

Clinical evaluation and serum lipid profile between individuals with acute hepatitis C

Abstract

Aim: Hepatitis C virus (HCV) exerts a profound influence on host lipid metabolism. It has been suggested that the synthesis of both fatty acids (FA) and cholesterol is dysregulated in HCV but this has not been directly quantified in humans.

Methodology: In present study the serum lipid and FA's profile (free and total) of hepatitis C patients (n=50) is evaluated in comparison to healthy controls (n=50). Blood hematology, serum proteins, enzymes, waste metabolites and nutrition status were also assessed by standard methods.

Results: The HCV patients have significantly lower lipid profile including triglycerides, cholesterol, HDL and LDL in relation to controls. Results of serum lipid FA's (total and free form) reveal elevated level of saturated fatty acid (SFA) and monounsaturated fatty acid (MUFA), with lower content of polyunsaturated fatty acid (PUFA) in hepatitis C patient as compared to controls. Among SFA, myristic and palmitic acid were increased and five unsaturated FAs including nervonic, linoleic, alpha-linolenic, docosahexaenoic and arachidonic acid were reduced than control subjects. Significantly higher ($P < 0.05$) alanine transaminase, alkaline phosphatase, direct bilirubin and globulin levels were found in HCV patients possibly due to viral infection.

Conclusion: This study provides direct evidence that lipogenesis is increased as evident from low serum PUFA and higher SFA as well as MUFA in patients with HCV compared to healthy controls and hence HCV patients have low PUFA levels, which suggests a role for dietary PUFA supplementation.

Keywords: complete picture of blood, proteins and enzymes, waste metabolites, lipids, GC – FID.

29

30 **Introduction**

31 The hepatitis C virus (HCV) is a linear, single strand RNA virus of the *flaviviridae*
32 family that was identified in 1989 and recognized as a major causal agent of non – A and B
33 hepatitis[1] . The World Health Organization (WHO) had estimated that every year, 3–4 million
34 people are infected with the hepatitis C virus. About 150 million people are chronically infected
35 and at risk of developing liver cirrhosis and/or liver cancer. More than 3.5 million people die
36 from hepatitis C-related liver diseases every year [2]. HCV is the one of the leading causes of
37 chronic liver disease worldwide, affecting 3% of world population. Prevalence of HCV infection
38 reported in various studies by the Pakistani authors ranges from 2.2-14%.Accordingly;
39 approximately 10 million people in Pakistan are infected with HCV [3].

40 The liver is the one of most important organ in energy metabolism. Most of the endogenous
41 lipids and lipoprotein are synthesized in liver. This depends on the integrity of liver cellular
42 function, which ensures homeostasis of lipid and lipoprotein metabolism [1]. HCV is a unique
43 virus targeted towards liver cells; this virus closely interacts with host lipoprotein metabolism.
44 The particles of HCV exist in binding from with beta lipoprotein [low density lipoprotein (LDL)
45 and very low density lipoprotein (VLDL)] and immunoglobulins. Complexing of the virus to
46 VLDL or LDL could promote the endocytosis of HCV via the LDL receptor [4]. Diagnosis and
47 treatment of HCV- related autoimmune features has become a clinical challenge in HCV-
48 infected patients, in whom chronic liver disease associated with severe autoimmune features may
49 contribute to a very poor prognosis. The natural history of HCV infection in terms of chronic
50 toxicity and disease progression seems to be largely determined by the host immune response to
51 virus-infected hepatocytes [5, 6].

52 Serum ALT has also been correlated with the degree of histological inflammation in the
53 liver. Long-term elevation in serum transaminases, especially alanine transaminase (ALT), was
54 once the principal indication of persistent HCV infection. Serum liver enzymes such as ALT,
55 alkaline phosphatase (ALP) tested routinely and automatically in current clinical settings [8].

56 Recently, fatty acids have been implicated in the pathogenesis of several diseases associated with
57 metabolic disorders (such as obesity, diabetes and cardiovascular disease) [9, 10] and in

immunological response [11]. In liver diseases, especially in non-alcoholic steatohepatitis, the effect of impaired peroxisomal polyunsaturated fatty acid metabolism and nonenzymatic oxidation on fatty acid constitution is associated with disease progression [12]. It has been reported that HCV core protein has effects on fatty acid synthesis, and that fatty droplets in the liver are related to development of disease [13, 14]. It has been suggested that the synthesis of both fatty acids and cholesterol is dysregulated in HCV but this has not been directly quantified in humans.

