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The Role of Plasma Lipid Levels as Markers of Recovery of Liver Function after Hepatectomy

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Authors' contributions

This work was carried out in collaboration between all authors. Authors SL and EDL designed the study, performed the statistical analysis with author GS wrote the protocol, and wrote the first draft of the manuscript. Authors PK and KB performed the 2nd part of the experiment and contributed to the writing of the draft. Author CK supervised all steps of the study and edited the final draft with author SL. All authors read and approved the final manuscript.

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ABSTRACT

Aims: Currently there is not a single conventional marker to reliably assess liver function recovery after hepatectomy. Our aim was to investigate the potential role of circulating lipid levels as markers of liver function recovery.
The study was conducted in the experimental laboratory of the Aretaieion Hospital.
Methodology: 48 male Wistar rats (240-350g) were assigned in 2 groups; the sham operated group (A=21) and the 70% hepatectomy group (B=27). There were 3 subgroups according to the day the animals were killed (1st, 3rd and 7th postoperative day [PO]). We measured the levels of AST, ALT, albumin, total protein, triglycerides, HDL and cholesterol in plasma and correlated them with the steps of liver regeneration
Results: Significant differences between the groups were observed in the levels of HDL (*P*=0.001) and cholesterol (*P*=0.001) on the 1st PO day, and in triglycerides (*P*=0.045) on the 7th PO day.
Conclusion: Cholesterol, and in a lesser degree HDL levels seem to reflect well liver function recovery after hepatectomy. In this experiment they were more sensitive than albumin in assessing the deterioration of liver function as well as the subsequent recovery during regeneration hence they may represent a more accurate marker of liver function. Rise in plasma triglyceride levels reflect the completion of the regenerative process.

Keywords: Hepatectomy; liver regeneration; plasma lipids; liver function.

ABBREVIATIONS

Postoperative (PO), Albumin (Alb), total protein (TP), high density lipoprotein (HDL), triglycerides (TG), alanine aminotransferase (ALT), aspartate aminotransferase (AST),cholesterol (Chol).

1. INTRODUCTION

After a major hepatectomy the liver can recover using complex regenerative mechanisms whilst maintaining its essential metabolic and detoxifying function [1]. The balance between cell proliferation and cell differentiation should ideally be maintained during the regenerative process, and the basic liver function should not be compromised until the original volume is restored [1-4]. Should the liver loose excessive volume, this may result in fatal liver failure. Henceforth, it is vital to assess liver function after liver surgery in order to predict and manage promptly an unexpected liver failure [5].

Traditionally, conventional liver function tests provide information on the integrity of hepatocytes and biliary epithelium, while more advanced techniques provide a dynamic assessment of hepatic function using quantitative tests, allowing an estimation of the functional reserve of the liver parenchyma [6].

Among others, the liver is recognized as the main organ responsible for plasma lipoprotein cholesterol homeostasis [7]. Moreover, during liver regeneration a transient liver steatosis has been observed which is characterized by a triglyceride accumulation in the liver [8,9]. Although the exact role of lipids in this process is still poorly understood, liver steatosis is a

vital step in the regenerative process since it has been shown that decreased hepatic fat accumulation is associated with impaired liver regeneration [10].

Isolated studies on patients who underwent liver transplantation have shown that serum cholesterol levels are good indicators of effective recovery of liver function after the operation [5]. Despite the fact that other liver products, like albumin, have been used for the assessment of liver function after hepatectomy, the role of lipids, especially cholesterol, has not been extensively studied.

The rodent partial hepatectomy model has been widely used to investigate the process of liver regeneration and the effects on various metabolic factors [4,11]. Studies have shown that cholesterol uptake from high density lipoprotein (HDL) is carried out to make up new cell membranes during hepatic regeneration [12]. Circulating cholesterol and HDL decrease in plasma during the first 12-24h following hepatectomy [13]. Moreover, during the regenerative process, an influx of fatty acids coming from adipose tissue to the hepatic remnant occurs [1,9]. The fatty acids synthesized in the liver remnant along with the ones that came from adipose tissue are incorporated into triglycerides resulting in enhancement of the triacylglycerol biosynthesis [8,9,11,13]. Since lipids seem to be actively involved in the process of liver regeneration there is a possibility that their plasma levels may correlate well with the progression and completion of the process. In this experimental model we investigated alterations of various lipids after hepatectomy in order to identify which lipids could represent recovery of liver function after hepatectomy.

