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*World J Gastroenterol* 2005;11(41):6422-6428
***World Journal of Gastroenterology*** ISSN 1007-9327




# Changes in lipid metabolism in chronic hepatitis C

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Supported by the grant from the Hungarian Ministry of Education, No.NKFP-1A/0023/2002 National Research Development Projects and grant from the Hungarian National Scientific Research Fund, No.OTKA T037838
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Received: 2004-10-29 Accepted: 2004-11-19

## Abstract

**AIM:** To investigate the relationship between certain biochemical parameters of lipid metabolism in the serum and steatosis in the liver.

**METHODS:** The grade of steatosis (0-3) and histological activity index (HAI, 0-18) in liver biopsy specimens were correlated with serum alanine aminotransferase (ALT), total cholesterol and triglyceride levels in 142 patients with chronic hepatitis C (CH-C), and 28 patients with non-alcoholic fatty liver disease (NAFLD) without hepatitis C virus (HCV) infection. The serum parameters were further correlated with 1 797 age and sex matched control patients without any liver diseases.

**RESULTS:** Steatosis was detected in 90 out of 142 specimens (63%) with CH-C. The ALT levels correlated with the grade of steatosis, both in patients with CH-C and NAFLD ($P<0.01$). Inserting the score values of steatosis as part of the HAI, correlation with the ALT level ($P<0.00001$) was found. The triglyceride and cholesterol levels were significantly lower in patients with CH-C (with and without steatosis), compared to the NAFLD group and to the virus-free control groups.

**CONCLUSION:** Our study confirms the importance of liver steatosis in CH-C which correlates with lower lipid levels in the sera. Inclusion of the score of steatosis into HAI, in case of CH-C might reflect the alterations in the liver tissue more precisely, while correlating with the ALT enzyme elevation.

**Key words:** Lipid metabolism; Chronic hepatitis C; NAFLD

Jármay K, Karácsony G, Nagy A, Schaff Z. Changes in lipid metabolism in chronic hepatitis C. *World J Gastroenterol* 2005; 11(41):6422-6428
http://www.wjgnet.com/1007-9327/11/6422.asp

## INTRODUCTION

Hepatic steatosis is a frequent and characteristic histological finding in patients with chronic hepatitis C (CH-C) in addition to bile duct damage and lymphoid follicles[1-5]. Fat accumulation in hepatocytes has been reported in 30-70% of liver biopsy specimens obtained from patients infected with hepatitis C virus (HCV)[1-4]. Whether steatosis is mainly related to host factors or to the viral cytopathic effect is still uncertain and a matter of dispute[6-15]. The pathogenesis of development of steatosis in CH-C is complex; host factors including alcohol consumption, exposure to other hepatotoxins, obesity, insulin resistance, type 2 diabetes mellitus, hypertriglyceridaemia, hypobetalipoproteinemia have all been identified as determinants[8,12,16-18].

The most important question concerning the clinical significance of steatosis in CH-C is whether lipid accumulation influences the liver disease progression, affects treatment, or is solely an innocent bystander[5,19]. A role of steatosis in the progression of CH-C is suggested by the finding that the severity of fat accumulation correlates with the stage of fibrosis[20,21] and liver cell apoptosis[5]. Treatment with peginterferon or IFNα-2b and ribavirin has been found to reduce steatosis in HCV genotype 3 patients[1]. On the other hand, no significant association between the stage of fibrosis and liver steatosis has been found by others[22]. *In vitro* studies and transgenic mouse model have both shown the HCV core protein to be capable of inducing steatosis by itself[23,24], which suggests a direct effect of specific viral sequences in the pathogenesis of lipid accumulation. An association of nonstructural protein 5A (NS5A) with lipid droplets and apoA1 has also been observed[11].

The aim of the present work was to study the changes in certain parameters of lipid metabolism in patients with CH-C and compare the findings with the accumulation of fat in liver biopsy specimens and with the histology activity index (HAI). For this reason the serum cholesterol and triglyceride levels of CH-C patients were examined. Patients were selected on the basis of the presence or absence of steatosis in their liver biopsy specimens, as well

as the absence of other risk factors for developing steatosis from a broader HCV-positive group referred to our center for treatment of chronic liver diseases. The results were compared to the lipid levels of patients with non-alcoholic fatty liver disease (NAFLD) verified histologically (NAFL, obesity, diabetes mellitus), and with data of a further control group: outpatients from the praxis without clinical and laboratory signs of liver disease, with or without history of alcohol abuse.