These findings suggests that examination of serum biochemical and metabolic profile including FA composition, which may be important for understand biological feature in hepatitis C infection and may help to select the suitable treatment.

Material and Methods

Fifty hepatitis C (PCR positive) patients along with age and sex matched healthy controls were included in the study. 10 ml intravenous blood was collected from the patients and healthy subjects; 5ml was transferred in cp vacate for complete picture (CP) of blood and analyzed by CP analyzer MS43e. 5ml blood was transferred in gel tube, serum was separated and immediately used for liver function test (ALT, ALP, Total, direct, and indirect bilirubin) and serum total protein (albumin, globulin) and lipid profile (cholesterol, triglyceride (TAG), high density lipoprotein (HDL), low density lipoprotein (LDL) total lipid using kit method based on spectrophotometric and microlab measurements. The kits are manufactured by Daisy's diagnostic system, Germany. Fatty acid composition was analyzed by Perkin Elmer Gas chromatograph 8700 equipped with FID detector by derivatization as reported by Liebich [15]. All solvents and reagents were used analytical grade.

Sample Preparation:

Fasting blood sample were instantly centrifuge at 3000 rpm for 15 minutes and stored at -80 °C before analysis. Total fatty acids were extracted from 200 µl serum by adding 4ml methanol/toluene (4:1, v/v) and 400 µl acetyl chloride. The mixture was reacted at 100 °C for 60 min in screw-cap vials on the heating/stirring module, and then neutralized by shaking with 5ml

87 of 6% aqueous potassium carbonate. Following the addition of 2ml toluene with subsequent
88 mixing the sample was centrifuged at 2000rpm for 10 minutes. The toluene phases with the FA
89 methyl esters (FAMES) were subjected to gas chromatographic (GC) analysis.

90 The free fatty acid were methylated and isolated from 200 µl serum by adding 5ml
91 methanol and 200 µl acetyl chloride. The mixtures were reacted at 40 °C for 45 minutes in
92 screw-cap vials on the heating/stirring module, and were then neutralized by shaking with 3ml of
93 6% aqueous potassium carbonate. Following the addition of 2ml hexane with subsequent mixing
94 the sample was centrifuge at 2000rpm for 10 minutes. The supernatant collected was analyzed by
95 GC.

96 **Gas chromatographic analysis of the total and free fatty acid**

97 Fatty acid analysis was performed on a model 8700 Perkin–Elmer Ltd.,
98 (Buckinghamshire, England) fitted with nonbonded biscynopropylsiloxane stationary-phase,
99 polar capillary column Rt-2560 (100 m × 0.25 mm) 0.2 µm film thickness (Supelco, PA, USA)
100 and an FID. Oxygen-free nitrogen was used as a carrier gas at a flow rate of 3.5 mL/min. The
101 initial oven temperature was 120 °C at rate of 4 min which was raised to 220 °C held for 20 min.
102 The injector and detector temperature were set at 260 °C and 270 °C, respectively. A sample
103 volume of 2.0 µl was injected.

104 Peaks were identified by authentic standards supplied by Fluka Chemika (Buchs, Switzerland).
105 The Fatty acid standards, myristic acid (C14:0), myristoleic acid (C14:1), palmitic acid (C16:0),
106 palmitoleic acid (C16:1w9), alpha-linolenic acid (ALA (C18:3w3), stearic acid (C18:0), linoleic
107 acid (C18:2w6), oleic acid and vaccenic acid (C18:1), eicosapentaenoic acid (EPA (C20:5w3),
108 arachidonic acid (C20:4w6) arachidic acid (C20:0), docosahexaenoic acid (DHA (C22:6w3),
109 docosenoic acid (C22:1), nervonic acid(C24:1), eicosatrienoic acid (C-20:3) All the
110 quantification was done by gas chromatography. The FA composition was reported as a relative
111 percentage of the total peak area.

