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# The role of the liver in the metabolism of adiponectin and proinsulin

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## Abstract

**Background**: Adiponectin and proinsulin are commonly used biomarkers in patients with metabolic syndrome. In individuals without cirrhosis adiponectin serum levels correlate inversely with insulin resistance and mortality. Proinsulin is a surrogate parameter of β-cell function, its level increases due to β-cell insufficiency while insulin resistance occurs. Metabolic syndrome is often associated with steatohepatitis, and thus liver cirrhosis. An increasing interest in adiponectin and proinsulin as a marker for metabolic syndrome associated morbidity and mortality might be observed in the literature. Still, a diagnostic value of both markers in cirrhotic state is not fully understood.

**Methods**: Eighteen recipients and donors undergoing living donor liver transplantation were included. Routine parameters, insulin resistance, adiponectin, and proinsulin serum levels were measured at evaluation, 10$^{th}$, 180$^{th}$, and 365$^{th}$ postoperative day (POD).

**Results**: Adiponectin levels before operation were lower in the donor than in the recipient group. Adiponectin levels in donors increased at the 10$^{th}$ and did not change until the 365$^{th}$ POD. In the recipient group levels improved already at 10$^{th}$ and increased slightly till 365$^{th}$ POD. Proinsulin levels in the recipient group were higher before transplantation and improved already at the 10$^{th}$ POD. It decreased continuously till 180$^{th}$ and rose slightly till 365$^{th}$ POD. Proinsulin levels in the donors remained constant regardless of the postoperative acute insulin resistance.

**Conclusions**: The grade of liver cirrhosis is positively correlated with the adiponectin serum level. In patients with liver cirrhosis, in contrast to liver healthy population, insulin resistance does not cause an adiponectin serum level down regulation. Therefore, the use of adiponectin as a marker of cardiovascular risk associated mortality in patients with cirrhosis is limited. Contrariwise, proinsulin might be used as a marker for hepatic insulin resistance in patients with liver cirrhosis. β-cell insufficiency seems not to play a major role in case of postoperative insulin resistance.

**Keywords**: Adiponectin, proinsulin, insulin resistance, liver function, liver cirrhosis

## Introduction

Adiponectin (APO) and proinsulin (P-INS) are commonly used biomarkers in patients with metabolic diseases such as diabetes mellitus (DM) and metabolic syndrome. Both markers have intensively been investigated for their role in metabolic disorders. The liver plays an important part during the pathogenesis of different metabolic diseases like i.e., nonalcoholic steatohepatitis and DM type 2. Till today the role of APO and P-INS in development of liver diseases, and even more importantly, the influence of liver dysfunction and cirrhosis on APO and P-INS blood levels are not well understood.

APO is a 30-kDa plasma protein, which is detectable in high levels in healthy subjects (serum level approximately 2-10 µg/ml) [1,2]. In contrast to other adipokines, serum levels of APO decrease in obese patients. It correlates negatively with percent body fat, fasting plasma insulin, and oral glucose tolerance. In patients with coronary artery disease levels of APO are lower than in a matched control group. This suggests a correlation between low APO levels and vasculopathy [3,4]. APO exerts a strong anti-inflammatory and atheroprotective as well as insulin sensitizing effect in tissues involved in glucose and lipid metabolism. A low APO level is characteristic for metabolic syndrome, thus it has a strong diagnostic potential to assess the cardiovascular risk associated with obesity and type II DM [5]. Because of this APO also tend to develop as a diagnostic tool and also therapeutic agent in a population suffering of metabolic syndrome. Liver has been proposed as the main site of APO clearance [6,7]. Moreover APO has been proposed as a marker of hepatic injury i.e., in steatohepatitis patients [8,9]. Hence, the influence of liver cirrhosis on APO metabolism and its

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diagnostic potential might lead to development of confusing results, since patients suffering from metabolic syndrome and steatohepatitis might consecutively develop liver cirrhosis [10]. An increased APO serum levels in patients with liver cirrhosis was described by some authors till now [11,12].