## MATERIALS AND METHODS

### Study population

A total of 142 patients with chronic HCV infection with no history of alcohol consumption were studied before the treatment with antiviral drugs (Table 1). CH-C was diagnosed on the basis of biochemical (serum aminotransferases greater than or equal to 1.5 times the upper normal value for at least 6 mo), serological (positivity for anti-HCV antibody, detection of HCV-RNA in the sera using the Amplicor HCV assay, Roche, Mannheim) and histological findings in liver biopsy specimens. Among the patients, 90 (63%) had micro- or macro-vesicular steatosis in their hepatocytes. This group comprised 46 females (26 patients younger than 44 years, average 38 years; 20 patients older than 45 years, average 52 years) and 44 males (29 patients younger than 44 years, average 38 years; 15 patients older than 45 years, average 51 years). There were 52 CH-C patients without steatosis, and this group comprised 17 females (10 patients younger than 44 years, average: 29 years; 7 patients older than 45 years, average: 53 years) and 45 males (24 patients younger than 44 years, average: 34 years; 11 patients older than 45 years, average: 49 years).

The control group was made up of 28 (16 males and 12 females) patients with NAFLD without HCV infection (Table 1).

A total of 1 797 patients from the outpatient praxis served as another control group which was matched to the CH-C patients with regard to age (Table 1). The 133 patients younger than 44-year-old female patients included 125 non-alcoholic and 8 alcoholic, the 819 patients older than 45-year-old females included 803 non-alcoholic and 16 alcoholic patients. Among the 146 patients younger than 44-year-old males, 60 were non-alcoholic and 86 were alcoholic patients. Out of the 699 patients older than 45-year-old males, 509 were non-alcoholic and 190 were alcoholic patients (Table 1).

### Laboratory, virology assessment

Blood samples for the evaluation of alanine aminotransferase (ALT), total cholesterol and triglyceride were obtained after overnight fasting. Routine biochemical tests were carried out using commercially available kits. The HCV-RNA was determined in the sera using an RT-PCR assay (Amplicor, Roche, Mannheim), with a sensitivity of 70 copies/mL.

### Histopathological evaluation

Liver biopsy was performed according to the Menghini technique. The biopsy specimens were formalin-fixed and paraffin-embedded, routinely stained with hematoxylin-eosin, picrosirius red, and a Berliner-blue reaction was carried out for the detection of iron. The slides were evaluated by two independent pathologists. A modified Knodell's histological activity index score[25,26] was applied to semiquantitatively score necroinflammatory activity (grade 0-18) and fibrosis (stage 0-4). Steatosis was graded according to the percentage of hepatocytes containing vacuoles in the cytoplasms (macro- or micro-vesicular steatosis). Steatosis was graded as follows, based on the percentage of fat containing hepatocytes[18]: none (0); mild (1: <10% of hepatocytes); moderate (2: 10-30% of hepatocytes); and severe (3: >30% of hepatocytes).

### Statistical analysis

One-way analysis of variance was used to differentiate the data among the groups. Pearson's correlation coefficient was applied to measure the degree of association between the variables. $P < 0.05$ values were corrected according to the Bonferroni method. All values were presented as mean ±SD.

## RESULTS

Steatosis was detected in 90 out of the 142 specimens (63%) with CH-C. The ALT levels were significantly higher in patients with grade 3 compared to grade 0 steatosis in the CH-C group ($P < 0.01$) (Figure 1). Significant correlation was found between the ALT level and HAI in the CH-C group without steatosis ($r = 0.556$, $P < 0.0002$) (Figure 2A), while this correlation could not be detected in the CH-C with steatosis subgroup (Figure 2B). Inserting the score values of steatosis (0-3) as part of the activity index (HAI)

**Table 1** Study population with or without HCV-infection

| | Male | | | Female | | | Total |
|---|---|---|---|---|---|---|---|
| | <44 (yr) | >45 (yr) | total | <44 (yr) | >45 (yr) | Total | |
| HCV-infection: | | | | | | | |
| With steatosis | 29 | 15 | 44 | 26 | 20 | 46 | 90 |
| Without steatosis | 24 | 11 | 45 | 10 | 7 | 17 | 52 |
| No HCV-infection | | | | | | | |
| NAFLD | 10 | 6 | 16 | 5 | 7 | 12 | 28 |
| No liver disease: | | | | | | | |
| Non alcoholic | 60 | 509 | 569 | 125 | 803 | 928 | 1 497 |
| Alcoholic | 86 | 190 | 276 | 8 | 16 | 24 | 300 |