112 **Results**

113 The clinical characteristics of HCV patients (n=50) blood samples and the healthy non – HCV
114 controls (n = 50) are shown in Table 1. The mean age of HCV patients and controls was 34.16

years (range, 17 – 57 years). Some clinical characteristics including random blood sugar, hemoglobin, mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), total leucocytes, lymphocytes, monocytes, platelets, serum ALT, ALP, direct and indirect bilirubin, urea, globulin, albumin globulin (A/G) ratio were severely affected by hepatitis C virus, which reveals marked differences in the number of abnormal liver chemistry test. Significantly higher ALT and ALP levels were found and total and direct bilirubin, albumin was similar in HCV patients and controls. However direct bilirubin, globulin and total leucocytes including lymphocytes was found significantly higher in HCV patients with significantly lower A/G ratio, urea, random blood sugar, hemoglobin, MCV, MCH, MCHC and platelets counts in HCV patients as Compared to controls.

The HCV patients have significantly lower LDL, HDL, TAG and total lipid in relation to controls (Table 2).

The Table 3 summarizes the TFA composition of HCV patients. Mean levels of individual FAs in two groups were subjected to pair-wise comparison. This revealed that there were significant differences in the levels of seven FAs between the samples from the HCV patients and controls. Two SFA myristic and palmitic acid were elevated and five unsaturated FAs including nervonic, linoleic, alpha-linolenic, DHA and arachidonic acid were reduced in HCV patients than controls.

The total FFA composition of HCV patient's serum in comparison to control is depicted in Table 4 ; the total free SFA and MUFA contents were higher in HCV patients, where as only one SFA arachidic acid and total free PUFA were found lower except eicosatrienoic acid in HCV patients as contrast of controls. The significant difference occurred in three saturated and three unsaturated FAs including myristic, palmitic, stearic, palmitoleic, linoleic acid and arachidonic acid.

Figure 1and 2 shows the detailed evaluation of total free form of fatty acids. The SFA, MUFA were elevated and PUFA was lower both total and free form in HCV patients with contrast of healthy subjects.

Discussion

Our study has shown significantly elevated level of ALT and ALP in HCV patients as compared to controls. ALT is a cytosolic enzyme, which is specific to the liver function and disease. Elevations in ALT levels should be interpreted as indicative of liver disease with only rare exceptions: severe rhabdomyolysis or systemic myopathies. ALP being more common with superadded viral infection [1].

In present study patients shows lower A/G ratio and increased globulin level that may reflect an increased burden on the liver. Umer et al. [16] has also found elevated levels of alpha-1, beta and gamma globulin levels in serum for combined anti- HCV and HBsAg positive group as compared to seronegative and anti-HCV positive groups. The significantly lower urea, random blood sugar, hemoglobin, MCV, MCH, MCHC and platelets counts were detected in HCV patients; similarly abnormal level of these clinical characteristics have been reported previously [17, 18]. The result shows higher total leucocytes including lymphocytes in HCV patients in contrast to control. It has been established that the extracellular binding of HCV-E2, stimulates the proliferation of hepatocytes and lymphocytes [19].

The present study shows significantly lower lipid profile in HCV patients. HCV induced steatosis occurs via different mechanism that interfere in the host lipid production for its benefit. The HCV interfere in the mevalonate pathway leading to decreased cholesterol production compensatory up regulation of LDL receptors leading to decrease LDL levels, comparable to the mechanism of HMG-CoA reductase inhibitors [20]. In addition hepatitis C associated with reduced level of microsomal triglyceride transferase protein (MTIP), the enzyme responsible for the production of VLDL, which is in turn accountable for decreased circulating LDL and cholesterol levels. Thus the decrease in non HDL cholesterol levels, a composite of principally VLDL and LDL, could reflect viral inhibition of MTIP [21].

The fatty acids were extracted from serum to describe de novo fatty acid synthesis in HCV patients in Comparison of healthy subjects. The current work provides evidence that lipogenesis is elevated and have produced higher levels of SFA and MUFA with reduced PUFA in HCV patients. The evidence is mounting from multiple sources to suggest lipogenesis is up-regulated in HCV and tied to viral progression. The lipogenesis increase the availability of saturated FA for cell membranes and consequently reduces plasma membrane PUFA content. Membrane lipid saturation is observed in cancer cells and is hypothesized to provide protection from

chemotherapy agents and cell death by reducing peroxidation, due to replacement of PUFA with saturated FA. Enhanced lipogenesis may also contribute to hepatic lipid droplets, which are necessary for HCV assembly [22-24].