2. MATERIALS AND METHODS

The study was approved by the ethical committee of Aretaieion Athens University Hospital and was performed in concordance with the European Union regulations (EU directive 86/609/EEC) for experimental animals. The animals received humane care in compliance with guidelines of National Institutes of Health (NIH) described in the “Guide for the Care and Use of Laboratory Animals”[14].

For the purpose of the study we used 48 male healthy Wistar rats (240-350g) from one colony (Institute Pasteur Hellenique). They were fed a standard chow diet (65% carbohydrate, 18% protein, 3% fat, 5% fiber, 5% minerals and vitamins) and maintained in a 12h light/12h dark cycle with free access to food and water until 12 hours before the operation. Body weight and the amount of food intake were determined for each animal at the beginning of the study and just before killing them. All operations were performed between 08:00 and 10:00h.

For the induction in anaesthesia 40mg/kg ketamine (Ketalar 10mg/ml) along with 1mg/kg of atropine (Atropine sulfate 1mg/ml) were injected intramuscularly. Moreover, in a different side 5mg/kg of midazolame (Dormicum 15mg/3ml) diluted to 0.4ml of Normal saline 0.9% were also injected in order to maintain long lasting anaesthesia of the animals undergoing liver resection.

In the sham-operated rats (Group A=21), laparotomy, exposure and extensive manipulation of the liver for the same amount of time were performed without further interventions.

In the hepatectomy group (Group B=27), the anterior lobes (left lateral and medial lobe) representing around 70% of liver parenchyma were resected as this has been described by Higgins and Anderson [15].

At the end of the procedure, 15mL/kg of warm 0.9% saline was inserted into the abdominal cavity to replenish fluid losses and prevent dehydration until complete recovery.

After wound closure, the animals were kept in special boxes with free access to food and water under standard conditions of controlled temperature, humidity, and light exposure.

Each third of the animals was re-operated with the same anaesthetic procedure at post operative (PO) day 1 (Subgroup PO1), PO day 3 (Subgroup PO3) and PO day 7 (Subgroup PO7) in order to collect blood and liver specimen.

Using the classical automatic methods for quantification of biochemical parameters in serum in a recently calibrated biochemical analyzer (Hitachi 902 Automatic analyzer, Roche Diagnostics, IN, USA) we measured the following parameters: Albumin (Alb):g/dl, total protein (TP):g/dl, HDL:mg/dL, triglycerides (TG):mg/dL, alanine aminotransferase (ALT):IU/L, aspartate aminotransferase (AST):IU/L, and cholesterol (Chol):mg/dL. In our study we used ALT and AST levels as indicators of liver injury.

The histological specimens of the liver were immediately placed in buffered formalin and subsequently embedded in paraffin. Liver sections were stained with hematoxylin and eosin (H&E) and the sections were seen by 2 pathologists using a semi-quantitative scoring system in order to assess and document: the presence of mitotic activity, the presence of ischemic changes, the number of mitoses, the presence and grade of liver steatosis and the morphology of the liver, in order to correlate the status of the liver with the outcomes.

2.1 Statistics

The statistical analysis was carried out using SPSS for Windows version 17 software package (Statistical Package for Social sciences; Inc, Chicago, IL).

For categorical variables we used Pearson Chi-square test and Fisher's exact test. For continuous variables we used independent sample t-test and non-parametric test (Mann-Whitney Wilcoxon-test) according to the type of distribution of the outcomes. For comparisons involving more than 2 groups we used analysis of variance (ANOVA) to identify statistically significant differences between the means of the outcome values of the groups. We further applied follow-up tests (post-hoc) to assess which groups were different from which other groups ("Tukey's" and "Bonferroni" post hoc tests) [16]. We considered significant differences those with *P* value less than 0.05. Binary logistic regression was used to assess if there were significant predictors of binary outcomes (e.g. presence or not of mitoses). For the demonstration of the curves over time we used combine box plots where the range, 95% confidence intervals and median are clearly demonstrated at each time point and direct comparisons between the 2 groups can be made.