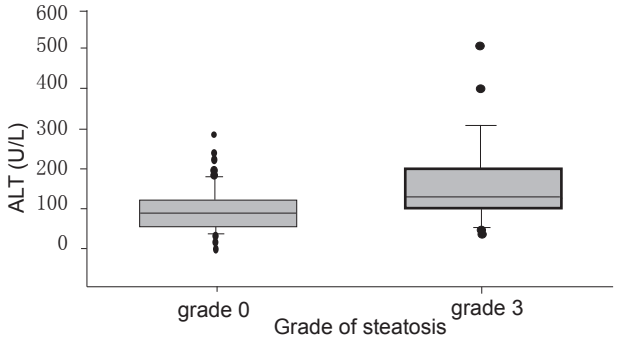


**Figure 1** Differences of ALT levels in CH-C patients without steatosis ($n$ = 52, grade 0) and with severe steatosis ($n$ = 28, grade 3) ($P$ < 0.01).

of chronic hepatitis, the correlation between the ALT level and histological scores of inflammatory activity grade was confirmed again in the 142 CH-C patients ($r$ = 0.41, $P$<0.00001) (Figure 2C). The ALT level also correlated with grade of steatosis in patients without HCV infection ($r$ = 0.533, $P$<0.005) (Figure 3), similarly to the CH-C patients. Upon comparing the ALT levels in patients with and without steatosis and with steatosis only, the highest ALT level was found in the steatotic CH-C group (Figure 4).

Triglyceride and cholesterol levels of CH-C patients were subgrouped according to age and sex, with and without steatosis. The lipid values of the CH-C groups were compared to the lipid values of the virus-free steatotic patients. The data of these subgroups were further compared based on their average age with the same age and sex group, as well as with the population from the outpatient praxis concerning drinking habits. The lipid levels of CH-C patients with liver steatosis were compared to the levels noted in the drinking outpatients, while these data involving the CH-C patients without liver steatosis were related to the non-drinking outpatients (Figures 5A and B; 6A and B; 7A and B; 8A and B)

The triglyceride levels were low in all the eight separated subgroups with CH-C (with and without steatosis, female, male, young, and elderly patients) (Figures 5A and B; 6A and B). These levels were conspicuously lower compared to the same values of the CH-C virus-free steatotic patients as well as to the drinker or nondrinker population.

Among the same groups, the cholesterol levels showed the same pattern as noted for the triglyceride levels. The only difference was that the young steatotic HCV infected male patients failed to show higher cholesterol levels, as was the case in relation to triglyceride (Figures 7A and B; 8A and B).

## DISCUSSION

Liver steatosis is an important hallmark of CH-C[1-4,8,10,13,27-29]. The mechanism of HCV-related steatosis, however, is not exactly clear. Both metabolic[8,16-18,29,30] and viral cytopathic effects[7,10,11,13,24] have been suggested to play a role in the pathogenesis. The HCV viral components, especially the HCV-core protein, have been found in association with steatosis. Lipid droplets on which the core accumulated have been found in HCV-core-transfected cell lines strongly expressing this protein[24]. Hepatic overexpression of the HCV-core protein has been shown to interfere with the hepatic assembly and secretion of triglyceride-rich very low density lipoproteins (VLDL)[31]. Fatty change was detected *in vivo* in core-expressing transgenic mice too[23]. Chimpanzees chronically infected with HCV developed steatosis similarly to human beings[24]. The degree of steatosis in chimpanzees correlated with the amount of viral load and ALT[32].

More recently the NS5A of HCV has been shown in association with lipid droplets and apoA1, suggesting that NS5A may contribute to the pathogenesis of steatosis commonly observed in HCV-infected livers[11]. It has, however, been demonstrated that neither HCV viral load nor HCV genotype is associated with the severity of steatosis in certain studies[29,33].

