2           | 0.070 (0.00)         | 4                    | 0.065 (0.00) | 0                    | 0.00       | 0.35         | 4                     | 0.065 (0.00)            |
| C24:0          | 4           | 0.065 (0.01)         | 20                   | 0.063 (0.006) | 5                   | 0.066 (0.008) | 0.21        | 25                     | 0.64 (0.00)             |
| MUFA           | 222         | 0.33 (0.38)          | 124                  | 1.36 (1.39) | 90                   | 1.76 (1.39) | 0.0001      | 214                    | 1.59 (1.27)*            |
| C14:1          | 1           | 0.00                 | 6                    | 0.07 (0.00) | 5                    | 0.07 (0.00) | 0.36         | 11                     | 0.071 (0.00)            |
| C16:1          | 120         | 0.08 (0.04)          | 98                   | 0.16 (0.12) | 68                   | 0.21 (0.25) | 0.0001      | 166                    | 0.183 (0.16)*           |
| C17:1          | 10          | 0.069 (0.00)         | 47                   | 0.069 (0.00) | 46                  | 0.072 (0.01) | 0.066        | 93                     | 0.070 (0.01)            |
| C18:1 elaidic  | 16          | 0.11 (0.05)          | 84                   | 0.102 (0.03) | 76                  | 0.108 (0.04) | 0.53         | 160                    | 0.106 (0.03)            |
| C18:1 oleic    | 215         | 0.14 (0.14)          | 124                  | 0.564 (0.54) | 90                  | 0.761 (0.58) | 0.0001      | 214                    | 0.63 (0.50)*            |
| C20:1          | 9           | 0.081 (0.012)        | 17                   | 0.084 (0.01) | 16                  | 0.088 (0.021) | 0.22        | 33                     | 0.084 (0.014)           |
| PUFA           | 222         | 0.082 (0.18)         | 124                  | 0.72 (0.47) | 90                   | 0.67 (0.41) | 0.0001      | 214                    | 0.69 (0.43)*            |
| C18:2n6c       | 209         | 0.30 (0.31)          | 122                  | 1.03 (1.06) | 90                   | 1.14 (0.82) | 0.0001      | 212                    | 1.06 (0.953)*           |
| C18:3n6        | 18          | 0.082 (0.01)         | 57                   | 0.083 (0.013) | 39                  | 0.084 (0.025) | 0.51        | 96                     | 0.084 (0.016)           |
| C18:3n3        | 46          | 0.078 (0.011)        | 64                   | 0.078 (0.015) | 46                  | 0.083 (0.017) | 0.013       | 110                    | 0.081 (0.016)*          |
| C20:2          | 17          | 0.081 (0.01)         | 58                   | 0.082 (0.007) | 41                  | 0.082 (0.00) | 0.42         | 99                     | 0.082 (0.007)           |
| C20:3n3        | 32          | 0.4 (0.32)           | 120                  | 0.38 (0.26) | 90                   | 0.40 (0.20) | 0.0001      | 210                    | 0.39 (0.23)             |
| C20:3n6        | 134         | 0.095 (0.04)         | 122                  | 0.139 (0.54) | 90                  | 0.15 (0.059) | 0.46         | 212                    | 0.143 (0.05)            |
| C22:2          | 19          | 0.079 (0.020)        | 8                    | 0.076 (0.00) | 31                  | 0.078 (0.012) | 0.48        | 39                     | 0.076 (0.011)           |
| C22:6n3        | 33          | 0.090 (0.02)         | 81                   | 0.090 (0.028) | 17                  | 0.087 (0.033) | 0.99        | 98                     | 0.089 (0.02)            |
| N3             | 222         | 0.00 (0.08)          | 124                  | 0.51 (0.36) | 90                   | 0.45 (0.27) | 0.0001      | 214                    | 0.48 (0.33)*            |
| N6             | 222         | 0.36 (0.35)          | 124                  | 1.20 (1.19) | 90                   | 1.2 (0.97)  | 0.0001      | 214                    | 1.22 (1.06)*            |

Values are expressed as median with range (IQR). The concentration of fatty acids was in mg/ml.
Interestingly, the mode of CVD subjects with hypertension is 52.4% and with high FBG is 35% (shown in Table 1). Also, the level of FFAs of individuals with hypertension was nearly double than of normal subjects (0.83 (1.20) and 0.42 (1.00) respectively) (p=0.007). Moreover, patients with high FBG showed a high level of FFAs circa two times more than the normal group (0.95 (1.24) and 0.4 (0.89) respectively).

**Discussion**

The major findings of our study revealed that the plasma level of fatty acids in patients with CVD is higher in comparison to healthy individuals which may be associated with some risk factors of heart diseases observed in patients (both angiography and non-angiography groups). Although some studies suggested that elevated plasma concentration of FFA has no relation with increasing the risk of ischemic heart disease and CVD mortality (8, 28), some other results reported that FFAs are independently related to cardiac mortality in patients with angiographic cardio artery diseases (26) and even is reported as a risk factor for future sudden death in such patients after nearly 7 years (29). Also, it was found high levels of FFAs play a role in arrhythmia and myocardial dysfunction (30-32).