3. RESULTS

All 21 animals from the Group A (100%) and 25 from the group B (92.6%) survived the procedure and completed the experiment in the predetermined postoperative day (i.e. 1st PO day, 3rd PO day, 7th PO day). In order to assess the heterogeneity between the experimental animals which were assigned to each subgroup, we compared the initial and final weights of the animals. Despite the range of animal weight, we found no statistically significant

difference in this parameter between the groups and at each time point (*P*=0.289) hence, the weight was not a confounding factor which could have interfere with our results (Table1).

We compared the outcome values of the subgroups according to the day they had completed the experiment, thus having 3 separate analyses (Table 1).

Table 1. Comparisons of the outcomes of the sham (group A) versus hepatectomy (Group B) for the 3 time points (1st, 3rd and 7th PO day)

	T-test	Mann-whitney	Mean difference	Mean
Subgroup PO1 (Group A=7, Group B=9)				
Mean animal body weight(g)	*P*=0.216	*P*=0.079	37	(A)=271 (B)=308
TP(g/dl)	*P*=0.002	*P*=0.007	0.968	(A)=6.51 (B)=5.54
HDL(mg/dL)	*P*=0.001	*P*=0.001	6.873	(A)=14.43 (B)=7.56
Chol(mg/dL)	*P*=0.001	*P*=0.002	26.143	(A)=62.14 (B)=40
LDL+VLDL (mg/dL)	*P*=0.003	*P*=0.002	17.492	(A)=47.71 (B)=30.22
AST(IU/L)	*P*=0.011	*P*= 0.007	351.603	(A)=345.29 (B)=696.89
ALT(IU/L)	*P*=0.006	*P*=0.004	32.525	(A)=14.59 (B)=47.11
Number of mitoses	*P*=0.014	*P*=0.0001	19.333	(A)=0/optical field (B)=19.33/optical field
Subgroup PO3 (Group A=7, Group B=7)				
Mean animal body weight(g)	*P*=0.083	*P*=0.142	26	(A)=270 (B)=296
AST(IU/L)	*P*=0.019	*P*=0.003	93.309	(A)=107.86 (B)=201.17
ALT(IU/L)	*P*=0.056	*P*=0.044	7.405	(A)=13.43 (B)=20.83
Number of mitoses	*P*<0.0001	*P*=0.001	10.333	(A)=0/optical field (B)=10.33/optical field
Subgroup PO7 (Group A=7, Group B=9)				
Mean animal body weight (g)	*P*=0.486	*P*=0.056	36	(A)=289 (B)=325
TG(mg/dL)	*P*=0.146	*P*=0.045	10.586	(A)=62.57 (B)=78.80
HDL(mg/dL)	*P*=0.012	*P*=0.024	1.223	(A)=13.29 (B)=9.80
AST(IU/L)	*P*=0.004	*P*=0.002	22.661	(A)=99.86 (B)=177.90

T-test and non-parametric test (Mann Whitney) were used. Only the weight and statistically significant outcomes are included. The means for each group and the mean difference are also displayed

3.1 Subgroup PO1 (1st PO Day)

In this analysis we included a total of 16 rats (Group A=7, Group B =9). Nine rats from group B (100%) and none from group A (0%) showed presence of mitoses in the liver specimen. Moreover, all 9 rats from group B had microscopic findings compatible with grade III, liver steatosis while none of the animals in group A had any signs of liver steatosis. These differences were statistically significant (X^2=16, *P*<0.0001). Two rats in group A (28.5%) and 2 rats in group B (22.2%) were found on histology to have findings compatible with liver ischemia in less than 5% of the specimen. Moreover, there were areas of focal ischemic necrosis in both rats of group B. The difference was not statistically significant (X^2=2.049, *P*=0.302).