The insulin resistance (IR) is a well-described independent risk factor in the development of cardiovascular disease in patients suffering of metabolic syndrome [13,14]. Since the impact of cardiovascular disease is high, its early and precise diagnosis is of certain importance. Furthermore, postoperative hyperglycemia, which develops partially due to the IR is associated with poor outcome [15]. However, the use of direct IR measurement i.e., using intravenous glucose tolerance test is expensive and time consuming [16-18], thus surrogate IR parameters like HOMA (Homeostasis Model Assessment) or P-INS have recently been intensively studied [19]. Like APO, P-INS has widely been used as a diagnostic marker in a metabolic syndrome population. Thus, it is of high importance to understand the role of liver cirrhosis in the P-INS metabolism to avoid its eventually confusing influence.

Although liver plays an important role in glucose and lipid metabolism [20,21], little is known about P-INS and APO homeostasis in patients with liver cirrhosis or undergoing liver surgery. The aim of this study was to evaluate the influence of liver function on levels of APO and P-INS in donors and recipients undergoing living donor liver transplantation. In this setting completely healthy donors who develop intermittent liver insufficiency after partial hepatectomy were compared to recipients with insufficient evolving to normal liver function after transplantation.

## Methods

### Study design

Data were collected from a prospective observational study, performed in the Department for General, Visceral, and Transplantation Surgery (Charité, Berlin) between January 2001 and Mai 2004. The study protocols had received prior approval by the faculty's ethical review board. The study was designed and performed in accordance with the guideline of the Declaration of Helsinki 1975. Since this is a non-interventional study, no registration in the public registry was performed.

Donors and recipients intended for living donor liver transplantation (LDLT) with an age between 18 and 65 years were included. For each patient written informed consent was obtained. Exclusion criteria were overt diabetes mellitus, previous transplantation, high-grade encephalopathy, catecholamine therapy or a need of hemodialysis.

LDLT of the right liver lobe was performed in a standardized manner as described by Settmacher et al., [22]. Immunosuppressive induction therapy was performed by IL-2R antibody (basiliximab) and continued with tacrolimus (target trough levels of 10-15 ng/ml for the first month and 5-10 ng/ml thereafter). Prednisolon was started directly after transplantation with 1 mg/kg and then tapered and withdrawn after 90 days.

### Study protocol

Data was collected during four study visits at different time points: shortly before LDLT, as well as at 10$^{th}$, 180$^{th}$ and 365$^{th}$ postoperative day (POD). Standard clinical parameters as well as the fasting levels of adiponectin (APO, radioimmunoassay, Linco, St Charles, MO, USA), intact proinsulin (P-INS, enzyme-linked immunosorbent assay, LincoResearch, St Charles, MO, USA), insulin (Sandwich-ELISA LKIN5 Immulite Insulin-Assay, DPC Biermann, Bad Nauenheim, Germany) and glucose (hexokinase method, Roche Diagnostics, Basel, Switzerland), as well as intravenous glucose tolerance tests were determined at every visit. P-INS and APO serum levels were measured in cooperation with the Institute for Clinical Science and Development, IKFE GmbH. Insulin resistance (HOMA-IR) and insulin sensitivity (QUICKI-IS) scores were calculated from the fasting glucose and insulin levels as described previously [23]: (HOMA-IR=$G_0 * I_0$/22,5; QUICKI-IS= 1/[log($I_0$)]+[log($G_0$)] ($G_0$ fasting glucose level (mmol/l), $I_0$ fasting insulin level (µIU/ml).