HCV is a lipid-containing virus which binds to low density lipoprotein, VLDL, IgG and to a minor degree to IgM and high density lipoprotein[34]. This is in correlation with the finding that HCV infection is associated with hypo-β-lipoproteinemia, which occurs in the early stages of HCV infection before the development of cirrhosis[16,35]. The correlation between apo B levels and HCV viral load confirms the interaction between HCV infection and β-lipoprotein metabolism. This suggests that β-lipoproteins competitively inhibit HCV infection, because both HCV and β-lipoprotein use the same LDL receptor[16].

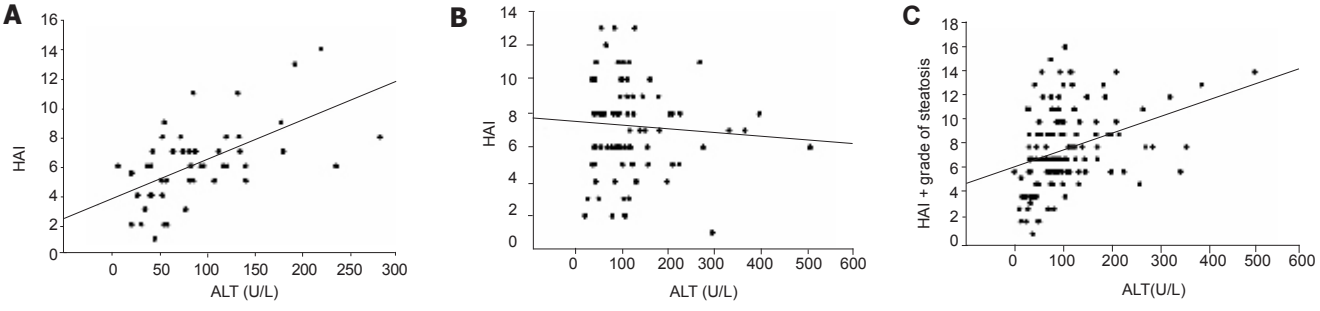


**Figure 2** Correlation between ALT levels and HAI/HAI+steatosis in CH-C patients. **A:** without steatosis ($n$ = 52, $r$ = 0.556, $P$ < 0.00002); **B:** with steatosis ($n$ = 90, NS); **C:** in CH-C patients ($n$ = 142, $r$ = 0.41, $P$ < 0.00001).

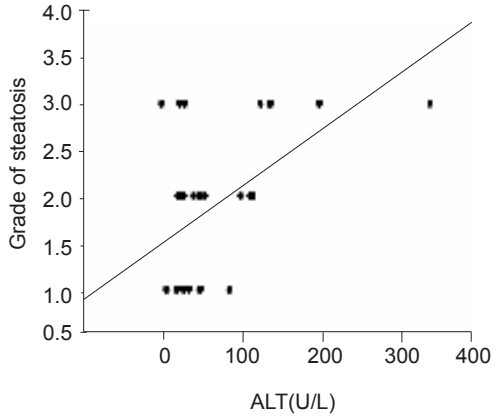


**Figure 3** Correlation between ALT levels and grade of steatosis in patients without CH-C ($n$ = 28, $r$ = 0.533, $P$<0.005).

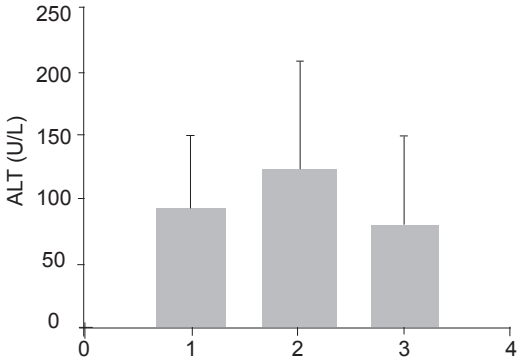


**Figure 4** ALT levels in CH-C patients without and with steatosis and in patients with steatosis without CH-C. Bar 1: CH-C patients without steatosis ($n$ = 52). Bar 2: CH-C patients with steatosis ($n$ = 90). Bar 3: Patients with liver steatosis without CH-C ($n$ = 28) Bar 1 $P$<0.02 *vs* Bar 2. Bar 2 $P$<0.02 *vs* Bar 3.