There were similar plasma levels of TG (*P*=0.823) between the 2 groups, but significantly lower levels of HDL (*P*=0.001), VLDL+LDL (*P*=0.002) and cholesterol (*P*=0.001) were observed in group B (Table 1). On the other hand in Group B animals, AST levels were twice as high (*P*=0.011) and ALT levels more than 3 times higher (*P*=0.006) than that of group A.

3.2 Subgroup PO3 (3rd PO Day)

In this analysis we included a total of 14 rats (Group A=7, Group B=7) since 2 rats from group B did not survive the procedure.

All seven rats which survived from group B (100%) and none from group A (0%) showed presence of mitoses in the liver specimen. This difference was statistically significant (X^2=13, *P*=0.001). No signs of ischemia were observed in any specimen of the 2 groups. Moreover, all 7 rats from group B had microscopic findings compatible with liver steatosis; 3 specimens had grade II and 4 specimens grade I steatosis while once more, none of the animals in group A had a similar picture. The difference between the 2 groups was statistically significant (X^2=7.583, *P*<0.0067).

At this time point there were no significant differences in the plasma levels of TG (*P*=0.071), cholesterol (*P*=0.93), HDL (*P*=0.455) and LDL+VLDL (*P*=0.178). Despite the lower actual levels of AST, group B had persistent twice as high levels as group A (*P*=0.019) while for ALT levels the difference decreased (*P*=0.056). The difference in ALT levels was only found to be significant with non parametric tests (*P*=0.044).

3.3 Subgroup PO7 (7th PO Day)

In this analysis we included a total of 16 rats (Group A=7, Group B=9). No mitosis and no signs of ischemia were observed in any specimen of the 2 groups. Moreover, only normal liver microscopic morphology was observed, indicating that their liver recovered completely from the postoperative stress and ischemia, the transient liver steatosis resolved and regeneration had been completed by this point.

At this time point, there were no significant differences in the plasma levels of cholesterol (p=0.581), LDL+VLDL (*P*=0.406) and TG (*P*=0.146). On the contrary, HDL levels were lower in group B (*P*=0.012). AST levels remained higher in group B (*P*=0.004) while ALT levels were equal in the 2 groups (*P*=0.187).

The significant results of all these comparisons are included in (Table 1).

For each group (group A and group B) we further compared independently the outcomes of the 3 time points (1st, 3rd and 7th PO day) and obtain curves over time for each outcome thus having 2 more comparisons (Table 2). For each outcome we created a combined box plot (Fig. 1).

Table 2. Comparisons of the outcomes of the three time points (1st, 3rd and 7th PO day) for sham (Group A) and hepatectomy (Group B)

	ANOVA	Bonferroni	Tukey-HSD	Means
Comparison of the results of the different PO days for Group A (sham)				
TG (mg/dL)	*P*=0.019	3rd >1st , *P*=0.024	*P*= 0.021	1st PO day=54.57 3rd PO day=95 7th PO day=62.57
LDL+VLDL (mg/dL)	*P*=0.032	3rd >1st , *P*=0.03	*P*=0.035	1st PO day=47.71 3rd PO day=61.57 7th PO day=56.71
AST (IU/L)	*P*<0.0001	1st >3rd , *P*=0.001 1st >7th, *P*<0.0001	*P*=0.001 *P*<0.0001	1st PO day=345.29 3rd PO day=107.86 7th PO day=99.86
Comparison of the results of the different PO days for Group B (Hepatectomy)				
TG (mg/dL)	*P*=0.046	7th >1st, *P*=0.043	*P*=0.036	1st PO day=52 3rd PO day=64.5 7th PO day=78.8
HDL(mg/dL)	*P*=0.020	3rd>1st, *P*=0.019	*P*=0.017	1st PO day=7.56 3rd PO day=11.83 7th, PO day=9.8
LDL+VLDL (mg/dL)	*P*<0.001	3rd >1st, *P*<0.000	*P*<0.000	1st PO day=30.22 3rd PO day=62.17 7th, PO day=63.80
ALT(IU/L)	*P*=0.001	1st>3rd, *P*=0.012 1st>7th, *P*=0.002	*P*=0.010 *P*=0.002	1st PO day=47.11 3rd PO day=20.83 7th, PO day=18.9
AST(IU/L)	*P*=0.015	1st>3rd, *P*<0.0001 1st>7th,, *P*<0.0001	*P*<0.0001 *P*<0.0001	1st PO day=696.89 3rd PO day=201.17 7th, PO day=177.9
Chol(mg/dL)	*P*<0.0001	3rd >1st, *P*<0.0001 7th, >1st, *P*<0.0001	*P*<0.0001 *P*<0.0001	1st PO day=36 3rd PO day=74 7th, PO day=73