### Intravenous glucose tolerance test

After overnight fasting, a polyethylene catheter was inserted into an antecubital vein for blood sampling. A second catheter was placed in the contralateral-arm vein for intravenous glucose and insulin injection. Three baseline samples for glucose, insulin, and C-peptide were obtained before starting frequently sampled intravenous glucose tolerance tests (FSIGTT). In addition, blood samples for routine investigations including tacrolimus trough levels were drawn. Glucose (300 mg/kg body weight, 50% solution) was administered intravenously within 2 minutes and blood samples were drawn over a 180 minutes period as described previously [24]. At 20 minutes, 0.035 U/kg insulin was injected [25]. Plasma glucose was measured by the glucose oxidase method (Modular System, Roche Diagnostics, Basel, Switzerland). Blood samples for plasma insulin and C-peptide were immediately centrifuged at 4°C and stored at -20°C until analysis. Insulin was measured by a solid-phase two-site chemiluminescent assay (Immulite, DPC Biermann, Bad Nauheim, Germany) and C-peptide by a solid-phase competitive chemiluminescent enzyme immunoassay (Immulite, DPC Biermann). Data analysis of the FSIGTT was performed by a blinded investigator and was based on the minimal model of glucose disappearance [26]. Calculations of insulin sensitivity and β-cell secretion were performed using the SAAM 1.2 software package (SAAM Institute, Seattle, WA) as described in detail before [24,27]. Insulin sensitivity (SI) measures the increase in fractional glucose clearance rate per unit change in plasma insulin.

### Statistical analysis

Median values with quartiles were used for all parameters. Non-parametric statistical tests were performed according to the group size. To evaluate statistical differences among the

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different time-points multivariate nonparametric repeated-measures analysis of variance (MANOVA) was used. First, with respect to differences in the groups (donors vs. recipients), then the time points were compared simultaneously on the corresponding curves. Univariate post-hoc analysis was performed by Mann-Whitney U-test for independent groups and Wilcoxon tests for paired observations to evaluate statistical differences between two different time-points. Spearman's correlation and simple linear regression analysis were used to analyze the correlation between APO, P-INS and liver function parameters as well as HOMA score. After normalization of the quantities, interactions between interesting parameters during the time course were tested by using a two-factorial nonparametric MANOVA test (1st factor: parameters, 2nd factor: time). Statistical significance was accepted at $p<0.05$. Calculations were performed with SPSS™ (version 19) and SAS™ (version 8.1) software, respectively.

## Results

Eighteen living donor liver transplantation patients (donors and recipients) were included in the study. The patient's characteristic are shown in (**Table 1**). One donor developed prolonged postoperative liver insufficiency with cholestasis, and therefore was excluded from postoperative analysis. Indications to the LDLT are shown in (**Table 1**). No patient had significant ascites at evaluation. The follow-up at 180$^{th}$ and 365$^{th}$ POD was missed for one donor and one recipient. In the postoperative course four recipients died due to intracranial bleeding, hemorrhagic shock by the bleeding from the arterial anastomosis, therapy resistant HCV re-infection and Klatskin tumor recurrence at the 19$^{th}$, 39$^{th}$, 93$^{th}$ and 341$^{th}$ POD respectively. Other complications occurred as follows: biliary tract leakage (6), bleeding (4), cholangitis (2), ulcus ventriculi perforation (1), Cushing Syndrome (1), scar hernia (2) and HCV re-infection of the graft in all HCV positive patients. The acute cellular rejection was diagnosed using fine needle biopsy and treated with 500 mg prednisolone/day over 3 days. In the recipient group one biliary leakage and one scar hernia occurred during the first postoperative year. In addition to our standard protocol, two patients received mycophenolate mofetil and three patients received sirolimus 10 days after transplantation. Six months after transplantation, five patients received mycophenolate mofetil and three received sirolimus. One year after transplantation five patients received mycophenolate mofetil (one with sirolimus) and four received sirolimus (one with mycophenolate mofetil and one sirolimus monotherapy).

### Immunsuppression

The tacrolimus daily dose was (in mg/kg/day: 0.15 [0.11-0.22] at 10$^{th}$ POD, 0.06 [0.03-0.09] at 180$^{th}$ POD and 0.04 [0.02-0.07] at POD 365$^{th}$. The serum trough level was (in ng/ml): 9,3 [7.3-13.4] at 10$^{th}$ POD, 7.1 [5.3-9.1] at 180$^{th}$ and 6.3 [4.1-7.7] at 365$^{th}$ POD. The patients were treated with followed prednisolone doses (in mg/day): 15 [15-20] (17 patients) at 10$^{th}$ POD, 8.8 [6.9-13.8] (4 patients) at 180$^{th}$ POD and 7.5 [6.3-8.8] (2 patients) at 365$^{th}$ POD.