In vitro and animal studies have shown that the HCV interfere FA metabolism by altering the expression of metabolically important genes including stearoyl-CoA desaturase (SCD). Therefore, it is conceivable that direct viral effects might contribute to the changes in hepatic FA profile. Variations in genes like FADS1 ($\Delta 5$ -desaturase) and FADS2 ($\Delta 6$ -desaturase) or SCD might also influence enzyme activity and FA acid composition [25]. However to our knowledge, there are currently no studies available investigating the fatty acid metabolism of HCV patients.

Conclusion

We found that level of hemoglobin, platelet, total leucocytes, monocytes , lymphocytes, random blood sugar, serum enzymes , proteins , waste metabolites, lipid profile and FA composition was altered in acute hepatitis C patients. The direct and indirect bilirubin, urea, globulin, albumin globulin ratio were severely affected by hepatitis C virus, the enzymes ALT and ALP were found significantly higher in hepatitis C patients. This study provides direct evidence that lipogenesis is increased as evident from low serum PUFA and higher SFA as well as MUFA in patients with HCV compared to healthy controls. Further study is necessary to resolve if these aberrations are related to development of cirrhosis, steatosis, and viral replication. The prospective for targeting lipid metabolism in HCV patients is high, given the plentiful evidence of dietary-related methods to reduce lipogenesis. In particular, dietary PUFA supplementation as adjuvant therapy has potential for numerous positive effects for HCV patients but further study is required to delineate exactly what benefits this therapy may hold.

Abbreviations

HCV: Hepatitis C virus, FA's: fatty acids, SFA: saturated fatty acid, MUFA: monounsaturated fatty acid, PUFA: poly unsaturated fatty acid, VLDL: very low density lipoprotein, LDL: low density lipoprotein, HDL: high density lipoprotein, TAG: triglyceride, ALT: transaminase, ALP: alkaline phosphatase, FAMES: FA methyl esters, GC: gas chromatography, FID: flame ionization detector, MCV: mean corpuscular volume, MCH: mean corpuscular hemoglobin,

202 MCHC: mean corpuscular hemoglobin concentration, MTIP: microsomal triglyceride transferase
203 protein, SCD: stearyl-CoA desaturase.

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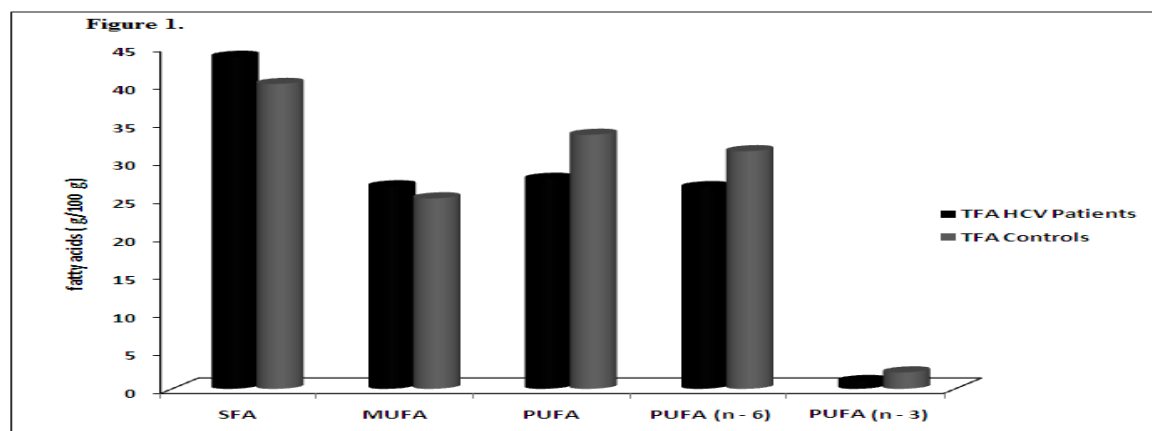
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277 Figure Captions