ANOVA was used to compare the outcomes between the different time points for each group. Post-hoc tests (Bonferroni and Tukey-HSD) were used for further analysis when significant differences were found in ANOVA. The means for each group are also displayed

For group A (sham operation), no significant differences between the time points were observed for the following outcomes: ALT, cholesterol, TP, and HDL while for group B (hepatectomy) there were significant differences between the time points for all the measured parameters. The significant results are included in (Table 2) and schematically presented in (Fig. 1).

Using binary logistic regression to find variables related to the presence or not of ischemia or mitosis failed to reveal any significant associations.

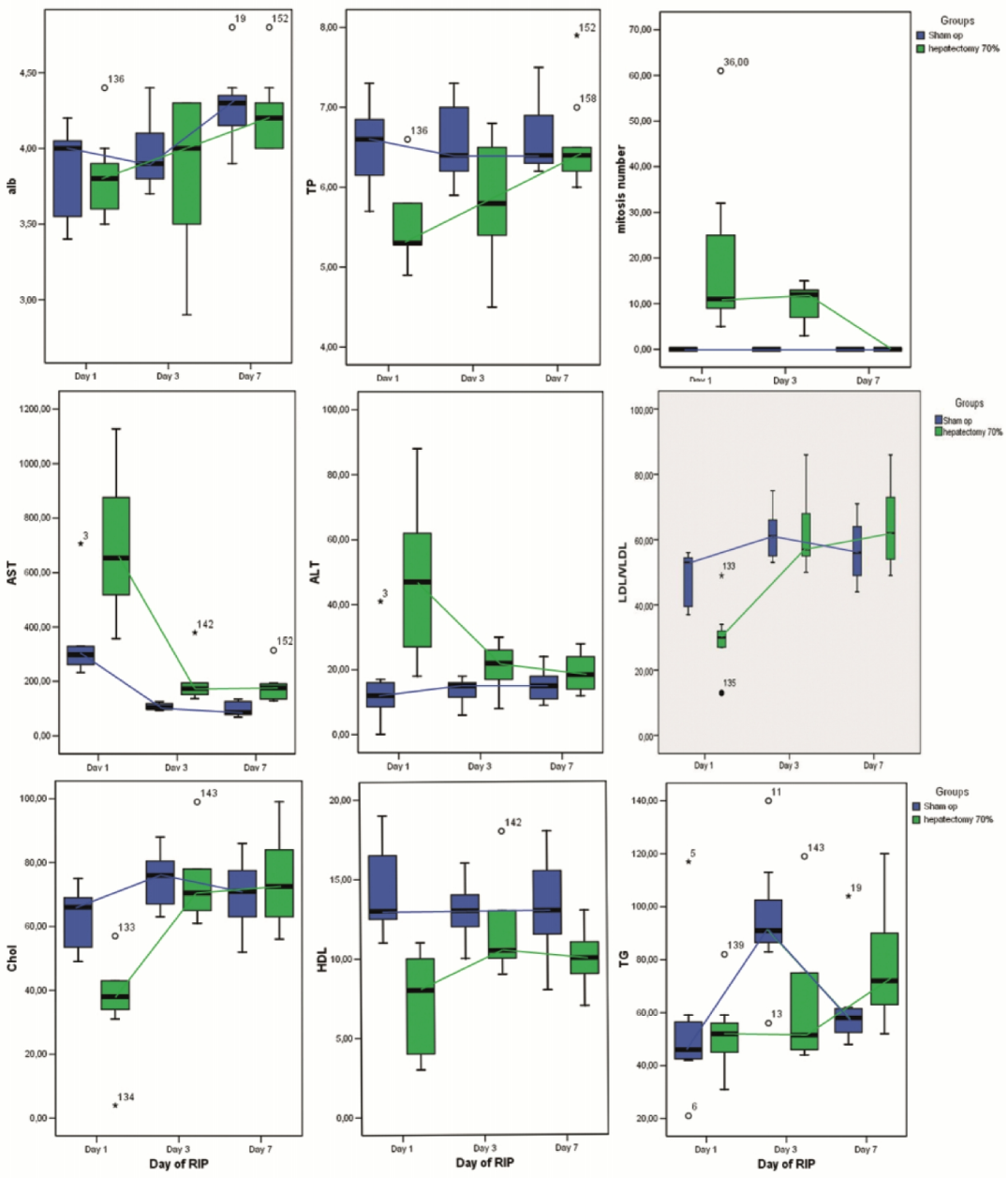

Fig. 1. Combined Box plots for the 2 groups for the main outcomes (AST, ALT, lipoproteins [excluded HDL], cholesterol, HDL and Triglycerides). The box for each time point and for each group along with the resulting curve is displayed
(Blue=sham, Green=hepatectomy)

4. DISCUSSION

Today, partial hepatic resection is considered a feasible and relatively safe procedure. The indications have expanded to include living donor hepatectomy for liver transplantation. Having expanded the indications to include healthy individuals there is always a concern about the risk of postoperative liver failure which ranges between 0.7% and 9.1% but in some cases may reach 32% of the operations. The major factors leading to liver failure is an inadequate quantity or quality of residual liver mass deteriorated by parenchymal congestion, ischemia, reperfusion injury and infection [17]. Although liver steatosis has also been considered a significant risk factor for postoperative complications after hepatectomy, studies have shown that fat accumulation in the liver is vital for the regeneration. There is even accumulating evidence that a mild preoperative steatosis may be beneficial for the patients since it may enhance the process [18,19].