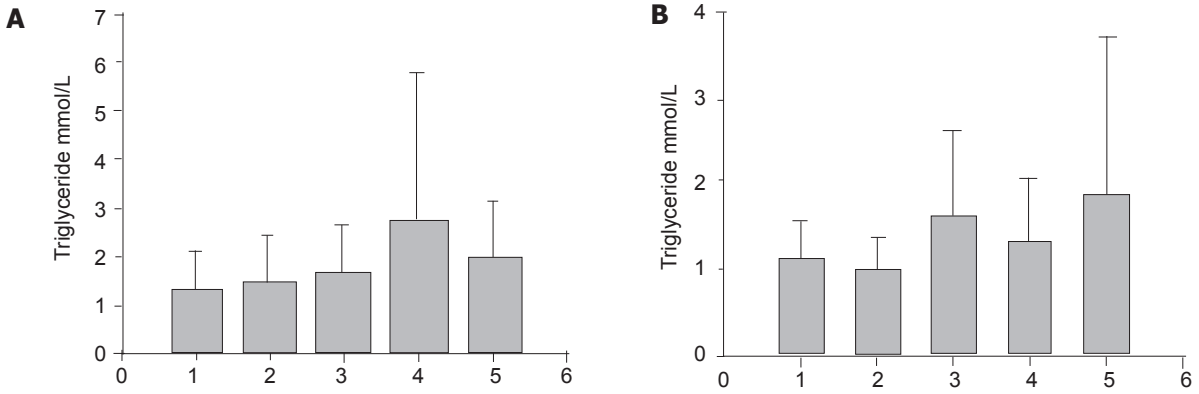


**Figure 5** Triglyceride levels in CH-C female patients. **A:** With steatosis compared to outpatients with alcoholic habits;
Bar 1: CH-C young female patients with steatosis ($n$ = 26, average age 38 years); Bar 2: CH-C elderly female patients with steatosis ($n$ = 20, average age 52 years);
Bar 3: Female patients with liver steatosis ($n$ = 12); Bar 4: outpatientswith alcoholic habits ($n$ = 8, age 38 years); Bar 5: outpatients with alcoholic habits ($n$ = 16, age 52 years). Bar 1 Ns *vs* Bar 2. Bar 1 and 2 NS *vs* Bar 3. Bar1 and 2 $P$<0.004 *vs* Bar 4. **B:** Without steatosis compared to outpatients without alcoholic habits.