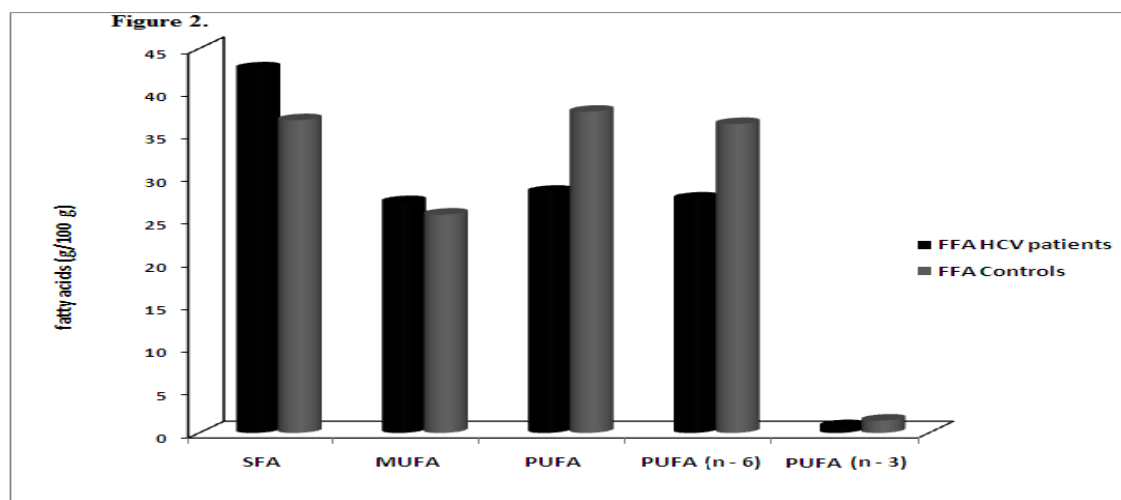
278 **Figure 1. Comparison of SFA, MUFA, PUFA including n-3 and n-6 fatty acids in total FA**
 279 **composition of Controls and HCV patients**



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282 **Figure 2. Comparison of Free SFA, MUFA, PUFA including n-3 and n-6 fatty acids in free**
 283 **FA composition of Controls and HCV patients**



298 **Table 1.Comparison of laboratory profile between HCV patients and healthy controls**

Clinical parameters	Healthy Controls	HCV Patients
Age (years)	34.2± 9.2 (17 – 57)	34.16 ± 9.1 (17 – 57)
Random Blood sugar (mg/dl)	140 ± 30* (100 – 170)	104.8 ± 26.4* (74 – 185)
Total Protein (g/dl)	7.1 ± 0.9 (6.2 – 8.0)	7.451 ± 0.7 (5.8 – 8.5)
Serum Albumin (g/dl)	4.5 ± 1.0 (3.5 – 5.5)	4.5 ± 0.6 (3.2 – 6.0)
Globulin (g/dl)	1.6 ± 0.5* (1.1 – 2.2)	2.9 ± 0.69* (0.6 – 4.2)
A/G Ratio	1.65 ± 0.55 (1.1 – 2.2)	1.29 ± 0.39* (0.9 – 2.1)
Serum Creatinine (mg/dl)	0.7 ± 0.2 (0.5 – 1.1)	0.8 ± 0.2 (0.3 – 1.1)
RBC (m/cmm)	4.7 ± 0.6 (4.0 – 5.5)	4.7 ± 0.6 (3.61 – 6.4)
Hemoglobin (g/dl)	14 ± 1.4* (12 – 16)	12.1 ± 1.9* (9.0 – 15.1)
MCV (μm3)	90 ± 10* (80 – 100)	78.0 ± 7.4* (53 – 86)
MCH (pg)	30 ± 4.0* (26 – 34)	25.8 ± 3.7* (15 – 32.2)
MCHC (g/dl)	34±3.0 (31 – 37)	32.7±2.4* (30 – 34.3)
Total Leucocytes count (m/cmm)	7 x 10 ⁹ /L ± 27* (4 – 10 x 10 ⁹ /L)	8 x 10 ⁹ /L ± 16* (6 – 11 x 10 ⁹ /L)
Neutrophil (%)	58 ± 4.0*(54 – 62)	66.5 ± 8.1* (40 – 78)
Esinophil (%)	2 ± 1 (1 – 3)	2.2 ± 1.4 (1 – 4)
Monocytes (%)	31.5 ± 8.0* (24 – 44)	2.6 ± 1.8* (1 – 9)
Lymphocytes (%)	4.5 ± 1.5* (3 – 6)	28.8 ± 7.7*(17 – 58)
Basophils (%)	0.6 ± 0.4 (0 – 1)	ND
Platelets (m/cmm)	300 x 10 ⁹ /L ± 150 (150 – 450 x 10 ⁹ /L)	245 x 10 ⁹ /L ± 66* (116 – 314 x 10 ⁹ /L)
Total bilirubin (mg/dl)	0.6 ± 0.5 (0.1 – 1.2)	0.7 ± 0.3 (0.5 – 1)
Direct bilirubin (mg/dl)	0.15 ± 0.15* (0 – 0.3)	0.4 ± 0.2*(0.2 – 1.2)
In direct bilirubin (mg/dl)	0.5 ± 0.2 (0.3 – 0.7)	0.374 ± 0.117(0.2 – 0.6)
Urea (mg/dl)	30 ± 14.1(20 – 40)	25 ± 8.3*(9 – 40)
SGPT or ALT (U/L)	25 ± 14*(11 – 39)	74.1 ± 34.2*(21 – 120)