After partial hepatectomy most of the residual differentiated quiescent hepatocytes in the remnant liver undergo a coordinated cellular activation and quickly proliferate leading to rapid restoration and replacement of liver mass [1,9,11,20,21].

In most vertebrates, including humans, regeneration through compensatory hyperplasia occurs in 6 to 8 days and does not require stem cells to replace the missing functional mass [1,9,11,20,21]. In humans, nearly two-thirds of liver regeneration is completed within only 2 weeks [5]. It has been shown that partial hepatectomy (PH) induces waves of hepatocyte replications beginning 24 hours postoperatively which are linked with waves of fat accumulation. The first wave is the most pronounced occurring 2.5 days after PH while hepatocyte proliferation is minimal after 4.5 days [9]. During the first wave (24-48h), when there is a rapid hepatocyte population expansion and a significant recovery of livers metabolic capacity, liver fat accumulation is a prominent event [9].

In our experiment there was no mitotic activity in group A. In group B the mitotic activity peaked on the first PO day, declined by the third PO day and was absent on the seventh PO day. There was a completely normal liver microscopic morphology on the seventh PO day and normalization of the liver enzymes (AST, ALT). These findings indicate that liver trauma was resolved and the process of regeneration was completed by this point. Our findings are consistent with the current literature data [9]. There was no difference in the percentage of ischemic changes between the groups indicating that any difference observed in the study between the groups could not be attributed to this factor.

Liver function includes the synthesis and degradation of glucose and glycogen, the synthesis of various proteins and the degradation of other serum proteins, the fatty acid metabolism, as well as the detoxification of toxins, and the degradation of bilirubin [22]. The hepatic synthetic ability during the regeneration process depends on the severity of liver disease and the volume of liver resection, both of which play an important role in maintaining the serum lipid level [5].