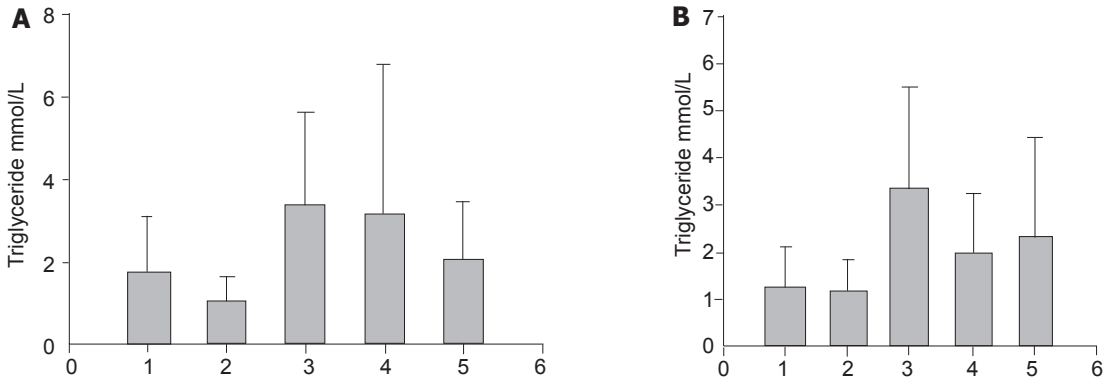


**Figure 6** Triglyceride levels in CH-C male patients. **A:** Steatosis compared to outpatients with alcoholic habits; Bar 1: CH-C young male patients with steatosis ($n$ = 29, average age 37 years); Bar 2: CH-C elderly male patients with steatosis ($n$ = 15, average age 52 years); Bar 3: Male patients with liver steatosis ($n$ = 16); Bar 4: Male outpatients with alcoholic habits ($n$ = 86, age 37 years); Bar 5: Male outpatients with alcoholic habits ($n$ = 190, age 52 years). Bar 1 $P$<0.0001 *vs* Bar 4. Bar 2 $P$<0.0001 *vs* Bar 4. Bar 4 $P$<0.0001 *vs* Bar 5. **B:** Without steatosis compared to outpatients without alcoholic habits. Bar 1: CH-C young male patients without steatosis ($n$ = 24, age 37 years); Bar2: CH-C elderly male patients without steatosis ($n$ = 11, average age 49 years); Bar 3: Male patients with liver steatosis ($n$ = 16); Bar 4: Male outpatients without alcoholic habits ($n$ = 60, age 37 years); Bar 5: Male outpatients without alcoholic habits ($n$ = 509, age 49 years). Bar 1 and 2 $P$<0.0005 *vs* Bar 3. Bar 3 $P$<0.0005 *vs* Bar 4.

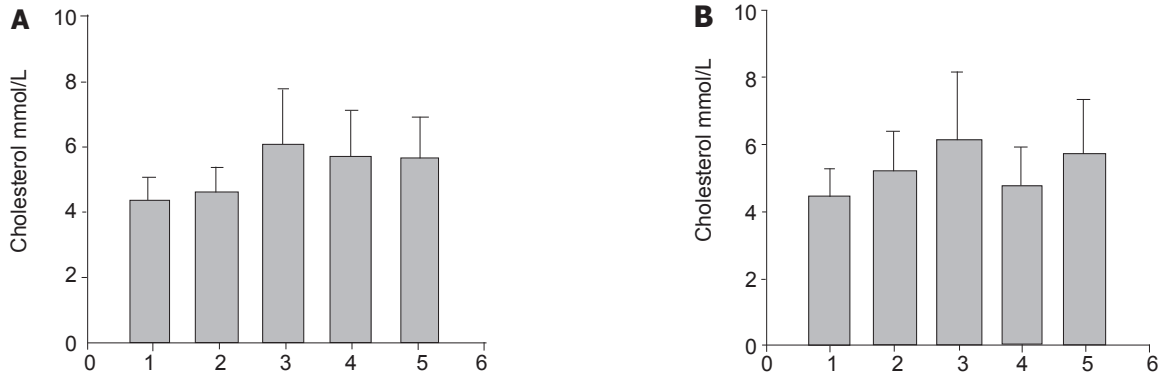


**Figure 7** Cholesterol levels in CH-C female patients. **A:** With steatosis compared to outpatients with alcoholic habits; Bar 1: CH-C young female patients with steatosis (*n* = 26, average age 38 years); Bar 2: CH-C elderly female patients with steatosis (*n* = 20,average age 52 years); Bar 3: female patients with liver steatosis (*n* = 12); Bar 4: female outpatients with alcoholic habits (*n* = 8, age 38 years); Bar 5: female outpatients with alcoholic habits (*n* = 16, age 52 years). Bar 1 NS *vs* Bar 2. Bar 1 and 2 *P*<0.0001 *vs* Bar 3. Bar 1 *P*<0.0001 *vs* Bar 4. Bar 1 and 2 *P*<0.0001 *vs* Bar 5. **B:** Without steatosis compared to outpatients without alcoholic habits. Bar 1: CH-C young female patients without steatosis (*n* = 10, average age 29 years); Bar 2: CH-C elderly female patients without steatosis (*n* = 7, average age 53 years); Bar 3: female patients with liver steatosis (*n* = 12); Bar 4: female outpatients without alcoholic habits (*n* = 125, age 29 years); Bar 5: female outpatients without alcoholic habits (*n* = 803, age 53 years). Bar 1 NS *vs* Bar 2. Bar 1 *P* < 0.0001 *vs* Bar 3. Bar 1 *P*<0.0001 *vs* Bar 5. Bar 3 *P* < 0.0001 *vs* Bar 4.