**Table 1. Patient characteristics.**

| | donors (n=18) | recipients (n=18) | p |
|---|---|---|---|
| age [years], median (IQR) | 39 (31-54) | 52 (42/55) | 0.171 |
| male gender (%) | 8 (44) | 7 (39) | 0.593 |
| BMI, median (IQR) | 24 (22-26) | 23 (20-25) | 0.584 |
| family history of DM (%) | 8 (44) | 8 (44) | 1.0 |
| **primary diagnosis** | | | |
| cirrhosis (%) | -- | 12 (67) | -- |
| hepatitis B (%) | -- | 0 (0) | -- |
| +HCC (%) | -- | 2 (11) | -- |
| hepatitis C (%) | -- | 1 (6) | -- |
| +HCC (%) | -- | 2 (11) | -- |
| alcoholic (%) | -- | 6 (33) | -- |
| autoimmune (%) | -- | 1 (6) | -- |
| primary biliary cirrhosis (%) | -- | 1 (6) | -- |
| primary sclerosing cholangitis (%) | -- | 1 (6) | -- |
| +Carcinoma (%) | -- | 2 (11) | -- |
| Budd-Chiari syndrome (%) | -- | 1 (6) | -- |
| malignancy (%) | -- | 1 (6) | -- |
| Child-Pugh [Points], median (IQR) | -- | 8 (5.5-11.8) | -- |
| **histology (explanted liver)** | | | |
| chronic hepatitis staging, median (IQR)** | -- | 2,5 (1-4) | -- |
| chronic hepatitis grading, median (IQR)** | -- | 2 (1-3) | -- |
| re-transplantation (%) | -- | 1 (6) | -- |
| dead until 1 Month (%) | 0 (0) | 4 (22) | -- |

Donor and recipient characteristics prior living donor liver transplantation. Variables do not significantly differ between groups (Chi-Square test for categorical variables, t-test for continuous variables). Chronic hepatitis score was assessed according to Batts and Ludwig [**25**].

### Insulin sensitivity

A significant decrease of the SI was found in recipients versus donors before operation ($p<.001$, **Figure 1**). This difference decreased during the first postoperative year. In the donor group a significant drop in SI values at 10$^{th}$ POD was observed ($p<.001$). It had improved at 180$^{th}$ POD. Similar findings were observed for the HOMA score (**Table 2**).

### Adiponectin

APO levels at the point of evaluation were low in the donor and high in the recipient group ($p<0.001$, **Figure 2**). At other time points, APO values were not significantly different between the groups. APO levels did not differ between major

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**Table 2. Liver function and glucose homeostasis during LDLT.**

| | before transplantation | 10th POD | 180th POD | 365th POD | p value |
|---|---|---|---|---|---|
| **n=donor/recipient** | 18/18 | 17/17 | 16/14 | 16/13 | -- |
| **HOMA Score** | | | | | |
| *Donors* | 1.43 (1.04-2.12) | 2.06 (1.50-2.61)* | 1.67 (1.35-2.01) | 1.46 (1.10-2.72) | 0.026 |
| *Recipients* | 3.09 (1.79-6.70)†‡ | 2.91 (1.76-3.46)† | 2.36 (1.46-2.84)* | 1.69 (1.47-3.17) | -- |
| **SI** | | | | | |
| *Donors* | 4.52(3.60-5.48) | 2.00 (1.20-3.84)† | 5.40 (2.99-7.15) | 4.20 (3.13-8.29) | 0.922 |
| *Recipients* | 1.52 (0.94-2.96)‡ | 2.00 (1.21-3.88)† | 3.90 (2.93-5.97) | 5.87 (3.23-8.77) | -- |
| **Bilirubin (total) [µmol/l]** | | | | | |
| *Donors* | 0.60 (0.40-0.73) | 1.00 (0.60-1.40) | 0.60 (0.40-0.80) | 0.60 (0.43-0.70) | 0.041 |
| *Recipients* | 2.60 (1.90-8.03)‡ | 1.90 (1.40-8.70)† | 0.70 (0.40-1.23) | 0.60 (0.50-0.70) | -- |
| **ALT [U/l]** | | | | | |
| *Donors* | 20 (14-22) | 96 (60-181)† | 23 (18-25) | 22 (19-33) | 0.148 |
| *Recipients* | 58 (26-180)‡ | 102 (84-261)† | 34 (20-42) | 29 (20-50) | -- |
| **AST [U/l]** | | | | | |
| *Donors* | 20 (18-24) | 61 (44-93)† | 27 (22-33) | 26 (25-31) | 0.171 |
| *Recipients* | 91 (46-162)‡ | 53 (33-106)† | 35 (27-42) | 32 (25-37) | -- |
| **pCHE [kU/l]** | | | | | |
| *Donors* | 9.27 (8.67-10.51) | 5.31 (4.84-5.91)* | 8.05 (7.14-9.38) | 8.97 (8.27-10.31) | 0.036 |
| *Recipients* | 3.52 (2.77-4.94)‡ | 3.60 (2.81-4.84)† | 7.81 (5.79-9.59) | 7.66 (6.27-9.79) | |
| **INR** | | | | | |
| *Donors* | 1.00 (0.97-1.03) | 1.03 (0.96-1.07) | 0.99 (0.96-1.03) | 0.99 (0.94-1.04) | 0.591 |
| *Recipients* | 1.18 (1.06-1.69) | 1.10 (0.97-1.13) | 1.00 (0.94-1.05) | 0.96 (0.93-1.07) | |