4.1 Evaluation of Liver Stress

Liver function evaluation is extremely important to predict liver failure and prepare for further intensive treatment.

Serum activity of transaminases, alkaline phosphatase and Gamma-Glutamyltransferase is non-specific for the evaluation of hepatic function. Their plasma levels are high in case of hepatocyte necrosis, increased hepatic activity or the presence of cholestasis [22,23].

Transaminase levels (ALT, AST) increase after hepatocyte collapse since they are released from the hepatocytes either due to parenchymal injury during transection, or due to partly ischemic liver remnant, indicating liver injury [5,23]. As it was expected, in this experiment, the levels of both the liver enzymes (AST and ALT) were higher in the hepatectomized group and the mean difference between the 2 groups was declining over time. AST was more sensitive than ALT in detecting operative stress, since it was able to identify the liver stress caused from the manipulations, even in group A animals.

In hepatectomized rats, the levels of AST and ALT, in a lesser degree, dropped dramatically by day 3 and low values (similar to the sham operated group) were maintained at day 7, indicating that by day 3 the liver recovered from the acute injury and stress (Fig.1).

4.2 Evaluation of Liver Function and Recovery

The integrity of hepatocytes and biliary epithelium as well as the synthetic and excretory liver function have been traditionally evaluated using conventional tests while today liver function can be quantitatively evaluated using a number of dynamic tests which assess hepatic clearance or conversion of xenobiotics [6]. Examples include the indocyanine green retention in 15min (ICGR15), the galactose elimination test, the lidocaine–monoethylglycinexylidide test (MEGX) and the 14C aminopyrine breath test [17,22,23].

Tests analyzing hepatic synthetic function (serum albumin and clotting factors) or excretory function (serum bilirubin), are able to detect hepatic dysfunction. These tests are now considered non-specific for the assessment of hepatic function. They are considered to be unable to reflect and predict the post-resectional outcome [17]. Moreover, in clinical practice these indicators could be modified by the transfusion of albumin and plasma and cannot reliably represent the liver function after surgery [5].

Nevertheless, since lipids are hardly affected by external sources during the acute postoperative period we tried to find a suitable single marker that could represent a recovery of liver function, or at least a marker that could correlate well with the progress of liver regeneration.

It is well known that post hepatectomy there is a rapid and marked hepatocellular fat accumulation which has not been observed after sham operations and is believed to be essential for the regeneration [4,10]. The observed accelerated adipogenesis and decreased liver binding capacity for lipoproteins [8,11,13] lead to steatosis which peaks by 12 hours and is maintained for 24 hours. After 48 hours the liver fat accumulation returns to normal [4]. Our results were in accordance with this, since a marked liver steatosis was universally observed in the hepatectomised but not sham operated animals on the 1st PO day. The steatosis was present but less pronounced on the 3rd PO day and completely resolved by day 7.

In liver transplant patients, it has been shown that serum cholesterol reflects well the effective recovery of liver function after the operation [24,25] while other studies found that LDL was also a good marker for liver recovery [5]. Moreover, studies in anhepatic animals have shown that despite the fact that free cholesterol is produced from peripheral tissues,

this hardly contributes to the levels of plasma cholesterol since most of the sterol was in the form of HDL. Meanwhile, all other lipids and apolipoproteins decrease following hepatic removal [7]. It has been shown that early in the process of regeneration the initial supply of lipids in the liver is provided by de novo lipogenesis (0-6 hours) followed by an uptake of lipolytic products from peripheral tissues leading to a drastically decrease in the levels of circulating lipids 24h after PH [10].

Studies have shown that circulating cholesterol and phospholipids associated with HDL and VLDL decrease in plasma during the first 12-24h following hepatectomy probably due to their influx into the hepatic remnant [11,13]. Despite the fact that other authors did not observe any fluctuations in the levels of the HDL during regeneration [5,13], we observed an initial decline at the 1st PO day and a recovery to normal values by day 3 following a curve similar to that of cholesterol (Fig.1). On the contrary HDL and cholesterol levels were not affected in sham operated animals.