According to evidence, high FFAs concentration is considered as toxic because of inducing oxidative stress (9, 13), subsequently leading to vascular endothelial dysfunction and inflammation (33). Both in-vivo and in-vitro studies reported that inflammatory cytokines such as IL-6 and TNF-α can be added to adipocytes which results in enhancing lipolysis and FFA levels (34). Elevating in circulating FFAs can active PKC-mediated inflammatory pathways and also increases the generation of oxidants (35, 36). All these results in endothelial dysfunction and inflammation (34-37). C-reactive protein is a highly sensitive biomarker of inflammation and tissue damage which is suggested for predicting major cardiovascular events (38, 39). In our study, we observed a statistical association between high levels of FFA and increased hs-CRP in patients who were in agreement with the known inflammatory action of elevated level FFA.

Based on some studies (40, 41) n-3 fatty acid intakes have developed effects on TG concentration, inflammatory factors, and arrhythmia, while n-6 PUFA is the main precursors of eicosanoids (C20) and has been considered as pro-inflammatory molecules (42). Our findings showed no significant difference in n-3 fatty acids between healthy and patient groups, however, the level of C20:3n6 (n-6 PUFA) was double in patients which can confirm the previous results.

The results of observational studies that investigated the association of FFA levels in the blood or adipose tissue to blood pressure are contradictory (43-47). It was reported no relation between serum fatty acid composition and blood pressure (46), while another found an inverse association between special FFA levels and blood pressure (43). Cholesterol ester stearic acid (18:0) has shown an inverse correlation with both systolic and diastolic pressure and particularly, the higher levels of stearic acid were related to lower levels of diastolic pressure (47, 48). Although Stearic acid is a saturated fatty acid (SFA) which is expected to raise blood pressure, it is less atherogenic than other SFAs and has an inverse association with blood pressure (49, 50). Also, dihomogammalinolenic acid (20:3) has an inverse relation with diastolic pressure, which results from lowering endothelial prostacyclin levels (48, 51). It was suggested that differences in the level of such prostaglandin may mediate the inverse correlation of DBP with dihomogammalinoenic (48). In agreement with such results, our findings showed that the level of stearic and dihomogammalinolenic acid in plasma of patients was more double in comparison with the control group. Likewise, diastolic blood pressure (DBP) was lower in patients. Some studies also reported that higher serum and adipose tissue levels of palmitoleic acid (16:1) are associated with heart diseases which refer to its independent correlation with a higher level of systolic blood pressure (48, 52). Palmitoleic acid, a monounsaturated fatty acid, is a metabolite of palmitic acid (16:0) and it’s relation to systolic blood pressure may deal with synthesis and metabolism of palmitic acid (48). Our data revealed high levels of plasma palmitic and palmitoleic acid in patients nearly three times more than a healthy group which may be the reason for the high level of systolic pressure in the patient group.
Elevated plasma FFAs play an important role to cause insulin resistance in non-diabetic people (53). FFAs are considered to exert atherogenic effects because of inducing oxidative stress which leads to apoptosis of endothelial and insulin resistance (9, 54). In the patient with heart diseases, a disruption of cardiac energy metabolism has often seen (25). B-oxidation blocks due to reducing transport of FFAs into the mitochondria (55). Some intermediates as fatty acyl-CoA and acylcarnitine accumulate in the cytosol are considered harmful (1, 55). They also increase mitochondrial uncoupling proteins which ultimately leads to less synthesis of efficient myocardial ATP (56). It also results in inhibition of Na+ K+ ATPase pump which reduces transport of glucose-4 into cells and the efficient myocardial glucose metabolism (57). According to such mechanism, high levels of FFAs can be effective on high levels of glucose and subsequently cardiovascular diseases (26). It is in agreement with our finding that higher levels of both FFAs and FBG observed in patients.

Our finding revealed the level of HDL-C, total cholesterol and LDL-C were lower in patients. A lower level of LDL-C can result from Statin treatment in patient groups. As reported, the main effect of statins is reducing plasma LDL-C concentrations (58). In addition, high levels of FFA can stimulate exchange the HDL and LDL which ultimately leads to low synthesis of efficient myocardial ATP, while the impacts of various FFAs can increase the formation of long-chain PUFAs (66) which was similar to our data of PUFAs levels. Nevertheless, statin type, treatment duration and dose can be effective on changes in lipid profiles and FFAs levels (67), then it is suggested to investigate more about such fields.

According to some results, high level of TG combined with increased WHR were considered as indicators for CVD (68) and the FFA level is relevant to them (69), whereas, in our study, the levels of TG, WHR and BMI in both groups showed no significant difference (26). Nevertheless, we investigated the correlation between FFAs levels and four main risk factors (shown in Table 1) in all subjects (healthy and patients). Our findings showed that hypertension and high level of FBG correlated significantly with higher FFAs levels which were in agreement with other studies (22, 23, 48). In spite of some previous reports (25, 26, 70, 71), no relation was observed in WHR and BMI with the level of FFAs. Although all these findings may be influenced by diet, medicine treatments, environmental and genetic factors and change in lifestyle in subjects.

**Conclusion**

The high level of fatty acids is considered as a risk factor for CVDs, while the impacts of various FFAs are different and each one can have specific influences on other known risk factors. The level of FBG and hypertension have a correlation with high levels of FFAs which can be considered an independent risk factor for CVDs. Regarding contradictory findings obtained from previous studies, more investigations are required to assess the association between Types of FFAs with CVD risk factors.

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