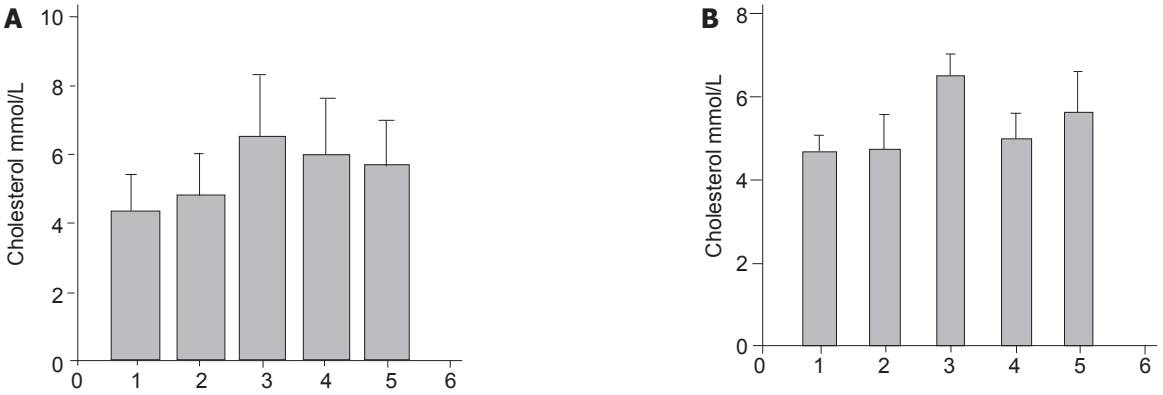


**Figure 8** Cholesterol levels in CH-C male patients. **A:** With steatosis compared to outpatients with alcoholic habits; Bar 1: CH-C young male patients with steatosis (*n* = 29, average age 37 years); Bar 2: CH-C elderly male patients with steatosis (*n* = 15, average age 51 years); Bar 3: male patients with liver steatosis (*n* = 16); Bar 4: male outpatients with alcoholic habits (*n* = 86, age 37 years); Bar 5: male outpatients with alcohollic habits (*n* = 190, age 51 years). Bar 1NS *vs* Bar 2. Bar 1 and 2 *P*<0.0001 *vs* Bar 3. Bar 1 and 2 *P*<0.0001 *vs* Bar 4. **B:** Without steatosis compared to outpatients without alcoholic habits. Bar 1: CH-C young male patients without steatosis (*n* = 24, average age 34 years); Bar 2: CH-C elderly male patients without steatosis (*n* = 11, average age 49 years); Bar 3: male patients with liver steatosis (*n* = 16); Bar 4: male outpatients without alcoholic habits (*n* = 60, age 34 years); Bar 5:Male outpatients without alcoholic habits (*n* = 509, age 49 years). Bar1NS *vs* Bar 2. Bar 1 and 2 *P*<0.001 *vs* Bar 3. Bar 1 and 2 *P*<0.001 *vs* Bar 5. Bar 3 P<0.001 *vs* Bar 4.

In certain studies, steatosis was found to accelerate the progression of liver disease and correlate with specific HCV genotype[36]. In our study steatosis was detected in 63% of CH-C patients. The ALT-levels were statistically in correlation with the grade of steatosis. Comparing the HAI and ALT levels, a significant correlation was found in the CH-C cases without steatosis, while no difference was found in the group with steatosis. Inserting the score values of steatosis (0-3) into the score of the grade of necroinflammation, a significant correlation was detected for HAI and ALT levels. This suggests that inclusion of the score of steatosis into the grade, for measuring the necroinflammatory reaction in CH-C, might better reflect the alteration in the liver tissue and correlate with ALT enzyme elevation. Our data strongly suggest the importance of steatosis in the virus related necroinflammatory process. Worsening of steatosis was found as an independent factor in fibrosis progression in CH-C[37].

Regarding the different patient subgroups, the triglyceride levels revealed a marked low degree in the C virus-infected patients in comparison to the levels seen in the non-viral steatotic patients and in the relatively large number of outpatients without clinical signs of liver diseases. This detected phenomenon was observable in relation to the age, sex, and drinking habits of the outpatients of the praxis, too.

The behavior of cholesterol levels was the same as in case of the triglyceride levels, while the young steatotic virus-infected male patients failed to show higher elevated levels.

In conclusion, a recent study has found an association

between liver cell apoptosis and steatosis in CH-C patients, which might contribute to the progression of liver injury and fibrosis in CH-C[5]. Our study confirms the importance of the presence of steatosis, which is associated with higher ALT- and lower lipid levels on the periphery in CH-C patients with steatosis. The peripheral lipid levels, however, do not reflect the severity of steatosis or liver damages. Our study suggests that the grade of steatosis in the liver is a substantial part of the determination of the grade of necroinflammation and should be incorporated into the evaluation of liver biopsy materials in CH-C patients.

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**Science Editor** Guo SY **Language Editor** Elsevier HK