Liver function and glucose homeostasis parameters in the perioperative course of living donor liver transplantation in donors and recipients are shown. HOMA score: insulin resistance score calculated of fasting insulin and glucose values, SI: insulin sensitivity score (FSIGTT); ALT: alanine aminotransferase; AST: aspartate aminotransferase; pCHE: pseudo-cholinesterase; INR: international normalized ratio. Median values (IQR), p value: significance level between donors and recipients was analyzed by multivariate, non-parametric testing for repeated measurements (10th till 365th POD). Comparison between the time points were performed using Mann-Whitney U-test: *p<0.05 vs. donors before operation; †p<0.01 vs. donors before operation; ‡p<0.05 for recipients vs. donors at the appropriate time.

diagnostic groups shown in (**Table 2**) (data not shown). The level correlated significantly with Child-Pugh score of the recipients before transplantation (p<.05), however not with the histologic cirrhosis grade or stage. In the donors APO levels increased at 10th POD (p=.002) and remained at that level until 365th POD. In the recipient group levels decreased already at the 10-th day and increased slightly till 365th POD (p=0.009) (**Figure 2**). APO levels correlated significantly with liver function parameters: cholinesterase (r=.358, p<.001), total bilirubin (r= .295, p<.003), INR (r=.477, p<.001), albumin serum level (at evaluation, 180-th and 365th POD, r=.633 and p<.001), and with the HOMA insulin resistance score (r=.325, p<.001). The APO levels correlated with SI only in the recipient group (r=.261, p=.045). Remarkably, APO serum level correlated positively with the body mass index (BMI) in the recipients before transplantation (p=.001, **Figure 3**), while no correlation with BMI of healthy donors was found.

## Proinsulin

P-INS levels before transplantation were higher in the recipient than in the donor group (p<0.01). P-INS levels did not differ between major diagnostic groups (data not shown). In the recipient group P-INS levels improved already at 10th POD, drop continuously till the 180th POD, than rose slightly till the 365th POD (p<.005). P-INS level in the donor group remained constant regardless the postoperative acute insulin resistance (**Figure 4**).

P-INS level correlated significantly with liver function parameters: cholinesterase (r=.278, p<.003), total bilirubin (r= .363, p<.001), INR (r=.519, p.001), with the HOMA insulin resistance score (r=.594, p<.001), and the insulin sensitivity score (SI, r=.270, p=.003).