We observed that the HDL levels were persistently higher in group A although only for the 1st PO day the difference was very significant. Despite a very severe and significant drop at the 1st PO day, cholesterol levels recovered fast to equalize in the two groups by day 3. When we analyzed the curve of the other lipoproteins excluding the HDL (VLDL and LDL) the results were similar, showing a significant acute drop in the lipoprotein levels on the 1st PO day, a fast recovery by the 3rd PO day, and high levels until the 7th PO day. Nevertheless, in this case, as opposed with the HDL and cholesterol curves, the fluctuation in the levels was also noticeable in the sham operated animals.

The pattern was different in the curve of triglycerides. It was observed that in group A there was a transient raise in TG plasma levels at 3rd PO day while in group B this peak was not observed. This is in accordance of other studies that demonstrated a marked accumulation of triglycerides in the liver in the early phase of regeneration hence the low plasma levels in group B [8]. In this group the levels of TG progressively rose to overcome those of group A on the 7th PO day. This may reflect a release in the circulation of the excess TG formed during the regeneration phase.

Hence, in this experiment we noticed that the acute liver injury, as it was demonstrated by transaminase curves, was almost resolved by day 3. During this time the plasma levels of cholesterol, HDL and other lipoproteins were inversely related to acute liver injury since they dropped significantly early after the PH and increased gradually. In the case of cholesterol the levels managed to return to normal. During the same period the plasma levels of albumin were similar in the 2 groups failing to detect any alteration in liver functional capacity as opposed to total protein levels which progressively recovered from a significant drop during the 7 days of the experiment. The low plasma TG levels on day 3, which increased on day 7, correlated well with the presence and resolution of the liver transient steatosis.

Considering these, after a liver resection or severe injury (when regeneration is expected to take place) we should expect that cholesterol, and HDL in a lesser degree, will initially drop significantly and return back to normal when the liver function and most of the producing capacity has recovered. TG levels are expected to drop initially, and then maintained in low levels during the persistence of fat accumulation. TG will increase in plasma, in higher levels than normal, as soon as the transient steatosis has completely resolved indicating the completion of the regenerating process and the release of the excess TG in the circulation. Since no similar studies have been shown this up to now, further research is necessary to confirm our observations.

If our findings are confirmed in humans then it should be a very useful and cost effective tool for liver surgeons. Nevertheless, further research should be conducted in animals with not normal livers (e.g steatosis) in order to assess if the findings of this experiment could be extended to such populations.

In conclusion, circulating lipid levels are affected during liver regeneration. HDL and cholesterol levels are reversely proportional to the liver stress and seem to recover fast after hepatectomy along with the recovery from acute injury. Triglyceride plasma levels recover later indicating that the regenerative process has been completed. Further research on humans may prove that plasma lipids can reflect well the regenerative process and be able to detect failure of the normal process.

4.3 Financial Disclosure

There was no financial or other form of support that has affected the design, writing and decision to publish this work. The authors declare they have not any commercial or proprietary interest in any drug, device, or equipment mentioned in the submitted article. None of the authors have any financial interest in any item mentioned in the article.

5. CONCLUSION

Circulating lipid levels are affected during liver regeneration. HDL and cholesterol levels are reversely proportional to the liver stress and seem to recover fast after hepatectomy along with the recovery from acute injury. Triglyceride plasma levels recover later indicating that the regenerative process has been completed. Further research on humans may prove that plasma lipids can reflect well the regenerative process and be able to detect failure of the normal process. This may be used as a non-expensive screening tool in these patients.

CONSENT

Not applicable.

ETHICAL APPROVAL

The study was approved by the ethical committee of Aretaieion Athens University Hospital and was performed in concordance with the European Union regulations (EU directive 86/609/EEC) for experimental animals. The animals received humane care in compliance with guidelines of National Institutes of Health (NIH) described in the “Guide for the Care and Use of Laboratory Animals”.

All authors hereby declare that "Principles of laboratory animal care" (NIH publication No. 85-23, revised 1985) were followed, as well as specific national laws where applicable. All experiments have been examined and approved by the appropriate ethics committee.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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