299 Values are median (range)*different from HCV patients with healthy controls, p<0.05 (T test).

Table 2. Lipid profile of HCV patients in comparison of controls subjects

Lipid profile	Control subjects	HCV Patients
Age	34.2 ± 9.2	34.2 ± 9.1
Cholesterol (mg/dl)	170.1 ± 9.7	159.7 ± 13.8*
LDL (mg/dl)	102.8 ± 5.2	82.9 ± 12.1*
HDL (mg/dl)	55.5 ± 7.8	30.3 ± 9.6*
Triglyceride (mg/dl)	126.5 ± 12.1	114.0 ± 1.3*
Total Lipid (mg/dl)	543.8 ± 34.2	496.3 ± 6.6*
Coronary risk (HDL/LDL Ratio	2.4 ± 0.86	5.1 ± 2.39*

Values are mean ± standard deviation*different from HCV patients with healthy controls, $p<0.05$ (T test).

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317 **Table 3. Total fatty acid composition of HCV patients in comparison of control Subjects**

Total fatty acids	Controls	HCV patients
C – 14:0	1.1 ± 3.9	2.4 ± 0.65*
C - 16 : 0	23.8 ± 5.3	27.2 ± 0.93*
C - 18 : 0	14.7 ± 5.5	13.9 ± 2.2
C - 20 : 0	0.4 ± 1.1	0.3 ± 0.1
C - 14 : 1	0.5 ± 0.98	0.3 ± 0.02
C - 16 : 1	2.6 ± 2.2	3.5 ± 0.4
C - 18 : 1	19.8 ± 4.6	20.3 ± 0.95
C - 22 : 1	1.9 ± 1.4	2.5 ± 0.33
C – 24 : 1	0.35 ± 0.73	ND*
C - 18 : 2	25.0 ± 5.4	21.3 ± 0.5*
C - 18 : 3 (n-3)	0.9 ± 1.3	0.5 ± 0.3*
C - 20 : 4 (n-6)	5.9 ± 2.2	5.2 ± 0.2*
C - 20 : 5 (n-3)	0.98 ± 1.7	0.5 ± 0.1
C - 20 : 3 (n-6)	0.3 ± 0.6	0.1 ± 0.2
C - 22 : 6	0.4 ± 0.7	ND*

318 ^aValues are mean ± standard deviation*different from HCV patients with healthy controls, $p<0.05$ (T test).

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328 **Table 4. Comparison Free fatty acid composition between HCV patients and control**
 329 **subjects**

Free fatty acids	Controls	HCV patients
C – 14:0	1.5 ± 0.6	2.05 ± 0.02*
C - 16 : 0	23.7 ± 3.6	27.2 ± 2.01*
C - 18 : 0	10.8 ± 4.1	12.03 ± 2.97*
C - 20 : 0	0.4 ± 0.9	0.11 ± 0.71
C - 14 : 1	0.12 ± 0.3	1.1 ± 0.2
C - 16 : 1	2.8 ± 1.4	3.7 ± 0.2*
C - 18 : 1	20.6 ± 2.9	20.7 ± 1.21
C - 22 : 1	2.1 ± 1.3	2.5 ± 0.4
C - 18 : 2	29.9 ± 4.7	22.5 ± 1.9*
C - 18 : 3 (n-3)	0.4 ± 1.1	0.3 ± 0.2
C - 20 : 4 (n-6)	6.1 ± 1.7	4.9 ± 0.6*
C - 20 : 5 (n-3)	1.0 ± 1.6	0.2 ± 0.3
C - 20 : 3 (n-6)	0.2 ± 0.7	0.3 ± 0.2

330 Values are mean ± standard deviation*different from HCV patients with healthy controls, $p < 0.05$ (T test).

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