## Discussion

In the present study we propose a novel interpretation for

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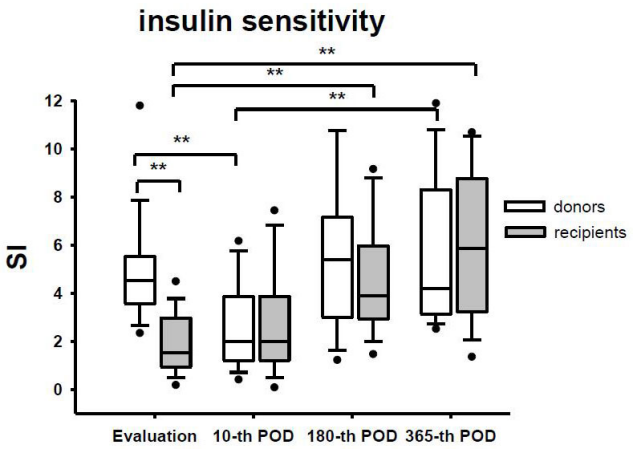


**Figure 1. Insulin sensitivity (SI).**
Insulin sensitivity (SI) measures the increase in fractional glucose clearance rate per unit change in plasma insulin. Donor and recipients SI score differs significantly (**, p<.001). After successful liver transplantation SI normalizes completely within 1 year. Donor group present an acute postoperative IR at 10th POD (**, p<.001).

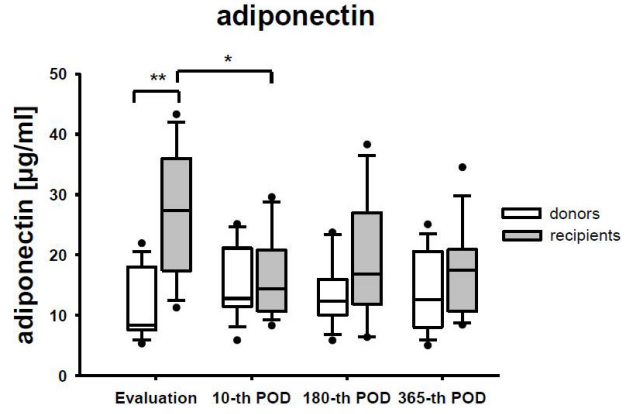


**Figure 2. Serum levels of adiponectin.**
Serum levels of adiponectin (APO) in the course of living donor liver transplantation by donors and recipients is presented. The significant difference between donor and recipient group (**, p<.001) at the point of evaluation indicate the influence of cirrhosis on the APO level. An improvement of APO level in transplanted patients confirms this observation (*, p=.007). (box-plot graph, no other significant differences were observed).

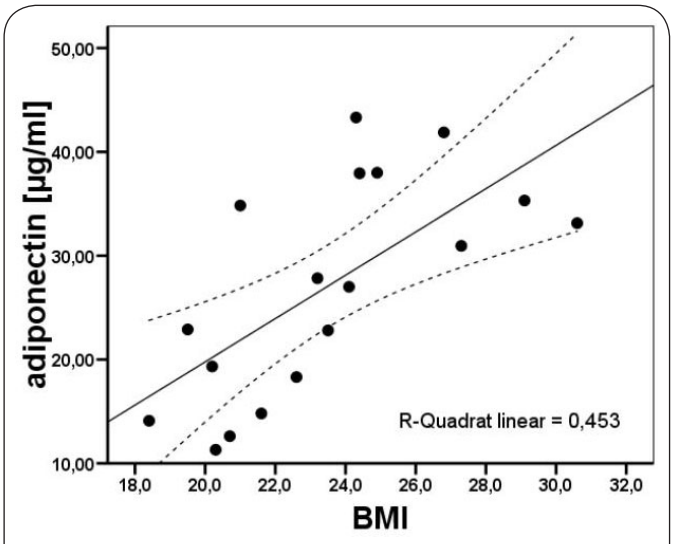


**Figure 3. Correlation between adiponectin serum level (APO) and body mass index (BMI) in cirrhotic patients.**
In cirrhotic patients (recipient group before transplantation) significant correlation between APO and BMI was found (p=.001, Pearson´s correlation). In healthy subjects an inverse correlation was described before (29, 30). However no significant correlation between BMI and APO serum level was found in the donor group.

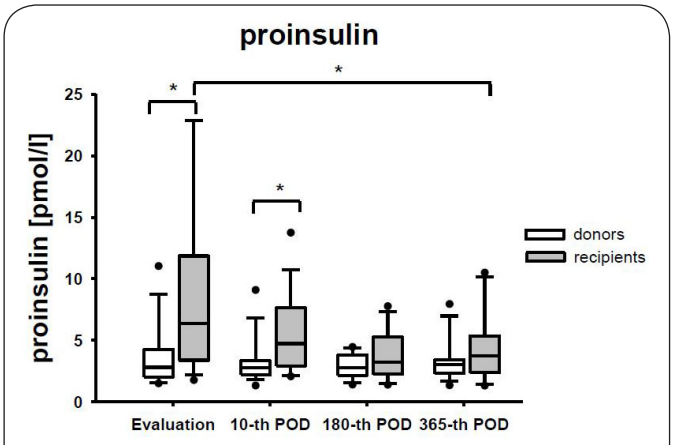


**Figure 4. Serum levels of proinsulin.**
Serum levels of proinsulin (P-INS) in course of living donor liver transplantation by donors and recipients is shown. The significant difference between donor and recipient group at the time point of evaluation and at 10th POD (*, p<.01) indicates the influence of liver function on P-INS level. P-INS level of transplanted patients improves after successful liver transplantation. No P-INS increase has been detected in the donors at 10th POD despite postoperative acute insulin resistance (**Figure 1**).

the paradoxical elevation of adiponectin (APO) and proinsulin (P-INS) levels observed in liver cirrhosis. The study setting was chosen, as it presents well the course of insulin resistance (IR) of patients with liver cirrhosis and after partial hepatectomy Stockmann et al., [**21**] already described the population presented in this study, and showed that liver function plays a major role in the development of IR. APO is known to act as an insulin-sensitizing agent und thus its low serum level is associated with obesity and metabolic syndrome [**2**]. However in case of liver dysfunction high levels of APO occur. In our population, patients with liver cirrhosis develope significant insulin resistance which correlates with high APO serum levels (**Figure 2**). Such phenomenon has already been described in experimental and clinical settings (**Table 3**). Kaser et al.,

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**Table 3. Adiponectin homeostasis in liver cirrhosis: literature overview.**

| reference | study group/ experimental setup | results/conclusions |
|---|---|---|
| Tietge et al., [7] | 20 patients with advanced cirrhosis | APO plasma levels and its splanchnic extraction correlates significantly with Child-Pugh score |
| Buechler et al., [11] | 76 alcoholic patients | the APO serum level increase in alcoholics, correlates with the amount of consumed alcohol and elevated aminotransferases; the alcohol abstinence cause reduction of the APO serum level |
| Neumeier et al., [38] | bile duct ligation in mice (cirrhosis model) | diminished expression of APO receptors in the cirrhotic mice |

analyzed 81 patients with different stages of liver cirrhosis and found an increased serum level of APO correlating with Child-Pugh stadium. In addition they described 7 patients with alcoholic steatohepatitis who reduced their APO serum level under anti-inflammatory treatment with anti-tumor necrosis factor alpha (TNF-α) antibody (infliximab) [12]. Interestingly, the authors found no correlation between APO serum levels and HOMA score, or liver function parameters in contrast to the present study. This result is however not fully explainable since the "clinical" (Child-Pugh) and laboratory liver function parameters should not vary as much. In our study correlation between APO level and all parameters of liver function as well as HOMA score and SI score (in the recipients group) were found. Beside this Asano et al., found increased TNF-α levels in APO-knockout mice compared to the wild type [28]. The APO-knockout mice developed hepatitis after 48 weeks, while the wild type did not. This suggests APO protective role against TNF-α induced inflammation, which is overwhelmed at some point by patients with alcoholic steatohepatitis. Tietge et al., analyzed APO kinetics in twenty patients with different stages of liver cirrhosis [7]. He found decreased APO clearance in patients with cirrhosis, and proposed that only the liver is a source of APO extraction. These results are confirmed by our data. Moreover, the normalization of APO levels after successful transplantation in parallel to improving liver function support the role of the liver in APO metabolism. Similar results were found by Buechler et al., in alcoholic patients [11]. The authors found increased APO serum levels due to alcohol consumption; moreover the correlation with the alcohol amount, serum level of transaminases and improvement by abstinence periods. Interestingly in the patients with cirrhosis APO serum levels correlated with the body mass index (BMI). This indicates that higher production of APO in the fat tissue is not counter regulated by the insufficient liver. In non-cirrhotic patients an inverse correlation of APO serum levels and BMI was observed previously [29,30]. We could not confirm this finding in our donor population. It might be due to too small number of patients. The acute IR after liver resection shown by the sensitive FSIGTT testing method had no significant influence on the APO level indicating that insulin resistance was not a determinant of APO levels.

Another explanation of paradoxically elevated APO levels in patients with cirrhosis is an increase of atrial natriuretic peptide (ANP) or brain natriuretic peptide (BNP) release as a consequence of altered hemodynamics [31-33]. ANP was previously shown to be a major determinant of APO levels and most likely explains the paradoxical association of APO with mortality [34]. This may also explain the paradoxical association with body weight which probably contributes to the elevation of ANP levels due to its hemodynamic effects in liver cirrhosis.

In our study a normalization of APO serum levels one year after transplantation occurs (**Figure 2**). This finding supports the important role of the liver in the APO metabolism [6,7]. Pro insulin (P-INS) is an insulin precursor, the serum concentrations of which increase if the β-cell insufficiency occurs. A level above 11 pmol/ml was proposed to indicate β-cell dysfunction [19]. In our study significant elevation of P-INS levels were observed in patients with liver cirrhosis who were insulin resistant indicating that liver dysfunction is associated with disturbed processing of insulin in β-cells. Remarkably, the insulin resistance induced by partial liver resection (**Figure 4**) did not increase P-INS indicating that acute insulin resistance does not rapidly induce β-cell dysfunction. This is readily explained by the capacity of intact β-cells to increase insulin secretion several fold [35]. Moreover, normalization of P-INS levels was observed upon improvement of hepatic function and regain of normal insulin sensitivity in the transplant recipients. This indicates that either prolonged insulin resistance caused secondary β-cell dysfunction or metabolic factors associated with liver cirrhosis impair β-cell function. However, this impairment was clearly reversible and P-INS was not a marker of irreversible β-cell decline in liver cirrhosis. This confirms our earlier observation that after major hepatic resection no severe β-cell dysfunction (stage III, [19]) occurs. Still, healthy patients with normal preoperative glucose homeostasis develop postoperatively significant IR. Thus this ought to occur due to the intraoperative management of the patient, or neuroendocrine stress response to the surgery [15], and not to β-cell insufficiency.

We could show a normalization of P-INS serum level as well as insulin resistance one year after transplantation (**Figures 4** and **1**). Increased insulin resistance and diminished insulin sensitivity are common in patients with liver cirrhosis [36]. Also its improvement in majority of cases after liver transplantation was described [37]. Our findings indicate, that also in those patients improved insulin resistance might be assessed using P-INS serum level.

In conclusion we confirm that APO levels are paradoxically increased in patients with liver cirrhosis. This is either due to

increased ANP or BNP release as a consequence of altered hemodynamics or lower hepatic APO uptake in the insufficient liver. P-INS might be used as a marker for hepatic IR in patients with liver cirrhosis. β-cell insufficiency seems not to play a major role in the development of postoperative IR.

## Competing interests

The authors declare that they have no competing interests.

## Authors' contributions

| Authors' contributions | MM | TK | SN | PM | DH | AP1 | AP2 | JL | AS | PN | MS |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Research concept and design | -- | ✓ | -- | -- | -- | -- | -- | -- | -- | -- | ✓ |
| Collection and/or assembly of data | ✓ | -- | ✓ | -- | ✓ | ✓ | -- | -- | -- | -- | -- |
| Data analysis and interpretation | ✓ | ✓ | ✓ | -- | ✓ | -- | ✓ | ✓ | ✓ | -- | ✓ |
| Writing the article | ✓ | -- | -- | -- | -- | -- | -- | -- | -- | -- | ✓ |
| Critical revision of the article | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ |
| Final approval of article | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ |
| Statistical analysis | ✓ | -- | -- | ✓ | -- | -- | -- | -- | -- | -- | ✓ |

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